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Antibacterial Potential of Different Parts of Aerva lanata (L.) Against Some Selected Clinical Isolates from Urinary Tract Infections

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Authors' contributions

This research work is part of first author RV's Ph. D work under the guidance of second author RU and it was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

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Original Research Article

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ABSTRACT

Aims: To investigate the antibacterial activity of different parts of *Aerva lanata* against *Staphylococcus saprophyticus, Streptococcus agalactiae, Acinetobacter baumannii, Xanthomonus citri, Klebsiella pneumoniae* and *Proteus vulgaris.*

Study Design: An experimental study.

Place and Duration of Study: This study was carried out in the Department Laboratory, Government Arts College (Autonomous), Kumbakonam-612 001, Tamilnadu, India, between November 2013 and April 2014.

Methodology: The different solvents like acetone, aqueous, benzene and ethyl acetate were used for the preparation of extracts. The antibacterial activity of the different solvent extracts of root, flower and leaf of *A. lanata* was determined by agar well diffusion method. Different concentration of extracts 5 mg / 25 μ l, 10 mg / 50 μ l, 15 mg / 75 μ l and 20 mg / 100 μ l were tested against

selected microorganisms

Results: All extracts have different level of antibacterial activity and it was compared with standard drug streptomycin. The maximum level of bacterial growth inhibition was seen in root extracts of *A. lanata.*

Conclusion: The present investigation clearly indicates that the different solvent extracts of root, flower and leaf of *A. lanata* exhibited broad-spectrum antibacterial activity. The ethyl acetate extract of root have higher activity when compared to flower and leaf extracts.

Keywords: Aerva lanata; antibacterial activity; acetone; aqueous; benzene; ethyl acetate; well diffusion method.

1. INTRODUCTION

Infectious diseases caused by bacteria, fungi, viruses and parasites are a major threat to public health despite tremendous growth in human chemotherapeutic medicine. Bacterial diseases and systemic mycoses are also difficult to medicate [1]. Nowadays, the increased incidence of severe opportunistic fungal and bacterial infections in immunologically deficient patients with the development of resistance among pathogenic Gram positive bacteria, Gram negative bacteria and fungi is an important problem. So, there is a great need in the determination of new classes of natural products that may be effective against antibiotic resistant bacteria and fungi. Natural products or semisynthetic derivatives provide novel example of such anti-infective drugs [2], because of resistance against antibiotics. Medicinal plants are a boon of nature to cure a number of aliments of human beings. In many part of the world medicinal plants were used against bacteria, virus and fungal infections [3].

Medicinal plants are used as a source for medicine to relief from illness can be traced from the back over five millennia written documents of the early civilization in China and India. Among the estimated 2,50,000-5,00,000 plant species, only a small percentage of plants and phytocompounds including their fractions submitted to biological or pharmacological activities. Pharmacological investigation of a medicinal plant will reveal only a very narrow spectrum of its constituents. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening process is an important tool in the discovery of new biologically active molecules and which has been most productive in the area of antibiotics [4,5]. A vast knowledge of how to use the plants against different illness may be expected to have accumulated in the areas,

where the use of plants is still great importance [6]. The medicinal value of plants lies in some chemical substances that produce definite physiological action on the human body. The important bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [7].

In the present study, *Aerva lanata* (Linn.) was selected for antimicrobial study based on the literature survey. It belongs to *Amaranthaceae* family, known as "Chaya" in Hindi, "Bhadram" in Sanskrit and "Palai" in Tamil [8]. *A. lanata* is a herbaccous perennial weed growing wild in the hot region of India. It has been claimed to be useful as diuretic, anthelmintic, antidiabetic, expectorant and hepatoprotective drug in the traditional system of medicine [9]. Antimicrobial and cytotoxicity activities of this plant were reported [10]. The plant is used for curing diabetes, lithiasis, cough, sore throat and wounds and it possess anti-inflammatory and nephroprotective properties [11].

Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases [12]. There are many studies on pharmacological activities of *A. lanata*, but there are a few studies on antimicrobial activity. In this study, we aimed to determine the antibacterial activity of different solvent extracts of root, flower and leaf of *A. lanata*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The medicinal plant *A. lanata* was collected from Mayiladuthurai, Nagapattinam District, Tamilnadu, India during the month of November 2013 and authenticated by the Botanist Dr. S. John Britto, Director, Rabinet Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli-620 002, Tamilnadu, India.

2.2 Preparation of Plant Extracts

The root, flower and leaves were separated from the collected plant and cleaned. The separated parts were dried under shade and then ground well into powder. 30 g powder of root, flower and leaves were taken separately in different conical flasks and labeled. 500 ml of solvent like acetone, benzene and ethyl acetate were added in each conical flask, shaken well, plugged with cotton and then kept at room temperature for 3 days. On the fourth day, the contents were shaken well and filtered through muslin cloth and then filtered again using Whatmann no. 1 filter paper. Then the filtrates were concentrated through hydro-distillation process. The extracts were dried until a constant weight of each was obtained [13]. 30 g powder of root, flower and leaves were soaked separately in distilled water for 12 to 16 hours and boiled and then it was filtered through muslin cloth and Whatmann no. 1 filter paper. The aqueous extracts were concentrated and made the final volume to onefifth of the original volume [14]. The extracts were stored in air tight containers at 4°C until the time of use.

2.3 Bacterial Strains

The bacterial strains Staphylococcus saprophyticus. Streptococcus agalactiae. Acinetobacter baumannii, Xanthomonus citri, Klebsiella pneumoniae, and Proteus vulgaris used in this study were obtained from Microbiology Laboratory, Doctors Diagnostic Centre Tiruchirappalli, Tamilnadu, India and where the bacterial strains isolated from urine cultures of urinary tract infected (UTIs) patients. Cultures were routinely maintained in nutrient agar slants at 4°C. For this study, the bacteria were sub-cultured in nutrient broth and incubated at 37°C for 24 hours before use.

2.4 Preparation of Medium

28 g of nutrient agar was dissolved in 1000 ml of distilled water and boiled to dissolve the agar completely and then sterilized by autoclaving at 15 lbs pressure at 121° C for 15 minutes (pH 7.4±0.2).

2.5 Antibacterial Activity [15]

The antibacterial activity of different parts of extracts of *A. lanata* was determined by agar well diffusion method. Nutrient agar plates were

seeded with 0.1 ml of bacterial culture (equivalent to 10⁷-10⁸ CFU/ml). The broth culture of each bacterium was inoculated in sterile molten nutrient agar at 45°C, and then allowed to solidify and well made by sterile standard cork borer (6 mm). 0.6 g of dried extract was dissolved in 3 ml of Dimethyl sulfoxide (DMSO) for the determination of antibacterial activity. Different concentrations like 5 mg/25µl, 10 mg/50µl, 15 mg/75µl and 20 mg/100µl of extracts were added in the well. Then the bacterial culture plates were incubated at 37°C for 24 hours. After incubation, the diameter of zone of inhibition was measured. DMSO and streptomycin (10 µg/µl) were used as negative and positive controls, respectively.

2.6 Statistical Analysis

All the results were subjected to statistical analysis and the results are expressed as mean ± standard deviation of three replicates.

3. RESULTS

The present study revealed that the tested extracts of different parts of *A. lanata* possess antibacterial activity against *S. saprophyticus*, *S. agalactiae*, *A. baumannii*, *X. citri*, *K. pneumoniae* and *P. vulgaris*. The extracts of different parts of *A. lanata* showed different degrees of antibacterial activity and the results are represented in Tables 1, 2 and 3 as well as in Figs. 1, 2 and 3.

3.1 Antibacterial Activity of Different Solvent Extracts of Root of *A. lanata*

The antibacterial activity of different solvents like acetone, aqueous, benzene and ethylacetate extracts of root of A. lanata was carried out and the results are represented in Table 1 and Fig. 1. The maximum level of antibacterial activity was observed in acetone extract against P. vulgaris (22±0.8 mm) at the concentration of 20 mg of root extract and there was no activity observed against S. saprophyticus.Benzene extract of root showed antibacterial activity against all the tested microorganisms, but higher level of bacterial growth inhibition was observed against S. saprophyticus (23±0.8 mm). Aqueous extract showed higher level of antibacterial activity against P. vulgaris (17.3±0.4 mm) at the concentration of 20 mg of root extract, but no activity was observed against A. baumannii. Ethylacetate extract exhibited the highest level of

antibacterial activity against *X. citri* (20 ± 0.8 mm) and the lowest activity was observed against *A. baumannii* (11 ± 0.8 mm).

3.2 Antibacterial Activity of Different Solvent Extracts of Flower of *A. lanata*

The antibacterial activity of different solvent extracts of flower of A. lanata is showed in Table 2 and Fig. 2. The acetone extract of flower of A. lanata showed highest antibacterial activity against A. baumannii (14.3±1.2 mm) and P. vulgaris (15.6±0.9 mm) at the concentration of 15 mg of extract, but no activity was observed against X. citri. The aqueous extract showed the highest zone of inhibition against A. baumannii (12.6±0.6 mm)and P. vulgaris (13±1.6 mm) and no activity was found against X. citri, The benzene extract exhibited higher level of antibacterial activity against X. citri (15±1.6 mm), but it has no activity against A. baumannii, K. pneumoniae and P. vulgaris. The ethylacetate flower extract showed highest zone of inhibition against A. baumannii (17±0.8 mm) followed by X. citri (16.3±1.2 mm) and there was no activity observed against K. pneumoniae and P. vulgaris.

3.3 Antibacterial Activity of Different Solvent Extracts of Leaf of *A. lanata*

The antibacterial activity of different solvent extracts of leaf of A. lanata was screened and the results are represented in Table 3 and Fig. 3. Acetone leaf extract of A. lanata showed more active against A. baumannii (11.3±0.4 mm) followed by S. saprophyticus (9±0.8 mm)at the concentration of 20 mg and no activity was observed against S. agalactiae, X. citri, K. pneumoniae and P. vulgaris. Aqueous extract showed maximum zone of inhibition 15±2.4 mm against S. saprophyticus followed by 10.6±1.2 mm against S. agalactiae and 9.0±1.6 mm against A. baumannii. There was no activity observed in aqueous extract against X. citri, K. pneumoniae and P. vulgaris. Benzeneleaf extract of A. lanata showed higher level of antibacterial activity 15±0.8 mm against S. agalactiae at the concentration of 20 mg and no activity was observed against X. citri, K. pneumoniae and P. vulgaris. The highest level of zone of inhibition 20.6±1.6 mm was observed in ethylacetate extract against S. saprophyticus followed by 14±0.8 mm against P. vulgaris at the concentration of 20 mg.

4. DISCUSSION

Plant is one of the best sources of development of new chemotherapeutic drugs and the first step towards this goal is the in vitro antibacterial activity assay [16]. More reports are available on the antiviral. antibacterial, antifungal. anthelmintic, antimolluscal and anti-inflammatory properties of medicinal plants [17-23]. Some of observations are helpful in the these establishment of active principle compounds responsible for pharmacological activities and which is useful in the development of new drugs for therapeutic purposes. An important function of plant extracts and their components is hydrophobicity. It is enables to partition the lipids of bacterial cell membrane and mitochondria and disturbing the cell structures and rendering them more permeable, which leads to extensive leakage of intercellular compounds from the bacterial cells or the exit of molecules and ions will lead to bacterial cell death [24].

In the present study, different solvent extracts of root, flower and leaf of *A. lanata* showed effective antibacterial activity against Gram positive bacteria like *S. saprophyticus* and *S. agalactiae* and Gram negative bacteria like *A. baumannii, X. citri, K. pneumoniae and P. vulgaris.* Similarly, Uma et al. [25] reported that the antibacterial activity of root of *Achranthes bidentata.*

The acetone and aqueous extracts of flower of A. lanata exhibited the highest zone of inhibition in all tested concentrations against P. vulgaris, but no activity was found in benzene and ethylacetate extract against P. vulguris and K. pneumonia. Murugan and Mohan [26] reported that no antibacterial activity in benzene and ethylacetate extract of whole plant of A. lanata against P. vulgaris and K. pneumonia. In the present study ethyl acetate extract of flower showed the highest level of zone of inhibition against S. agalactiae, A. baumannii and X. citri. but the lowest level of zone of inhibition was observed in the acetone extracts. Benzene extract of flower showed higher activity against S. agalactiae and X. citri, but no activity was observed against other four tested microorganisms. Kalirajan et al. [27] reported that the alkaloids present in the flower extracts of A. lanata may be the major reason for its potential antimicrobial activity.



Vidhya and Udayakumar; BMRJ, 7(1): 35-47, 2015; Article no.BMRJ.2015.093

Fig 1. Antibacterial activity of different solvent extracts of root of *A. lanata* AcEt-Acetone extract, AqEt – Aqueous extract, BeEt – Benzene extract, EtEt-Ethylacetate extract, NC-Negative control, PC-Positive control



Vidhya and Udayakumar; BMRJ, 7(1): 35-47, 2015; Article no.BMRJ.2015.093

Fig. 2. Antibacterial activity of different solvent extracts of flower of *A. lanata* AcEt-Acetone extract, AqEt – Aqueous extract, BeEt – Benzene extract, EtEt-Ethylacetate extract, NC-Negative control, PC-Positive control



Vidhya and Udayakumar; BMRJ, 7(1): 35-47, 2015; Article no.BMRJ.2015.093

Fig. 3. Antibacterial activity of different solvent extracts of leaf of *A. lanata* AcEt-Acetone extract, AqEt – Aqueous extract, BeEt – Benzene extract, EtEt-Ethylacetate extract, NC-Negative control, PC-Positive control

Name of solvent extracts	Concentration of plant extracts	Diameter of zone of inhibition (mm) Name of the microorganims						
		DMSO	NC	-	-	-	-	-
Acetone	25 µl(5 mg)	-	9.3±1.2	9.3±1.2	10.6±1.2	-	12.3±1.2	
	50 µl(10 mg)	-	9±0.8	11±0.8	10.3±1.2	9.6±0.9	13.6±1.2	
	75 µl(15 mg)	-	11.6±1.2	12.3±1.2	12±0.8	13.6±0.9	18±0.8	
	100 µl(20 mg)	-	12.6±1.2	15±1.6	15±1.6	14±0.8	22±0.8	
Aqueous	25 µl(5 mg)	9±0.8	-	-	-	9.3±1.2	-	
Aqueous	50 µl(10 mg)	10±0.8	10.6±1.2	-	-	11±1.6	9.6±0.9	
	75 µl(15 mg)	11±0.8	11.6±1.2	-	10±0.8	13±2.1	11.6±1.2	
	100 µl(20 mg)	13±0.8	13.3±1.6	-	12±0.8	11.6±1.6	17.3±0.4	
Benzene	25 µl(5 mg)	10±0.8	9.3±1.2	-	9.3±1.2	10.3±1.2	11.6±1.2	
	50 µl(10 mg)	14.6±0.4	10.3±1.2	11±1.6	9.3±0.9	10.3±1.2	11±0.8	
	75 µl(15 mg)	21.3±1.2	12±0.8	11±0.8	12±0.8	13±1.6	12.6±1.2	
	100 µl(20 mg)	23±0.8	14±0.8	12.6±0.4	19±0.8	14±1.6	19.3±1.2	
Ethylacetate	25 µl(5 mg)	-	-	-	9±0.8	9.3±1.2	9.3±1.2	
	50 µl (10 mg)	7.6±0.4	11.6±1.6	9±0.8	11.3±0.4	9.3±1.2	10.3±1.2	
	75 µl (15 mg)	12±1.6	11.0±1.6	9±1.6	13.3±1.2	12±1.6	11.6±0.4	
	100 µl(20 mg)	18.3±1.2	19.6±1.2	11±0.8	14±0.8	15±1.6	18±0.8	
Streptomycin	PC(10 µg)	20.3±1.2	19.6±1.2	22±1.6	16.3±0.9	20±0.8	21±0.8	

Table 1. Antibacterial activity of different solvents like acetone, aqueous, benzene and ethylacetate extracts of root of A. lanata

NC - Negative control, PC - Positive control (Streptomycin), - No activity Values are expressed as mean zone of inhibition (mm) ± Standard deviation of three replicates

Name of solvent	Concentration of plant extracts	Diameter of zone of inhibition (mm) Name of the microorganims						
extracts								
		Staphylococcus saprophytius	Streptococcus agalactiae	Acinetobaecter baumannii	Xanthomonus citri	Klbesiella pneumonia	Proteus vulgaris	
DMSO	NC	-	-	-	-	-	-	
Acetone	25 µl (5 mg)	-	-	9.6±0.4	-	-	12±1.6	
	50 µl (10 mg)	-	-	11±1.4	-	-	14±1.6	
	75 µl (15 mg)	9.6±1.6	10.6±1.2	14.3±1.2	-	-	15.6±0.9	
	100 µl (20 mg)	10.3±1.2	15±0.8	12.6±1.2	-	12±1.6	15.3±0.4	
Aqueous	25 µl (5 mg)	-	-	-	-	-	9.6±1.2	
	50 µl (10 mg)	-	9.3±0.4	-	-	-	9.3±1.2	
	75 µl (15 mg)	-	11±1.6	11±1.8	-	-	9.3±0.4	
	100 µl (20 mg)	11±0.8	11.6±1.2	12.6±0.6	-	9±0.8	13±1.6	
Benzene	25 µl (5 mg)	-	-	-	-	-	-	
	50 µl (10 mg)	-	12±1.6	-	11±1.6	-	-	
	75 µl (15 mg)	-	13.6±1.2	-	12.6±2.0	-	-	
	100 µl (20 mg)	10.3±1.2	14±1.6	-	15±1.6	-	-	
Ethylacetate	25 µl (5 mg)	-	12±0.8	10.6±1.2	11±0.8	-	-	
	50 µl (10 mg)	-	13±1.6	11.6±0.4	13.3±1.2	-	-	
	75 µl (15 mg)	-	15±1.6	15.6±1.2	13±0.8	-	-	
	100 µl (20 mg)	10.3±1.2	17±0.8	16.3±1.2	13.3±1.2	-	-	
Streptomycin	PC (10 µg)	20.3±1.2	19.6±1.2	22±1.6	16.3±0.9	20±0.8	21±0.8	

Table 2. Antibacterial activity of different solvents like acetone, aqueous, benzene and ethylacetate extracts of flower of A. lanata

NC - Negative control, PC - Positive control (Streptomycin), - No activity Values are expressed as mean zone of inhibition (mm) ± Standard deviation of three replicates

Name of solvent	Concentration of plant extracts	Diameter of zone of inhibition (mm) Name of the microorganims						
extracts								
	-	Staphylococcus saprophytius	Streptococcus agalactiae	Acinetobaecter baumannii	Xanthomonus citri	Klbesiella pneumonia	Proteus vulgaris	
DMSO	NC	-	-	-	-	-	-	
Acetone	25 µl (5 mg)	-	-	-	-	-	-	
	50 µl (10 mg)	-	-	-	-	-	-	
	75 µl (15 mg)	-	-	-	-	-	-	
	100 µl (20 mg)	9±0.8	-	11.3±0.4	-	-	-	
Aqueous	25 µl (5 mg)	11±1.4	-	-	-	-	-	
	50 µl (10 mg)	13±2.1	-	-	-	-	-	
	75 µl (15 mg)	13.6±2.0	-	-	-	-	-	
	100 µl (20 mg)	15±2.4	10.6±1.2	9±1.6	-	-	-	
Benzene	25 µl (5 mg)	13.3±1.2	11±0.8	-	-	-	-	
	50 µl (10 mg)	13±1.4	12.3±1.2	-	-	-	-	
	75 µl (15 mg)	11.3±1.2	12.3±2.0	9±1.6	-	-	-	
	100 µl (20 mg)	10±0.8	15±0.8	10±0.8	-	-	-	
Ethylacetate	25 µl (5 mg)	10.3±1.2	-	-	-	-	-	
	50 µl (10 mg)	11±0.8	10±0.8	-	12±1.6	-	13±1.6	
	75 µl (15 mg)	14.3±1.2	12±1.6	-	11.3±1.2	11.6±2.4	12±0.8	
	100 µl (20 mg)	20.6±1.6	13±1.6	-	12.3±1.2	9.6±1.2	14±0.8	
Streptomycin	PC (10 µg)	20.3±1.2	19.6±1.2	22±1.6	16.3±0.9	20±0.8	21±0.8	

Table 3. Antibacterial activity of different solvents like acetone, aqueous, benzene and ethylacetate extracts of leaf of A. lanata

NC - Negative control, PC - Positive control (Streptomycin), - No activity. Values are expressed as mean zone of inhibition (mm) ± Standard deviation of three replicates

In the present study, the antibacterial effect of different solvent extracts of leaf of A. lanata was exhibited different level of zone of inhibition against selected microorganisms. Ethylacetate extract of leaf showed higher activity against S. saprophyticus and P. vulgaris. Similarly, the antibacterial activity of different parts of Wrightia tomentosa was studied and reported that the higher level of antibacterial activity in ethylacetate extract of leaf [28]. Benzene and acetone extracts of leaf of A. lanata were showed antibacterial activity against S. agalactiae and A. baumannii. Aqueous extract of leaf showed lower level of antibacterial activity against S. saprophyticus and S. agalactiae, but there was no activity observed in other tested bacterial species. The results of the present study clearly indicates that the organic solvent extracts showed better activity against the tested bacterial species than aqueous extracts of different parts of A. lanata. Similarly, the antibacterial activity of Amaranthaceae plants [29] and Achyranthes aspera [30] were reported. The presence of phytoconstituents in the organic solvent extracts of A. lanata may be responsible for the antimicrobial activity [31]. The present study results are accordance with other studies in that the antimicrobial activity of medicinal plant Chenopodium album [32] and phytocompound flavonoids [33] were reported. Similarly. Parameswari et al. [34] reported that the in vitro antibacterial activity of extracts of Solanum nigrum. The antibacterial activity of ethylacetate and ethanol extracts of stem of A. lanata at the concentration of 1000 µg/ml against Gram positive bacteria Staphylococcus aureus and Bacillus subtilius and Gram negative bacteria Escherichia coli and Klebseilia pneumonia was reported [35].

The antibacterial activity of aqueous and ethanol extracts of leaf and stem of A. lanata against both Gram positive and Gram negative organisms was also reported [36]. The researchers reported that the ethyl acetate and methanol extracts of whole plant of A. lanata have antimicrobial properties due to the presence of steroids, terpenes and flavonoids [37]. The antibacterial activity of the methanol extracts of leaves of Tridax procumbens and A. lanata was reported [38]. Yamunadevi et al. [39] reported that the methanolic extract of stem, leaf, root and reproductive parts of A. lanata contained alkaloids, tannins. saponins, flavonoids, carbohydrates, glycosides, phenols, steroids, phlobatannins, cardiac glycosides, proteins and resins. The secondary metabolites

like saponins, cardiac glycosides, tannins, flavonoids and phenolic compounds were identified in root, flower and leaf of A. lanata (data not shown) and which are possessed antimicrobial properties were already reported [40-44]. In the present study, we determined that the antibacterial activity of different solvent extracts of root, flower and leaf of A. lanata against S. saprophyticus, S. agalactiae, A. baumannii, X. citri, K. pneumoniae and P. vulaaris. So. the above mentioned phytocompounds may be responsible for the antibacterial activity of different solvent extracts of root, flower and leaf of A. lanata against selected bacterial species.

5. CONCLUSION

A. lanata, an important medicinal plantis useful in the treatment of wide range of disorders. The present investigation clearly indicates that the different solvent extracts of root, flower and leaf of *A. lanata* exhibited antibacterial activity. Hence, the isolation of antimicrobial compounds from root, flower and leaf of *A. lanata* with possible mechanism would be a better option for the synthesis of new antimicrobial drugs to treat infectious diseases caused by microorganisms. This may prove to be a right replacement for many of the synthetic drugs in future.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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