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Full Length Research Paper

Antioxidant, antibacterial, antifungal activities and phytochemical analysis of dagger (*Yucca aloifolia*) leaves extracts

Sobia[1], Muhammad Zubair[1]*, Nasir Rasool[1], Asim Mansha[1], Fozia Anjum[1], Munawar Iqbal[2], Muhammad Mushtaq[2] and Muhammad Shahid[1]

[1]Department of Chemistry, Government College University, Faisalabad-38000, Pakistan.
[2]Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan.

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The leaves of *Yucca aloifolia* were extracted using methanol; fractions with organic solvents were analyzed for their biological activities and photochemical analysis. Total phenolic and flavonoid contents were determined using different colorimeteric assays. The antioxidant potential was evaluated by measuring reducing power, % inhibition of linoleic acid peroxide, and scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical while antimicrobial and antifungal activities were evaluated by disc diffusion assay against a set of bacterial and fungal strains. Methanolic extract showed the superior yield of bioactive (%), total phenolics and total flavonoids, and phytochemical analysis revealed the presence of alkaloids, tannins, steroids, saponins and flavonoids. The *Y. aloifolia* leaves extract showed considerable antioxidant activity but were solvent-dependent. In biological assay, the extracts showed the antimicrobial activity comparable with standard antibiotics.

Key words: *Yucca aloifolia*, antimicrobial activity, DPPH, total flavonoid content (TFC), minimum inhibitory concentration (MIC), antioxidant activity.

INTRODUCTION

Medicinal plants correspond to a wealthy source of biological agents which play vital role in a number of functional and regulation mechanisms occurring in plant as well as in animal body. The plant materials used in traditional medicine are readily available in rural areas and relatively cheaper than modern medicine (Mann et al., 2008; Mahesh and Satish, 2008).

Plants naturally produce a wide variety of secondary metabolites which have a good antibacterial, antifungal, anti-pests effect and hence are used for pharmaceutical, nutracuetical and cosmo-nutracuetical purposes. The presence of such bioactive ingredients also impart unique properties to the plant for example, terpenoids give specific order, quinones and tannins are responsible for color, and flavor in chili peppers is due to terpenoid capsaicin.

These compounds offer a defensive mechanism against oxidants produced in living systems such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), and regulate the process of oxidation (Gulcin et al., 2011, 2012).

The ROS and RNS are produced as by-products in aerobic organisms and have been implicated in the pathology of a vast variety of diseases including cancer, atherosclerosis, diabetic mellitus, hypertension, Acquired immune deficiency syndrome (AIDS) and ageing. Therefore, antioxidant activity is important in view of the free radical theory of ageing and associated diseases (Wallace, 1999; Lee et al., 2000; Biglari et al., 2008; Govindarajan et al., 2006).

According to world health organization report on infectious diseases, overcoming antibiotic resistance is the major issue for the next millennium. Hence, the last

*Corresponding author: E-mail: zubairmkn@yahoo.com. Phone: +923214680271.

decade witnessed an increase in the investigations on plants as a source of human disease management (Aiyelaagbe, 2000; Prashanth et al., 2001; Mounissamy et al., 2002; Woldemichael et al., 2003; Satish et al., 1999; Bisignano et al., 1996).

Dagger (*Yucca aloifolia*) is a small tree, with distribution from the North Carolina coast to central Florida, along the Gulf Coastal Plain to Louisiana, West Indies and south eastern Mexico. The *Y. aloifolia* plants are stiff, having evergreen, sword-shaped leaves around a stout trunk. They have a dense terminal flower head (panicle) that faintly resembles a candle. The flowers are of white or greenish color. The name *Yucca* applies to as many as 40 species of trees and shrubs found mostly in arid areas (Leung, 1980).

Hayashi et al. (992) reported the presence of steroidal saponin glycosides, while Olas et al. (2008) reported phenolic and stilbene constituents and Piacente et al. (2005) observed the higher free radical scavenging activity in *Y. aloifolia* plant extracts as compared to quercetin (standard). Pushpa and Paresh (1983) revealed the presence of sapogenins, smilagenin, sarsasapogenin, tigogenin, hecogenin, gitogenin, and chlorogenin, while Mortada (1994) reported the molluscicidal activity of *Y. aloifolia* against *Biomphalaria alexandrina and B. truncatus.* Aqueous alcoholic extracts of *Yucca* flowers have exhibited antitumor activity against B16 melanoma in mice (Ali et al., 1978).

In another study, a protective effect has been observed against nitrate-induced oxidative stress in rats fed with *Y. aloifolia* extract. According to World Health Organization (WHO), the medicinal plants are the best sources to obtain a variety of newer herbal drugs. Many plants have already been investigated for their medicinal importance (Rizwan et al., 2012; Bari et al 2012). Being a medicinal plant, the information relevant to the antimicrobial and antioxidant activity of *Yucca* leaves is not so common, and in this regards we designed the present research work to evaluate the antimicrobial activity, antioxidant potential and phytochemical constituents in leave extracts of *Yucca* native to Pakistan.

MATERIAL AND METHODS

Collection of plant material

The leaves of the plant *Y. aloifolia* were collected from Botanical Garden, University of Agriculture Faisalabad, Pakistan and further identified by a taxonomist, Dr. Mansoor Hameed from Department of Botany, University of Agriculture Faisalabad, Pakistan where a voucher specimen has been deposited (voucher specimen no: 21138).

Sample preparation

The dried leaves of *Y. aloifolia* were grinded and extracted with 100% methanol using an orbital shaker (Gallenkamp, UK) for 8 h at room temperature. The extract was filtered and the process was repeated three times with intervals of four days. Finally, the extract (200 ml) was concentrated in rotatory evaporator (N-N Series, Eyela, Rikakikai, Tokyo, Japan) until 80 ml and dissolved in distilled water and fractioned with different polarity based solvents; *n*-hexane, $CHCl_3$, Ethyl acetate (EtOAc) and *n*-butyl alcohol (*n*-BuOH), and obtained extract fractions were stored at -4°C in refrigerator till further analysis.

Phytochemical analysis

The samples for phytochemical tests were prepared as precisely described (Sofowara, 1993). For tannins determination, 0.5 ml of extract was dissolved in 1 ml of water and 1 to 2 drops of ferric chloride. A blue color was observed for gallic tannins and green black for catecholic tannins (Iyengar, 1995). For Saponin, foam test was applied by shaking the extract with small amount of water for 10 min, with the appearance of foam revealing the presence of saponin (Roopashree et al., 2008). Flavonoids were tested by alkaline reagent test by treating extracts with few drops of sodium hydroxide solution with the formation of intense yellow color which became colorless on addition of dilute acid indicating the presence of flavonoids (Roopashree et al., 2008). For steroids, 2 m of acetic anhydride was added to 0.5 ml extract along with 2 ml H_2SO_4. The color changed from violet to blue or green, indicating the presence of steroids. Alkaloids were measured by Mayer's reagents. The alcoholic extract was evaporated to dryness and the residue was heated in a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation (Siddiqui and Ali, 1197).

Determination of total phenolic and total flavonoid contents

The total phenolic contents (TPC) were assessed using Folin-Ciocalteu reagent as already described (Sultana et al., 2007), total flavonoid contents (TFC) were determined following the method of Dewanto et al. (2002).

Antioxidant activity

A number of colorimetric assays were used to assess the antioxidant activity of methanolic and fractioned extracts of *Y. aloifolia* leaves. Antioxidant activity in linoleic acid system (% inhibition of peroxidation) was determined as described previously by Iqbal and Bhanger (2005), while the reducing power of *Y. aloifolia* leaves extract was determined according to the procedure of Yen et al. (2000). For DPPH, radical scavenging activity assay method (Bozin et al., 2006) was adopted by adding 5 ml of freshly prepared DPPH having concentration 0.025 g/L (0.0050 g DPPH in 200 ml CH_3OH) to 1 mg/ml of extracts and absorbance was measured at 517 nm after 10 min along with blank (5 ml DPPH + 1 ml methanol).

Antimicrobial activity

Microbial strains and inoculum preparation

The extracts of *Y. aloifolia* were individually tested against a set of fungal and bacterial strains; *Aspergillus niger, A. flavus, Alternaria altrnata, Ganoderma lucidum, Pasturella multocida, Escherichia coli,* and *Staphylococcus aureus.* The pure fungal and bacterial strains were obtained from the Department of Veternary Microbiology, University of Agriculture, Faisalabad.

Table 1. Percentage yield of bioactive, TPC and TFC different extracts of *Y. aloifolia* leaves.

Solvent	Yield%[m]	Total phenolic[n]	Total flavonoids[o]
Methanol	16.5±0.4[d]	158±0.61[d]	331±0.8[d]
n-hexane	6.1±0.23[b]	23.3±0.47[ab]	44±0.81[a]
Chloroform	3.5±0.41[a]	38.2 ±0.28[b]	81.9±1.21[b]
Ethyl acetate	4.3±0.47[a]	42.5±0.73[c]	144±1.21[c]
n-butanol	5.1±0.23[b]	17.4±0.31[a]	66.1±0.26[ab]

[m]Values are mean weight (g/100 g) ± standard deviation (SD) triplicate experiment,[n]values are mean (mg gallic acid equivalen/100 g of dry matter) ± SD of triplicate experiments, [o]values are mean (mg as catechin equivalent/100 g of dry matter) ± SD of triplicate experiments as catechin equivalent.Small alphabets in superscript within the same column indicated significance difference ($p < 0.05$) among solvents used.

Disc diffusion assay

Nutrient agar and potato dextrose were used for bacterial and fungal growth, respectively. The antimicrobial activity of extracts/fractions was determined using disc diffusion method of The Clinical and Laboratory Standards Institute (CLSI) (2007). Nutrientagar/potato dextrose agar (Oxoid, UK) was suspended in distilled water, mixed well and distributed homogenously. The medium was sterilized by autoclaving at 121°C for 15 min. Before the medium was transferred to Petri plates, inoculum (100 µl/100 ml) was added to the medium and poured in sterilized Petri plates. Now, small filter paper discs were laid flat on growth medium containing 100 µl of extracts. The Petri plates were then incubated (37°C for bacteria and 30°C for fungus) for 24 h. The extracts having antimicrobial activity inhibited the bacterial and fungal growth, and clear zones were formed after 24 h which were measured in mm using zone reader. Rifampcin and fluconazole were used as control against bacteria and fungus, respectively.

MIC determination using resazurin microtitre-plate assay

The MIC value was determined by resazurin microtitre-plate assay to obtain quantitative results. In 96 well plates, the first row was filled with 100 µ of sample and reference solution, and 50 µl of Muller-Hinton agar broth and nutrient broth for fungus and bacteria, respectively which were poured in all wells except the first column of plate, and finally dilutions was performed. Then 10 µl resazurine solution was added as an indicator in each well. In the end, 10 µl of fungal or bacterial strain were added and plates were wrapped with aluminum foil. The plates were then incubated at 28°C for fungus for 48 h and 37°C for bacteria for 24 h (Sarker et al., 2007).

Statistical analysis

One way Analysis of variance (ANOVA) was performed using Statistica (version 8.1. Stat soft Inc, Tulsa Okahoma, USA) at 95% confidence interval of mean, and all the experiments were seeded in triplicate and data thus obtained was reported as mean ± standard deviation (SD) (Steel et al., 1996).

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of *Y. aloifolia* l eaves in methanol and other fractionized solvents is given in Table 1. The highest yield was obtained from methanol (16.5%) and lowest yield in chloroform (3.5%). The yield (%) of other fractionized solvent was; MeOH > *n*-hexane > *n*-BuOH > EtOAc > $CHCl_3$. Effect of the different solvents, on the yield, was found to be significant ($p < 0.05$). Variations in the yield of extracts from *Y. aloifolia* plant leaves might be attributed to the ability of solvent, which depends upon the chemical composition of the plant material, extraction procedure and condtions and polarity of the extracting solvent (Halvorsen et al., 2002; Piacente et al., 2005; Wanga et al., 2006; Sultana et al., 2008).

Phytochemical composition

The phytochemical analysis of *Y. aloifolia* is given in Table 2. The alkaloid was found in all extracts except $CHCl_3$ and *n*-BuOH, flavonoid were present in water, MeOH, EtOAc and *n*-BuOH, while in $CHCl_3$ and *n*-hexane it was not. The tannins were found to be present in methanolic, EtOAc and *n*-BuOH fractions, while steroids were only in $CHCl_3$ and *n*-hexane and water. MeOH, EtOAc and *n*-BuOH revealed the presence of saponins. It is revealed that the phytochemicals are well known due to their medicinal, antimicrobial and antioxidant activity. Flavonoids have been reported to possess antibacterial, antioxidant, anti-inflammatory, antiallergic, antimutagenic, and vasodilatory activity (Alan and Miller, 1996). Saponins showed hypocholesterolemic and antidiabetic properties, while steroids are well known due to analgesic properties (Rupasinghe et al., 2003; Sayyah et al., 2004; Malairajan et al., 2006). The presence of biologically important phytochemicals in the *Y. aloifolia* leaves extracts, as tested in our study, contribute to their medicinal value and point to potential sources for useful drugs. Further phytochemical and pharmacological investigations, as well as characterization of the active compounds from *Y. aloifolia* should be conducted for traditional uses and potential therapeutic applications.

Table 2. Phytochemical constituents of *Y. aloifolia* in different solvents.

Parameter	Water	Methanol	*n-hexane*	Chloroform	Ethyl acetate	*n*-butanol
Alkaloids	-	-	-	+	-	+
Flavonoids	+	+	-	-	+	+
Tannins	-	+	-	-	+	+
Steroids	-	-	+	+	-	-
Saponins	+	+	-	-	+	+

+ = Present, - = Absent.

Total phenolic and total flavonoid contents

A large number of aromatic herbs, spicies, medicinal and other plants exhibits the different types of biological activities which are attributed to the presence of bioactive components, mostly polyphenols. Numerous studies were carried out on some of these plants, which resulted in a development of natural antioxidant formulations for food, cosmetic and other applications. However, scientific information on antioxidant properties of various plants, particularly those that are less widely used in culinary and medicine is still rather scarce. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals (Miliauskas et al., 2004; Gulcin et al., 2010 a, b).

The TPC of *Y. aloifolia* plant extract in methanol (MeOH), *n*-hexane, n-buthanol (*n*-BuOH), ethyl acetate (EtOAc) and $CHCl_3$ were found to range from 17 to 158 gallic acid equivalent (GAE) mg/100 g as shown in Table 1. The ability of different solvents to extract TPC was found as: methanol > *n*-BuOH > EtOAc > $CHCl_3$ > *n*-hexane. The higher phenolic contents extracted as observed in the case of methanolic extracts of *Y. aloifolia* might be attributed to the higher polarity of methanol as compared to other solvents investigated during the present study. Furthermore, the results of present study are consistent with Halvorsen et al., (2002) and TPC range observed for the methanolic extract of *Y. aloifolia* were higher than those reported earlier by Kamran et al. (2009) for different medicinal plants and gull et al. (2012) for guava fruit. Similarly, TFC were found to range from 44 to 331 caffeic acid equivalent (CAE) mg/100 g. The observed value of TFC varied significantly ($p < 0.05$) for different solvents as follows: MeOH (331) > *n*-BuOH (144) > EtOAc (81) > *n*-hexane (66) > $CHCl_3$ (44). To the best of our knowledge, reports in the literature regarding the antioxidant components of *Y. aloifolia* for comparison have not been found. However, the extraction potential of different solvent towards the phenolic and flavonoids attributes was consistent (Halvorsen et al., 2002)

DPPH$^{\cdot}$ scavenging activity

For antioxidant activity , different assays were used, including scavenging activity on DPPH radicals, reducing power and inhibition of lipid peroxidation, and also phenolic and flavonoids content were also measured and is given in Table 1. The DPPH radical is a stable free radical which is successfully used to estimate free radical-scavenging activity. Antioxidants neutralize DPPH by donating hydrogen or electron (Archana et al., 2005). The DPPH radical scavenging activity of methanolic extract and different fractions of *Y. aloifolia* leaves is shown in Table 3. The DPPH radical scavenging activity of extract (10 mg/ml) ranged from 51 to 74%. Methanolic extract exhibited more scavenging activity than all the fractions. Among the solvent, MeOH, $CHCl_3$, EtOAc, *n*-BuOH and *n*-hexane showed 74.19, 60.25, 61.15, 65.24 and 51.19% activity versus control butylated hydroxyltoluene (BHT). The higher DPPH$^{\cdot}$ scavenging activity of methanolic extract of *Y. aloifolia* leaves might be attributed to the higher amount of phenolic bioactives extracted with relatively high polarity based solvent.

Inhibition of linoleic acid

The inhibition (%) of linoleic acid oxidation as exhibited by methanolic extract and other fractions of *Y. aloifolia* leaves is given in Table 3. MeOH extract offered significantly ($p < 0.05$) higher inhibition of peroxidation (64.17%) versus $CHCl_3$ (25.25%), EtOAc (32.33%), n-BuOH (41.8%) and n-hexane (10.16%). In case of reducing power, MeOH extract showed significantly ($p < 0.05$) higher reducing power (0.439 %) versus $CHCl_3$ (0.049%), EtOAc (0.331%), *n*-BuOH (0.056%) and *n*-hexane (0.024%) (Table 3).

Antimicrobial activity

The antimicrobial activities of *Y. aloifolia* plant leaves extracts method are shown in Table 4. The antibacterial activity was determined in comparison with rifampcin, while antifungal activity was compared with fluconazole. Against *E. coli,* the higher activity was shown by *n*-BuOH extract followed by EtOAc, $CHCl_3$ and *n*-hexane, while MeOH did not show any activity against *E. coli.* For *P. multocida,* $CHCl_3$ extract showed better activity followed

Table 3. Antioxidant activities of *Yucca aloifolia* methanolic extract and other organic fractions[a].

Antioxidant assays	Methanol	Chloroform	Ethyl acetate	*n*-butanol	*n*-hexane	BHT	Ascorbic acid
DPPH radical scavenging activity	74.19±0.2	60.25±0.36	61.15±0.21	65.24±0.34	51.19±0.26	82.2±0.28	-
% Inhibition of linoleic acid peroxidation	64.17±0.21	25.25±0.11	32.33±0.17	41.8±0.05	10.16±0.23	-	82.2±0.28
Reducing power (absorbance at 700 nm)	0.439±0.17	0.049±0.01	0.331±0.01	0.056±0.01	0.024±0.01	-	0.8±0.00

[a]Values are mean ± SD of triplicate experiment.

Table 4. *In vitro* antimicrobial activity of *Y. aloifolia* against selected bacterial and fungal strains[a].

Tested microorganism	Methanol	Chloroform	Ethyl acetate	*n*-butanol	*n*-hexane	Rifampcin	Fluconazole
E. coli	-	9.18±0.26	11.11±0.15	16.47±0.40	9.16±0.23	25±0.11	-
P. multocida	10.16±0.23	13.12±0.17	-	12.14±0.20	12.26±0.37	25±0.77	-
S. aureus	9.19±0.26	9.12±0.17	20.2±0.21	27	-	28.16±0.23	-
A. niger	14.2 ±0.32	25.54±0.50		16.11±0.1	17.2±0.30	-	27.16±0.23
A. flavus	25.16±0.23	11.11±0.14	9.16±0.23	10.2±0.29	15.29±0.41	-	27.3±0.24
A. alternata	25.43±0.51	12.44±0.58	-	9.40±0.55	14.8±0.38	-	28.4±0.49
G. lucidum	28.13±0.18	10.12±0.17	-	11.09±0.13	13.26±0.36	-	30.16±0.2

[a]Values are mean inhibition zone ± standard deviation (SD) of three separate experiments.

by n-hexane, *n*-BuOH and MeOH extract, EtOAc was found to be neutral. Against *S. aureus, n*-BuOH showed highest activity which is also statistically comparable with rifampcin, and EtOAc also showed good activity against *S. aureus* followed by MeOH and $CHCl_3$. Similarly, *Y. aloifolia* plant leaves also showed a considerable antifungal activity against a set of fungal strains. The $CHCl_3$ extract showed the highest activity which is also comparable with the standard, followed by *n*-hexane, *n*-BuOH and MeOH, while EtOAc did not show activity against *A. niger* and the MeOH extract showed high potential, which was also comparable with fluconazole standard against *A. flavus*. The order of other extracts was found as; *n*-hexane > $CHCl_3$ > *n*-BuOH > EtOAc.

MIC of the *Y. aloifolia* leaves extracts was determined against a set of bacterial and fungal strain (Table 5).

The MIC value against *E. coli* was found in the following order; $CHCl_3$ = *n*-hexane > EtOAc > *n*-BuOH, *P. multocida*; MeOH > *n*-BuOH > CHCl3 > *n*-hexane, while against *S. aureus;* MeOH = CHCl3 > EtOAc > *n*-BuOH. The MIC value was also determined against a set of fungal strains and found in the following order: *A niger,* MeOH > *alternata*, *n*-BuOH > $CHCl_3$ > *n*-hexane > MeOH and *G. lucidum,* $CHCl_3$ > n-BuOH > n-hexane > MeOH. The lowest MIC against *E. coli, P. multocida* and *S. aureus* was found in *n*-BuOH, *n*-hexane and *n*-BuOH, respectively, while against *A. niger* it was lower in $CHCl_3$ extracts, and *A. flavus*, *A. alternata* and *G. lucidum* showed the lowest MIC values in MeOH.

According to Moghadam et al. (2010), the antimicrobial activity of medicinal plant material would probability be due to the presence of alkaloids, and flavonoides such as harmine/harmadine/harmadol in *P. hermala*, and also in these compounds or most probably soluble in organic polar solvent. So, the variation in the antimicrobial activity of *Y. aloifolia* plant leaves used in this study might be attributed to the different nature of solvent. From the last few decades, there has been considerable interest in extracts from plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms.

Table 5. Minimum inhibitory concentration (MIC, mg/ml) of different extracts of *Y. aloifolia* against selected bacterial and fungal strains[a].

Tested microorganism	Methanol	Chloroform	Ethyl acetate	*n*-butanol	*n*-hexane	Rifampcin	Fluconazole
E. coli	-	270.5±0.41	250±0.47	124.6±0.47	270±0.81	62±0.76	-
P. multocida	261.2±0.23	154.5±0.40	-	164±0.62	139.6±0.25	62±0.77	-
S. aureus	269.6±0.47	269.1±0.62	98.6±0.47	41±0.41	-	30.3±0.47	-
A. niger	142±0.81	62±0.47	-	124±0.47	115±0.47	-	41±0.62
A. flavus	61±0.41	250±0.47	270±0.81	260 ±0.47	138±0.41	-	41±0.81
A.alternata	41±0.81	165±0.41	-	270±0.23	143±0.23	-	31.4±0.16
G. lucidum	31±0.16	261±0.29	-	250±0.26	155±0.41	-	14±0.10

[a]Values are mean ± SD of triplicate experiment.

Our studies have shown that *Y. aloifolia* might be a good candidate for this due to quite good results of different extracts against a set of bacterial and fungal strain.

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus, there has been a continuing search for new and more potent antibiotics.

Conclusion

From present study, it was concluded that the *Y. aloifolia* plant leaves extract have antibacterial and antifungal activities comparable with standard antibiotic and the leaves extracts can be successfully used as an antibacterial and antifungal agent, practically against the microorganisms studied in the present work. Antioxidant activity of *Y. aloifolia* plant leaves suggest its use for stabilization of different foods after a preliminary experiment, separation of nutracuetical and como-nutracueticals. Furthermore, It was observed that methanol is an efficient solvent for extracting bioactives from *Y. aloifolia* and there is further need to identify and quantify the potent compound responsible for antimicrobial and antioxidant activity and their possible application.

n-BuOH > *n*-hexane > $CHCl_3$; *A. flavus,* EtOAc > *n*-BuOH > $CHCl_3$ > *n*-hexane > MeOH; *A.*

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