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Original Research

Antibacterial Activity of *Aloe barbadensis* Mill

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Abstract

Plant secondary metabolites are rich source of medication ever since millennium time and can efficiently alienate biological actions. *Aloe vera* has been enchanted as a matchless cure for ailments globally ever since the historical epochs due to its ample biological actions. In the present study, ethanolic extract of *A. vera* leaves gel and its fractions (n-Hexane, Petroleum Ether, Chloroform, Dichloromethane, Acetone, Methanol and Aqueous) were being used for phytochemical analysis. In qualitative phytochemical exploration, the ethanolic extract and various fractions have shown the presence of numerous secondary metabolites including phenolics, flavonoids and alkaloids in all samples. In quantitative phytochemical investigation, the explicit appearance of the total phenolic, flavonoids and alkaloids content was recorded in methanol, dichloromethane and aqueous fractions. The ethanolic *A. vera* extract and its fractions were used to evaluate their antibacterial activity. *A. vera* ethanolic extract, methanol and aqueous fraction have exhibited greater antibacterial activity rather than other fractions. Thus, *A. vera* encompasses plentiful quantity of phytochemicals indicating its importance for exploitation as a herbal remedy for different diseases and as antibacterial agent.

Keywords: ethanolic extract, fractionation, phytochemical, antibacterial activity, *A. vera*

Introduction

In modern medicine, antibiotics are the essential agents to fight the microbes known as the marvel drugs but in reality, these are the major threats globally to our health, food protection and industries. They are not just used for pharmaceutical purposes but also being implemented prophylactically in animal farming and in agricultural industries. The augmented usage of antibiotics has led to devastating circumstances leading to fueling of antibiotic resistance (AR) in micro-organisms [1-3]. Although antibiotics are the revolutionary pharmaceutics, curing fatal infections, but their extensive usage is responsible for alarmingly increasing resistance of bacteria leading to deadly infections untreatable [4]. The emerging resistome of these magic bullets have given rise to resistant strains due to the poor sanitation, usage in clinics and disposal structure, quarantine of traveling, excellence of drugs and their diagnostics [5]. Bacteria develop resistance against antibiotics through numerous factors such as chromosomal mutations, intrinsic resistance genes development and acquiring certain genetic elements such as vectors, plasmids and transposons which work as vectors. Plasmids are the key components driving resistance and can be easily transferred from one to other bacteria by means of lateral DNA transfer (LDT) mechanisms. Resistance occurs through natural selection when bacteria are exposed to some antibiotics for prolong period of time [2, 5-7].

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A large number of natural anti-oxidant compounds exist in *A. vera* such as ascorbic acid, carotenoids, flavonoids, glutathione peroxidase, phenolics, superoxide dismutase, tannins and vitamins C and E. It mechanically works as reducing the oxidative stress of cell devastation and also reduces physiological and biochemical changes [8-12]. The antiseptic capability of *A. vera* is basically due to the presence of six novel compounds specifically cinnamomic acid, lupeol, phenol, salicylic acid, sulfur and urea nitrogen. Their assortments have obstructive action on various diseases, organisms and parasites [13, 14]. The antiseptic agents such as lignin is abundantly present in its pulp along with other components such as saponins and these including glucosides with them possessing cleansing properties are involved in penetration into epithelial tissues dealing with various skin issues also dental problems [15].

In the present study, *A. vera* leaves gel is used to evaluate its antibacterial potential through its various polar and non-polar fractions along with its qualitative and quantitative phytochemical analysis.

Material and Methods

Plant Material

The *A. vera* plant was grown using department facilities in the Botanical Garden of Botany Department, PU. Fresh leaves were collected from *A. vera* plant for each extraction and the outside greenish rind was removed after which the transparent gel exudate present in the leaves of *A. vera* was used for the preparation of extract.

Preparation of Extract and Fractionation

The preparation of *A. vera* ethanolic extract was done by using the mechanical shaking method [16]. Primarily the clear gel removed from the leaves was homogenized by means of grinder which was mixed with ethanol. Then the solvent-gel mixture was placed in mechanical shaker for 24 hours which was then filtered using Whatmann No. 1 filter paper and finally the filtrate was oven dried at 40ºC. After ethanolic extraction, the extract residues were then further sequentially utilized for the preparation of different fractions following the method based on the polarity gradient solvents i.e., n-Hexane (F1), Petroleum Ether (F2), Chloroform (F3), Dichloromethane (F4), Acetone (F5), Methanol (F6) and Aqueous (F7) fractions.

Phytochemicals Exploration

The *A. vera* ethanolic extract and its fractions were examined for the qualitative presence of different phytochemical compounds i.e., tannins, phenolics, saponins, alkaloids, flavonoids, steroids, tri-terpenes and glycosides [17-19].

Quantitative screening of phenolics, flavonoids and alkaloids of *A. vera* ethanolic extract and its fractions was carried out through colorimetric protocol. The stock solutions for estimation of total phenolic content, total flavonoid content and total alkaloid content were set at 1mg/ml for ethanolic extract and its fractions [19].

Total Phenolic Content (TPC)

For the evaluation of total phenolic content, Folin-Ciocalteu reagent methodology was used which was based on the principle that in alkaline circumstances, reduction of phosphotungstate-phoshomolybdate compound indicated the existence of phenolics and articulated as mg GAE/g weight of the dry extract [19].

Total Flavonoid Content (TFC)

The screening of total flavonoid content was done through the aluminium chloride protocol and articulated by mg QE/g of the weight of the dry extract [20].

Total Alkaloid Content (TAC)

The colorimetrical examination of the total alkaloid content is reliable on the reactivity between bromocresol green (BCG) and an alkaloid which was expressed by means of mg AE/g of the dry extracts weight [21].

Antibacterial Assay

The antibacterial potential of ethanolic extract and its fractions was done by means of disc diffusion method [22]. Nine bacterial strains were used for the activity i.e., *Aeromonas* sp. (TR2a, TR2e, TS2j), *Pseudomonas* sp. (TR2c, TR2f, TR1k, TF1d), *Pseudomonas picketti* (TS3d), and *Chryseobacterium meningosepticum* (TR2g). The activity was carried out in two categories i.e., control and experimental group. The control group comprises of positive and negative control. In positive control, commercially prepared cefadroxil drug (30 mg) was used while negative control contains only DMSO, whereas in experimental group, the ethanolic extract and its fractions were being evaluated [23], after liquefying them in DMSO (0.5 g/ml) for the analysis. The streaking of bacterial cultures was being done on L-agar plates and the discs i.e., control/experimental were positioned at equivalent intervals on plates and were left for 24 hours at 37ºC for incubation. After incubation, the plates were perceived for the bacterial resistivity and sensitivity. The antibacterial activity of *A. vera* ethanolic extract and its fractions was carried out in replicates on each plate and the mean zone of inhibition was calculated for each bacterial isolate.

Table 1. Qualitative Phytochemical analysis of *A. vera* ethanolic extract and its fractions.

Phyto-chemicals	Ethanolic ext.	n-Hexane (F1)	PET (F2)	$ChCl_3$ (F3)	DCM (F4)	Acetone (F5)	MeOH (F6)	Aqueous (F7)
Tannins	++	--	+	+	++	+	+	--
Phenolics	++	+++	++	+	+++	+	+++	+++
Saponins	--	--	--	++	++	++	--	--
Alkaloids	+++	++	+++	+++	+++	+++	+++	+++
Flavonoids	+++	+	+++	+	+++	+++	+	+
Steroids	--	--	--	--	--	--	--	--
Tri-terpenes	++	--	--	--	--	+	+	--
Glycosides	--	--	--	--	--	--	--	--

+++ intensely present, ++ moderately present, + mildly present, -- absent

Results and Discussion

Phytochemicals Exploration

The qualitative exploration of phytochemicals demonstrated the influential expression of phenolics, flavonoids and alkaloids in ethanolic *A. vera* extract and it's all fractions, tannins were also present except in n-Hexane (F1) and Aqueous (F7) fractions while saponins were present in Chloroform (F3), DCM (F4) and Acetone (F5) fractions. Triterpenes were only present in ethanolic extract, Acetone (F5) and Methanol (F6) fractions. However, steroids and glycosides were not present in any samples (Table 1).

Total Phenolic Content (TPC)

In quantitative phytochemical screening, the phenolic content varied between 53.6 mg/g to 398.0 mg/g. Marked presence of phenols was recorded in MeOH (F6) fraction while the lowest content of phenols was found in Acetone (F5) fraction (Fig. 1). The orderly array of concentration of phenolic content according to each fraction is as following:

MeOH>DCM>Aqueous>n-Hexane>Ethanolic ext.
>PET>Chloroform>Acetone

Total Flavonoid Content (TFC)

A. vera flavonoid content ranged from 0.53 mg/g to 776.7 mg/g. Maximum flavonoid content was recorded in DCM (F4) fraction and the minimum quantity was observed in Aqueous (F7) fraction (Fig. 1). The serial assortment of flavonoid content in each fraction is described as:

DCM>PET>Acetone>Ethanolic ext.
>Chloroform>Methanol>n-Hexane>Aqueous

Total Alkaloid Content (TAC)

A. vera alkaloids content varies between 1483.6 mg/g to 1670.6 mg/g, correspondingly. The aqueous (F4) fraction has displayed extravagant content of alkaloids and DCM (F4) fraction has expressed the least alkaloids, amongst all fractions (Fig. 1). The orderly array of alkaloids as per each fractions is as follows:

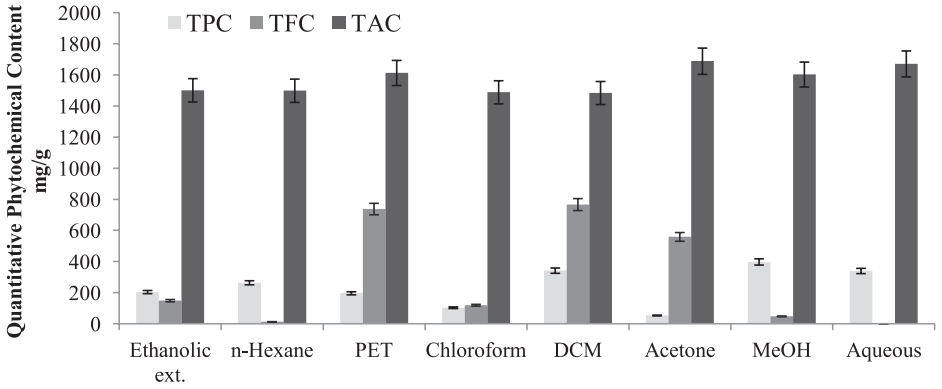

Fig. 1. Estimation of Total Phenolic content, Total flavonoid content and Total Alkaloid content of ethanolic *A. vera* extract and its fractions.

Table 2. Antibacterial activity of *A. vera* ethanolic extract and its fractions.

Bacterial Strains	Mean diameter of inhibition zone (mm)								
	Cefadroxil	Ethanolic ext.	n-Hexane (F1)	PET (F2)	$CHCl_3$ (F3)	DCM (F4)	Acetone (F5)	MeOH (F6)	Aqueous (F7)
TF1d	4.00±0.7	4.1±0.91	0.75±0.46	1.00±0.0	1.00± 0.0	0.87±0.35	1.00± 0.0	1.87±0.35	1.56±0.62
TR1k	1.00±0.0	2.2±0.46	0.62±0.51	0.75±0.46	0.87±0.23	0.75±0.41	1.00± 0.0	3.00±0.75	2.00± 0.0
TR2a	4.00±0.0	7.7±0.88	0.60±0.51	0.75±0.88	1.00± 0.0	1.00± 0.0	1.00± 0.0	1.75±0.70	2.00±0.75
TR2c	2.00±0.0	1.0±0.0	0.25±0.46	0.87±0.35	1.06±0.17	1.00± 0.0	0.25±0.46	2.12±0.35	1.75±0.70
TR2e	16.00±0.0	9.6±1.06	0.75±0.88	--	0.37±0.44	1.12±0.35	1.00± 0.0	--	0.93±0.56
TR2f	2.00± 0.0	6.1±0.83	1.00± 0.0	0.77±0.48	0.81±0.37	0.87±0.35	1.00± 0.0	1.00± 0.0	--
TR2g	15.00±0.0	2.1±0.35	0.12±0.35	0.62±0.44	0.75±0.46	2.00± 0.0	1.00±1.06	3.75±1.03	--
TS2j	--	6.2±0.88	1.00± 0.0	1.00± 0.0	0.75±0.46	1.25±0.46	1.00± 0.0	2.62±1.30	1.25±0.70
TS3d	1.00±0.0	4.0±0.75	0.75±0.46	--	1.00± 0.0	0.87±0.35	1.00± 0.0	1.00± 0.0	1.75±0.88

Aqueous>Acetone>PET>MeOH>Ethanolic ext. >n-Hexane>Chloroform>DCM

Antibacterial Assay

In *A. vera* ethanolic extract and its fractions, significant zones of inhibition were being expressed by ethanolic extract and MeOH (F6) fraction, amongst the other fractions. The aqueous (F7) fraction has also expressed noteworthy zones of inhibition. However, the DCM (F4) and Acetone (F5) fractions have exhibited better antibacterial potential then other non-polar fractions (Table 2). The Cefadroxil (positive control) has expressed remarkable zones of inhibition from 1mm to 16mm in size. *A. vera* ethanolic extract has proved to be very effective against the isolate *Aeromonas* sp. (TR2e) with inhibition zone of 9.6mm which was comparable to the inhibition zone produced in the presence of the control treatment using cefadroxil (Fig. 2).

Among all the *A. vera* extracts and its fractions, the biggest zone of inhibition was produced by the ethanolic extract i.e., 9.6mm while the n-Hexane (F1) fraction showed the least anti-bacterial potential with inhibition zone of 0.12mm. Other non-polar fractions i.e., PET (F2), Chloroform (F3), DCM (F4) and Acetone (F5) fractions inhibition zones were assorted between 0.25 to 2 mm. The polar MeOH (F6) and Aqueous (F7) fractions produced array of zones between 0.93 to 3.75 mm. Among the bacterial strains, the *Aeromonas* sp. (TS2j) isolate was resilient towards the Cefadroxil (positive control) and did not express any zone of inhibition. Similarly in the *A. vera* plant fractions the *Aeromonas* sp. (TR2e) and *Pseudomonas picketti* (TS3d) strains was resistive in the PET (F2) fraction and in methanol (F6) fraction, TR2e was resistant only. The *Pseudomonas* sp. (TR2f) and *Chryseobacterium meningosepticum* (TR2g) strains also did not demonstrated inhibition zone in Aqueous (F7) fraction (Table 2).

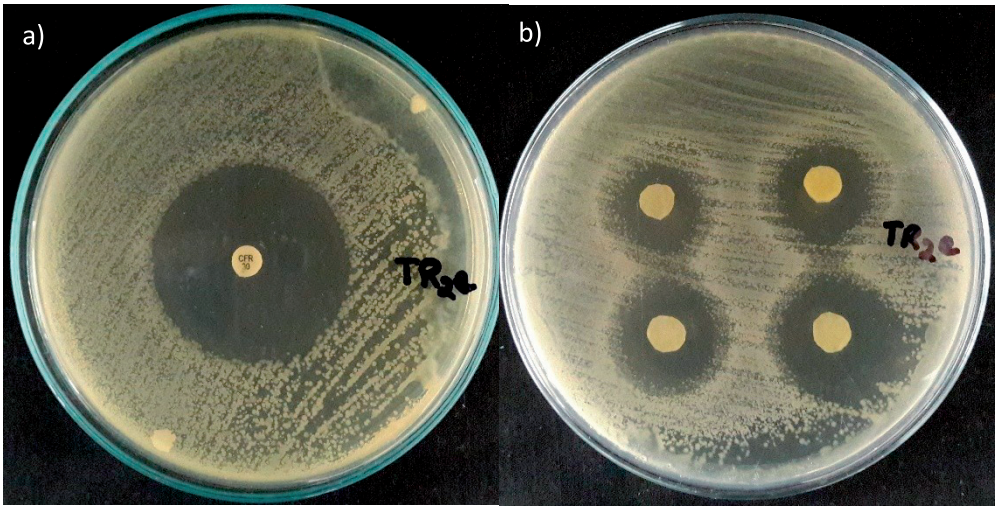

Fig. 2. Antibacterial activity against TR2e strain: a) Cefadroxil and b) *A. vera* ethanolic extract.

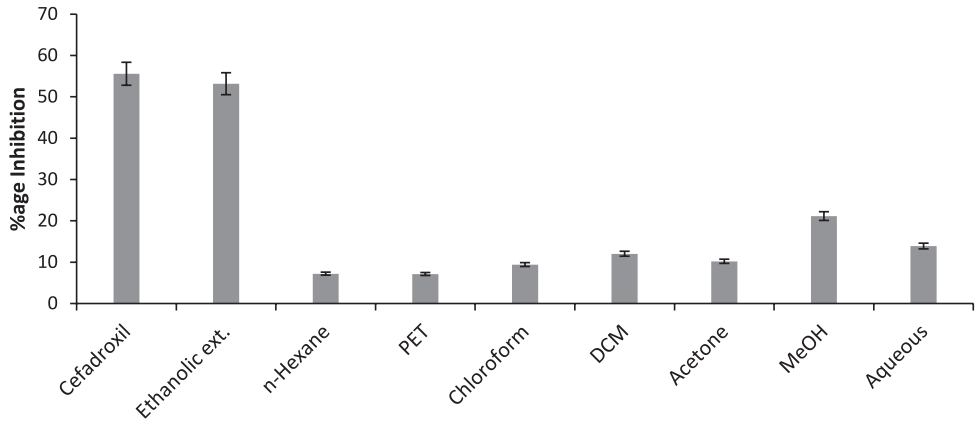

Fig. 3. Comparison of %age Inhibition of bacterial growth in *A. vera* ethanolic extract and its fractions with Cefadroxil.

The DMSO (negative control) had no influence on the antibacterial activity and did not show any zone of inhibition. The total percentage inhibition competency of positive control and the *A. vera* ethanolic extract and its fractions among all strains revealed that the highest percentage inhibition was of cefadroxil (positive control) which was 55%. However, amongst the *A. vera* ethanolic extract and its fractions, the utmost percentage was of ethanolic extract and the minimum percentage of inhibition potential was of n-Hexane (F1) fraction i.e., 53.14 and 7.11%, correspondingly (Fig. 3). The percentage antibacterial activity of ethanolic extract and its fractions was in the following order:

Ethanolic ext.>MeOH>Aqueous>DCM >Acetone>Chloroform>n-Hexane>PET

A. vera is one of the most commonly used medicinal plant in the human society since ancient times being used as a core source of herbal medication in Unani, Ayurvedic, Allopathic, Siddha and Homeopathic as rivulets of remedy. Its leaves are flooded with plentiful compounds, natural sugars, vitamins, enzymes, minerals, amino acids etc. The gel present in its leaves is being administered for numerous purposes such as in pharmaceutics, food supplements, nutraceuticals, and profligately in cosmetic industry. Literature has reported its exploitation even since time periods of Cleopatra, Egyptian queens, Nefertiti, the great Alexander, Christopher Columbus alongside multiple regions in the world [24, 25]. However, *in vitro* analysis and organic analysis was needed specifically for its anti-bacterial potential and phytochemical analysis. Phytochemical analysis of *A. vera* ethanolic extract and its various fractions exhibited the presence of tannins, phenolics, saponins, alkaloids, flavonoids, and triterpenes whereas steroids and glycosides were absent in the ethanolic extract and all fractions, although there is slight variability according to the presence and absence of these compounds with respect to their extraction solvents [26-29]. The quantitative phytochemical analysis of total phenolic content, total flavonoid content and total alkaloid contents expressed variable presence of these substances in ethanolic *A. vera* extract and its fractions, the supreme most was the total alkaloid content among all these. Our data is also supported by the research work of other scientists who reported the variable presence of TPC, TFC and TAC in *A. vera* various extracts [27, 29, 30].

Terpenoids are one of the prime groups of secondary metabolites involving almost 50,000 of the natural plant products. They are insoluble in water and they contribute to the odoriferous attributes of plants [31, 32]. Tannins are the polyphenolic compounds which are the key ingredients in tannin industry because these have the capability of protein precipitation thus; they enhance the protein cross-linkage. Also tannins strongly influence bactericidal and fungal activities [33]. Alkaloids are the heterocyclic nitrogenous class of compounds, naturally synthesized from amino acids and are chiefly involved in protective actions such as anesthesia, anti-biotic, analgesia, cardiac stimulant, anti-cancer, respiratory relaxant and stimulant, vasoconstriction, antineoplastic, cytotoxic, anti-microbial, insecticidal etc. Phenolic compounds are the biggest category of plant secondary metabolites comprising of phenolic group, majorly involved in defensive mechanisms, coloration, growth and reproduction of plants. They are pervasive among plants pharmacologically and involved in multiple biological activities such as anti-inflammatory, anti-tumor, anti-microbial and anti-mutagenic etc. Flavonoids are basically the largest collection of hydroxylated polyphenolic components, and are present in all vascular plants especially in the regions of leaves, roots, stems, flowers, fruits and wood and are to be synthesized in response to microbial infections. They are involved in various biological activities such as anti-tumor, anti-inflammatory, anti-allergic, gastric disorder, anti-thrombotic etc. The secondary metabolites belonging to a group of polycyclic aglycone moiety that possess different chemical compound classes are the carbohydrates, steroids and triterpenoids. They

play their part in numerous biological activities such as anti-microbial, anti-tumor, analgesic, molluscicidal, expectorant, nematocidal, sedative activities [31, 33].

From current study of *A. vera*, the presence of vast variety of phytochemicals has been proved that possesses various biological activities. The ethanolic extract has shown highest percentage inhibitory capability among all other fractions and much closer to the positive control (Cefadroxil) while among other polar fractions; MeOH (F6) and Aqueous (F7) fractions have represented considerable inhibition. As mentioned above the activities of different phytochemicals in literature provide the correlated evidence regarding antibacterial activity of *A. vera* extracts and fractions because from results it has been observed that the ethanolic extract possess the maximum variety of qualitative phytochemicals which can be directly related to its antibacterial potential correspondingly in MeOH (F6) and Aqueous (F7) fractions the abundant presence of alkaloids and phenolics contents. Although all the fractions have shown ample total alkaloid content but the total flavonoid content was much better in non-polar fractions especially in PET (F2), DCM (F4) and Acetone (F5) fractions among the other polar fractions although they did not articulate much greater zones of inhibition. However. the total phenolic content presence was in an array among all *A. vera* plant samples. Also the qualitative appearance of phytochemicals was variable. Finally the total resistivity of some bacterial isolates i.e., TR2e, TR2f, TR2g and TS3d not representing any zone of inhibition in different *A. vera* fractions i.e., PET (F2), MeOH (F6) and Aqueous (F7) fractions even though the variable phytochemicals are being present in them. So at this point it is difficult to mention a key phytochemical compound being accountable for antibacterial activity of *A. vera*. Because there has been a pervasive outlook that not a solo phytochemical be responsible for any biological activity. All the phytochemicals work synergistically influencing each other. Further analysis of this study is still recommended to be carried out at one step ahead towards molecular level to view the comprehensive actions of phytochemical compounds and also answering the confrontation of bacterial strains and modifications of the *A. vera* to perform as a pharmaceutical element better than the antibiotics. Plant secondary metabolites are the key rulers of every day and in each field of our lives, being exploited in pharmaceutical, industrial, nutraceutical, cosmetics, food applications, fragrances and also in defensive sections because of the existence of bioactive compounds that are synthesized by plants in response to the biotic pressures as well as the physiological activities [32, 33, 34]. Antibacterial potential of *A. vera* gel extracts has also been reported against various pathogenic strains such as *Bacillus subtilis, Escherichia coli, Salmonella typhi,* and *Staphylococcus aureus.* Maximum inhibitory potential was observed by ethanol extract [35].

Conclusion

In conclusion, the current study has revealed the significant antibacterial activity by the polar fractions of *A. vera* i.e., ethanolic extract and methanol fraction owing to the presence of various metabolites, which is quite important with reference to the currently prevailing antibiotic resistance among the bacteria. The bacterial antibiotic resistance is expected to increase day by day leading to alarming situation especially in the post covid-pandemic era. Hence, this magical plant with reference to its various fractions can be exploited to cure a wide variety of human diseases and health issues particularly its antibacterial potential which can help the mankind to minimize the ever increasing risk of bacterial diseases organically without the intervention of chemical substances.

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Conflict of Interest

The authors declare no conflict of interest.

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