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Isolation of Flavonoid (Z)-2-(3-Acetyl-4-Hydroxy Benzylidine)-6-Hydroxy-4-Methoxybenzofuran-3(2h)-One of Rheum Rhabarbarum and Evaluation of Antimicrobial Activity against a Panel of Human Pathogens

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Abstract **- Rheum rhabarbarum, often referred to as rhubarb, has been utilized as a healing herb in various nations. Particularly, its stems are recognized as a traditional remedy in various cultures. Rhubarbs contain numerous bioactive substances, belonging to diverse groups of phytochemicals, e.g. stilbenes, anthraquinones and flavonoids. In this study Phytochemical screening of Rheum rhabarbarum stems revealed the presence of flavonoids, alkaloids, sterols, saponins and glycosides. Stems Rheum rhabarbarum were macerated with 95% ethanol to prepare for isolation. The method used for isolation was a combination column chromatography (CC) followed by thin layer chromatography (TLC), which gave flavonoid (z)-2-(3-acetyl-4-hydroxy benzylidine)-6-hydroxy-4-methoxybenzofuran-3(2H)-one. And the structures of the isolate were elucidated via a combination of spectral techniques. Also study the antimicrobial activity of ethanol extract of R. rhabarbarum stems against bacterial and fungal strain. And the results showed that the ethanolic extract was very active against Staphylococcus aureus, Bacillus subtilis, and the fungus Candida albicans, and active against Pseudomonas aeruginosa, and inactive against Escherichia coli.**

Keywords: Rheum rhabarbarum, medicinal herb, Flavonoid, Antimicrobial, TLC.

I. INTRODUCTION

Rheum rhabarbarum is a perennial vegetable belonging to the Polygonaceous family. It is considered to be a common vegetable known as sibiric rhubarb ab [1]. The color of the stalks varies according to the variety. Rhubarb stalk can be red, pink, or green [2,3]. Rhubarb is a source of biologically active ingredients necessary for the treatment and prevention of lifestyle-related diseases due to its laxative, diuretic, antibacterial, anti-inflammatory, and anticancer properties [4,5]. Rhubarb roots are used as oriental laxative medicine and an antipsoriatic drug, also used against diarrhoea, as well as stomachic, antiemetic, haemorrhoids, measles, smallpox, and cholagogue [6,7]. Rhubarb also showed the protective effect against liver injury and fatty liver [8,9]. Flavonoids are natural compounds of plant origin that represent, with their abundance, the largest group of polyphenolic compounds [10]. Their common natural sources are mainly different sorts of fruits, vegetables, and cereals. In nature, they can exist as aglycons, but more often they form glycosides [11]. Many studies have shown an inverse correlation between intake of food rich in flavonoids and a risk of cardiovascular, cancer, and other diseases associated with ageing [12-14]. Flavonoids are the secondary plant metabolites with diversities of pharmacological activities like antioxidant, anticancer, anti-inflammatory, hepatoprotective, and also antimicrobial [15]. Natural products showed more antimicrobial activity than conventional drugs, so flavonoids could be an effective treatment of pathogenic infections [16]. The flavonoid isolated from the leaf extraction of Hymenonsporum flavum gives a lead structure for drug development strategies against cancer [17]. Flavonoid and total phenolic contents of Salacca zalacca combined with Bacillus velezensis B-27 gave the highest antioxidant [18]. The flavonoid extract from Costus afer showed activity against Staphylococcus aureus, Escherichia coli, and Pseudomonas spp [19]. The flavonoid from the ethanolic extract of the leaves of snake grass has antioxidant and antibacterial activity [20]. Antimicrobial and antioxidant activites of the phenolic compound and flavonoid extract from Ganoderma lucidum were increased by increasing the concentration. Staphylococcus aureus shows the highest sensitivity to Ganoderma extract with the minimum inhibitory concentration [21]. The ethanolic extracts of the leaves and pulp of Campomanesia xanthocarpa have a greater number of flavonoids and are more bioactive [22]. Flavonoids Isolate from leaves of Derris Scandisk and performed screening for

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bioactive secondary metabolites in Sri medicinal plant by a process known as microfractionation showed MICs of 23 g Ml-1 against S. aureus also exhibited relevant MICs on species of various Gram-negative bacteria such as E. coli and Pseudomonas aeruginosa [23]. Isolated hesperetin-A from Cordia sebestena flower extract; it has antibacterial activity [24]. 3,4′,5-trihydroxy-3′,7-dimethoxyflavone Isolated from surface exudates of the leaves of Dodo- naea Angustifolia showed broad- spectrum antibacterial activities against E. coli and B. pu- milus with minimum inhibition concentration (MIC) [25]. Antibacterial activity of flavonoids isolated from Croton piauhiensi mull active against S. aureus and E. coli [26]. Musa acuminata acuminata leaf is rich in bioactive flavonoids such as kaempferol-3-O-rutinoside and rutin, with relatively high antioxidative, antidiabetic, and anti-inflammatory activities [27]. More than 40 phenolic compounds of the peel of different banana species. It has potent antioxidant and antimicrobial activities [28]. The whole plant of Coccinia grandis having pharmacological activities like analgesic, antipyretic, anti-inflammatory, antimicrobial, antiulcer, antidiabetic, antioxidant, hypoglycemic, hepatoprotective, antimalarial, antidyslipidemic, anticancer, antitussive, mutagenic [29]. The ethanol extract of the stems of the Cambodian plant (Plumeria alba L.) inactive antioxidant. The ethanol extract flowers and leaves are active antioxidants [30]. The objective of this study is to evaluate antimicrobial activity and the isolation of flavonoid (z). -2-(3-acetyl-4-hydroxy benzylidine)-6-hydroxy-4-methoxybenzofuran-3(2H)-one of Rheum rhabarbarum stems by column chromatography followed by thin layer chromatography. The structures of these isolates were elucidated via a combination of spectral techniques.

II. MATERIALS AND METHODS

2.1 Plant material and sample preparation

Stems of Rheum rhabarbarum were purchased from the local market - Khartoum. taken to the Laboratory and prepared to worked on it. The plant was authenticated by direct comparison with a herbarium sample.

2.1.1 Solvents

All solvents used are of analytical grade. Methanol HPLC grade is used for spectroscopic purposes (BDH, England).

2.1.2 Ethanolic Extract

Preparation of plant extract for phytochemical screening (200g) of powdered shade-dried stems of Rheum rhabarbarum were extracted with 80% ethanol (soxhlet) for 6 hours. Thereafter, the cooled solution was filtered through a Whatmann filter paper No. 42 (125mm) and subsequently through cotton wool. The extract was then concentrated using a rotary evaporator with the water bath set at 40oC was used to concentrate extract and finally with a freeze drier. This was followed by storage of dried residue at 4ºC. The crude extract residue were then weighed and dissolved in distilled water for experimental analysis.

2.1.3 Phytochemical Screening

Tests were carried out on the prepared extract to identify of flavonoids, saponins, tannins, alkaloids, carbohydrates and/or glycosides, and triterpenes and/or sterols by phytochemical screening.

III. QUALITATIVE ANALYSIS OF THE PHYTOCHEMICALS

3.1 Test for saponins (Froth test)

The dried extract was dissolved in water, transferred into a test tube, and shaken vigorously. Then it was left to stand for 30 minutes. When a thick, persistent froth appears and persists for one hour, saponins are present.

3.1.1 Test for alkaloids

(50 mg) of extract was dissolved in 50 ml of methanol in a water bath for 20 minutes; the extract was then filtered off and allowed to cool. Two ml of the extract was poured into test tubes. Drage Dorff's or Mayer's reagent was added to the tube, and the presence or absence of colours or any precipitates was noted.

3.1.2 Test for tannins

(50 mg) of extract was dissolved in 50 ml of water. The aqueous extract was then treated with a 15% ferric chloride solution. A blue colour indicates condensed tannins; a green colour indicates hydrolysable tannins.

3.1.3 Test for sterols and triterpenes (Liebermann-Burchard test)

1 ml of glacial acetic acid was added to (1 ml) chloroform and cooled to 0 °C, then one drop of concentrated sulphuric acid was added to the cooled mixture, followed by the extract.

3.1.4 Test for flavonoids

(50 mg) of extract was defatted with petroleum ether, and the residue was dissolved in (30 ml) 80% methanol and filtered. The filtrate was used for the following tests: (1) To 30 ml of the filtrate, a few drops of methanolic aluminium chloride were added. A dark yellow colour was observed. (2) To 30 ml of the filtrate, a few drops of potassium hydroxide

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solution were added. A dark yellow colour was observed. (3) To 30 ml of the filtrate, a few drops of ferric chloride solution were added. A blue colouration was observed.

3.1.5 Isolation of flavonoids

Analytical (TLC) was carried out using aluminium sheets precoated with Kiesel gel 60F 254 of 0.2 mm thickness. The aim was to detect a suitable solvent system for separation of flavonoid to monitor fractions from the column and to determine the purity of the isolate. The spotted (TLC) sheets were developed in the saturated vapour chromatographic tanks by using suitable solvent systems. The spots were then viewed in UV light using both (max. 366 nm and max. 254 nm) with and without exposure to NH3 vapour. An open column (80 4 cm) was used for fractionation of the ethanolic extracts of rhubarb roots. Silica gel with particle size 100–200 mesh from LOBA chemicals was used as the stationary phase. The composition of the mobile phase (acetic acid 60%) was determined by TLC analysis. The column was packed with a slurry of silica gel with acetic acid and then allowed to equilibrate for two hours before use. The ethanolic extract of rhubarb roots (3 g) was mixed with 10 g of silica gel and then applied on the top of the column. Elution commenced by acetic acid 60% Fractions of 10 ml were collected. Depending on their TLC pattern fractions, F2–FF6 were pooled together. Further fractionation on TLC plates using the solvent system ethyl acetate: hexane (2:3; v: v) gave compound I. The purity of the isolate was checked by TLC using 3 different solvent systems.

3.2 Antimicrobial assay

The ethanol extract of Rheum rhabarbarum was evaluated for antimicrobial activity against four human pathogenic bacterial strains: Gram-positive (Staphylococus aureus and Bacillus subtilis), Gram-negative (Pseudomonas aeruginosa and Escherichia coli), and fungal species (Candida albicans). The cup plate agar diffusion bioassay was used.

3.2.1 Testing of antibacterial susceptibility

The cup plate agar diffusion assay was used to screen the antibacterial activity of plant extract and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 108 cfu/ml (turbidity = McFarland standard 0.5). One hundred microlitres of bacterial suspension were swabbed uniformly on the surface of MHA, and the inoculum was allowed to dry for 5 minutes. Sterilised filter paper discs (Whatman No. 1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of plant extract. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured in duplicates and averaged.

3.2.2 Testing of antifungal susceptibility

The above-mentioned method was adopted for antifungal activity, but instead of agar, potato dextrose agar was used. Samples were used here at the same concentrations used above.

IV. RESULTS AND DISCUSSION

Phytochemical screening of Rheum rhabarbarum stems revealed the presence of flavonoids, alkaloids, sterols, saponins and glycosides. Stems of this species were macerated with 95% ethanol.

4.1 UV-Vis Spectroscopy

UV-Vis spectroscopic study of flavonoids is a very useful tool to elucidate the structure of flavonoids and give tentative information about different subclasses of flavonoids and their substituents in the structures [31]. Absorptions due to the benzene rings A and B and their possible conjugations to ring C give rise to the UV-Vis spectra of flavonoids. All flavonoids show an absorption maximum at around 240-290 nm (band II), which varies by the conjugation of the ring A benzoyl system and its substitution pattern. Band I is considered to be associated with absorption due to the B-ring cinnamoyl system absorbance maximum of (band I) flavone 300-350 nm, flavonol 350-385 nm, flavanone 300-330 nm, and isoflavones 300-340 Chalcones and aurones having an elongated conjugation system from their ring B show a second absorption maximum around 345-390 nm for chalcones and at 390-430 nm for aurones, an absorption maximum around 460-560 nm for anthocyanins [32]. In the UV absorbs (Fig. 1) at λmax 279,414nm. Such absoption is characteristic of aurones. Very significant structural features may also be obtained by utilising a combination of UV shift reagents: sodium acetate, aluminium chloride, and boric acid. These reagents produce shifts. In the UV absorption maxima in accordance with the location of the various hydroxyl groups in the flavonoid nucleus.

Sodium acetate is a weaker base and ionizes only the more acidic hydroxyl groups in flavonoid 3-,7-, and 4'-hydroxy groups. The ionisation of the 7-hydroxy group mainly affects band II. Particularly NaOAc is a useful diagnostic reagent for the specific detection of the 7-hydroxyl group. Certain 3' and 4' dioxygenated derivatives without the 7-OH group showed bathochromic shifts of 20–25 nm. The sodium acetate spectrum of (z)-2-(3-acetyl-4-hydroxy benzylidine)-6-hydroxy-4-methoxybenzofuran-3(2H)-one revealed a

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bathochromic shift suggesting a free 6-OH function (Fig. 2). Also, the sodium methoxide spectrum revealed a bathochromic shift diagnostic of a 4'-OH group (Fig. 3). However, the aluminium chloride spectrum of the compound (Fig. 4) did not reveal any bathochromic shift diagnostic of 5-, 3-OH, or catechol systems. Likewise, the boric acid spectrum, which is diagnostic of catechol systems, did not show any detectable bathochromic shift (Fig. 5). Flavonoids with 3-, 5-OH, and catechol systems can form complexes with the shift reagent, aluminium chloride. The aluminium chloride complexes with catechols are not stable in acidic media, but hydroxyl groups at C-3 or C-5 can form acid stable complexes. Such acid-stable and acid-labile complexes are shown in (Fig. 6).

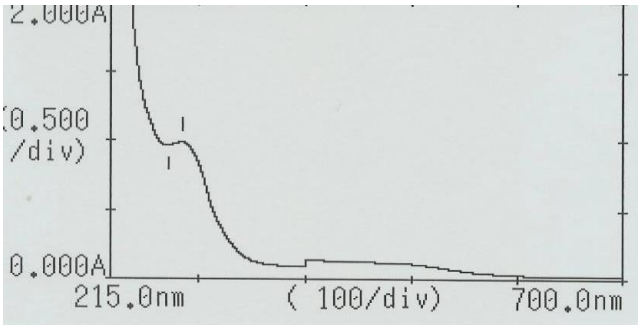

Figure (1): The UV spectrum of (z)-2-(3-acetyl-4-hydroxy benzylidine)-6-hydroxy-4-methoxybenzofuran-3(2H)-one

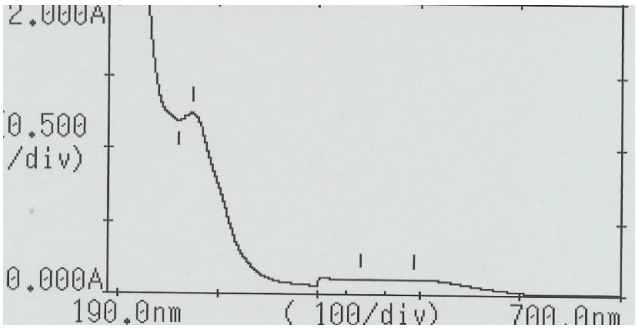

Figure (2): Sodium acetate spectrum of (z)-2-(3-acetyl-4-hydroxy benzylidine)-6-hydroxy-4-methoxybenzofuran-3(2H)-one

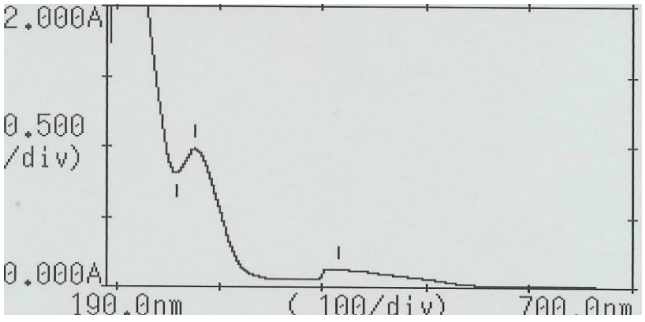

Figure (3): Sodium methoxide spectrum of (z)-2-(3-acetyl-4-hydroxy benzylidine)-6-hydroxy-4-methoxybenzofuran-3(2H)-one

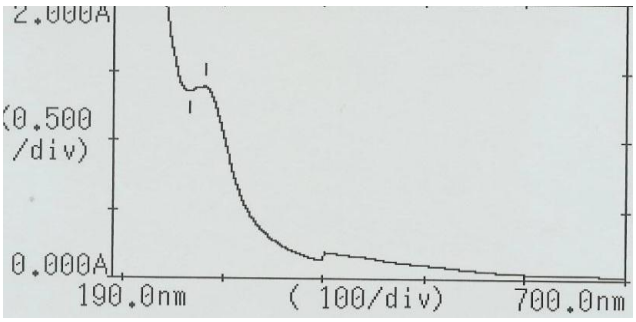

Figure (4): Aluminium chloride spectrum of (z)-2-(3-acetyl-4-hydroxy benzylidine)-6-hydroxy-4-methoxybenzofuran-3(2H)-one

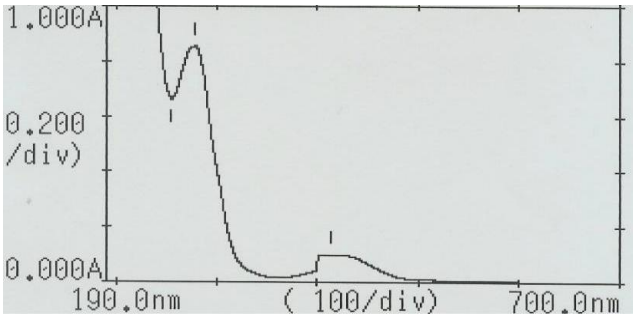

Figure (5): Boric acid spectrum of (z)-2-(3-acetyl-4-hydroxy benzylidine)-6-hydroxy-4-methoxybenzofuran

Figure (6): Formation of flavonoids-alumiinium chloride complex

4.2 Nuclear magnetic resonance 1H NMR spectrum

The H1NMR the give resonance at δ 2.20, δ 3.4, δ 3.6, δ 7.2, δ 7.60 and δ 8.10 (Fig.7).

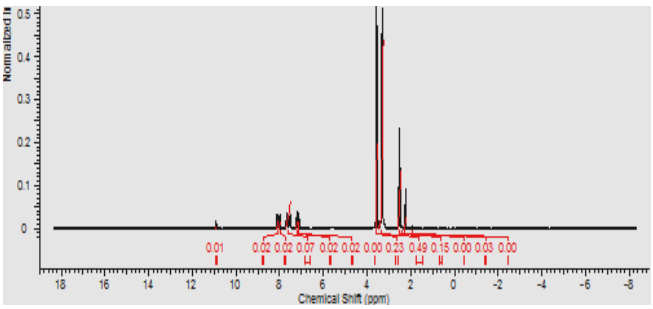

Figure (7): 1HNMR spectrum of (z)-2-(3-acetyl-4-hydroxy benzylidine)-6-hydroxy-4-methoxybenzofuran-3(2H)-one

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4.3 Mass spectrometer MS

The mass spectrum (Fig. 8) gave m/z325 corresponding to (M+- H). On the basis of the above cumulative data, the following partial structure was suggested for the compound (Fig 9).

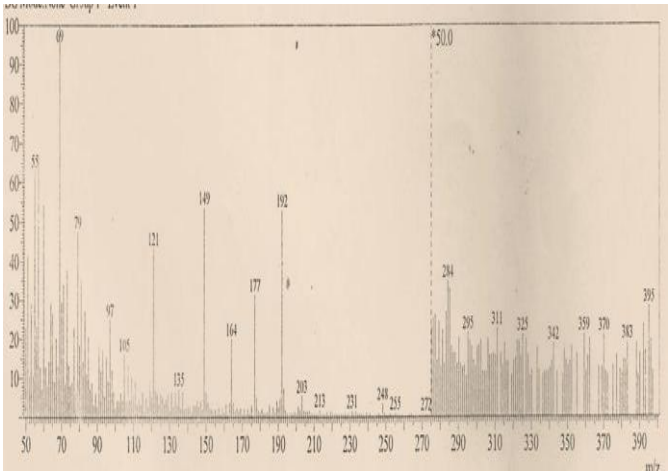

Figure (8): Mass spectrum of (z)-2-(3-acetyl-4-hydroxy benzylidine)-6-hydroxy-4-methoxybenzofuran-3(2H)-one

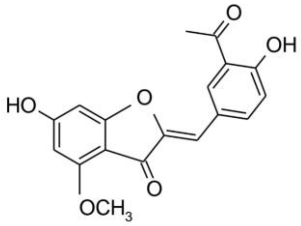

Figure (9): (z)-2-(3-acetyl-4-hydroxy benzylidine)-6-hydroxy-4-methoxybenzofuran-3(2H)-one

4.4 Antibacterial activity

Antibacterial activity of Rheum rhabarbarum ethanolic extracts the diameters average of the growth inhibition zones are shown in Table (1). Ethanolic extract of Rheum rhabarbarum were screened for their antimicrobial activity against five standard human pathogens (Table 2). And the results were interpreted in commonly used terms (< 9mm inactive; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Tables (3) and (4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi, respectively [33]. The results showed that the ethanolic extract was very active against Staphylococcus aureus, Bacillus subtilis, and the fungus Candida albicans, active against Pseudomonas aeruginosa, And inactive against Escherichia coli.

Table (1): Antibacterial activity of Rheum rhabarbarum ethanolic extract

Fraction	Conc.(mg/ml)	Escherichia coli	Pseudomonas aeruginosa	Staphylococcus aureus	Bacillus subtilis	Candida albicans
Ethanolic extract	100	-	14	25	23	18

Table (2): Test organisms

Ser. No	Micro organism	Type	Source
1	*Bacillus subtillus*	G+ve	ATCC 2836
2	*Staphylococcus aureus*	G+ve	ATCC 29213
3	*Pseudomonas aeroginosa*	G-ve	NCTC 27853
4	*Escherichia coli*	G-ve	ATCC 25922
5	*Candida albicans*	fungi	ATCC 7596

* NCTC. National collection of type culture, Colindale. England

** ATCC. American type culture collection, Maryland, USA

Table (3): Antibacterial activity of standard chemotherapeutic agents: M.D.I.Z (mm)

Drug	Conc. (mg/ml	Bacillus subtilis	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa
Ampicillin	40	15	30	-	-
	20	14	25	-	-

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	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table (4): Antifungal activity of standard chemotherapeutic agents against standard fungi

Drug	Conc. mg/ml	Candida albicans
Clotrimazole	30	38
	15	31
	7.5	29

M.D.I.Z: Mean diameter or growth inhibition zone (mm).

V. CONCLUSION

The results of this study Phytochemical screening of Rheum rhabarbarum stems revealed the presence of flavonoids, alkaloids, sterols, saponins and glycosides. The flavonoid (z)-2-(3-acetyl-4-hydroxy benzylidine)-6-hydroxy-4-methoxybenzofuran-3(2H)-one is isolated from stems of Rheum rhabarbarum. The structure of the isolate is elucidated via a combination of spectral techniques. The ethanolic extract is evaluated for antimicrobial activity with significant results.

DECLARATION OF COMPETING INTEREST

We are the authors declare that we are worked together in this work in experimental, written the result. We worked together in all the part of paper.

FUNDING

The Authors received NO FUNDING for this work.

DATA AVAILABILITY

The data sets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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