**A Comparative Evaluation of phytochemicals and Antioxidant Activity of *Sida acuta* L. and *Sida cordifolia* Linn.**

**ABSTRACT**

 *Sida acuta* L. and *Sida cordifolia* Linn. (Malavaceae) have a long history of being used for their medicinal properties. The Present study was carried out to compare phytochemical constituents and antioxidant activity of *Sida acuta* L and *Sida cordifolia* Linn. For qualitative analysis, methanol and ethyl acetate extracts were tested for various phytoconstitents, while for quantitative analysis and antioxidant activity, methanolic extracts of leaf and stem were subjected for various tests. The results of qualitative analysis showed that the methanolic extracts of both plants were able to extract more phytoconstitents compare to ethyl acetate. For quantitative analysis, leaf extract of *Sida cordifolia* Linn. was found to have the highest amount of total phenolic content (0.0852±0.006 mgGAE/g), total flavonoids content (0.1194±0.097mgQE/g), and lipid (1.66±0.016mg/g) while *Sida acuta* L. leaf extract possessed highest amount of alkaloids (1.257± 0.086mg/g). The antioxidant activity was found highest in *Sida cordifolia* Linn. leaf (70.59%±0.0017) at 1mg/ml. Above study shows that *Sida cordifolia* Linn. is rich in secondary metabolites and possesses the highest antioxidant activity.

**Keyword**: Phytochemical, Methanol, Ethyl acetate, Total phenolic content, total flavonoid content, , antioxidant activity

**INTRODUCTION**

The use of medicinal plants in modern medicine system proves the veracity behind the traditional claims of herbal products in human health care. Various plants have been utilized worldwide over the generation for their remedial purposes1. Plants have been considered for use in Ayuveda, Traditional Chinese medicine, Indigenous healing practices in the form of crude extract, homemade tea, dry powder and many more. A report by WHO states that around 80% of the global population relies on herbal medicine2. This is due to their affordability and availability compared to synthetic drugs. This has led to the extensive study of plant extracts against diseases worldwide3. Around 70,000 medicinal plants have been utilized for therapeutic purposes in Asian counties, and 14000 of these are found in India alone4. Moreover, medicinal plants serves as a fundamental source of information for a variety of chemical compounds that could be used in the designing of modern drugs5.These compounds are phytochemicals released in plants in response to stress but seldom play any important role in plant growth. They don’t have any nutritional value but contribute to the colour and savour of food. They are generally categorized as phenolic compounds, terpenoids, nitrogen containing compounds and many more6. Many of these phytochemicals possess significant antioxidant activity, which decreases the occurrence of several health disorders such as cardiovascular diseases, diabetes, cancer, Alzheimer’s and Parkinson’s disease. Antioxidants are molecules work by delaying or inhibiting the negative impact of free radicals that leads to the damage of cellular compartments. These free radicals are released as by-products during the basic essential metabolic pathways in human systems7. Present study was conducted to compare phytochemical constituents and antioxidant activity of the medicinal plant *Sida acuta* L. and *Sida cordifolia* Linn.

*Sida acuta* L and *Sida cordifolia* Linn are wild plants belonging to the genus Sida L. of the family malvaceae. Two morphological characters of this genus make it different from other genera of the same family are: 1.a calyx with 10 veins, and 2. Schizocarp fruits with 5-10 one- seeded mericarp8.Genus Sida L. has 200 species of herbaceous plants, distributed in tropical and subtropical regions of the world. This has been reported in various studies that plants of this genus have a long history of being used to treat neurological disorders, uterine disorders, headaches, tuberculosis, diabetes, malarial fever, piles, ulcers, wounds, rheumatism, diarrhoea, dysentery and skin diseases9.

*Sida acuta* L., also known as “wireweed,” “Morning Mallow,” and “Common Fanpetals,” is native to Central America and is found throughout the tropics and sub-tropics in Asia, Africa, and the Pacific10, 11. Tremendous studies have reported that people living in tropical places have used this plant to cure health problems like rheumatic affections, respiratory diseases, azoospermia, and oligospermia 12. The Plant has been found to possess antioxidant potential and can be used to manage liver and kidney dysfunction13. *Sida acuta* L. is often found flourishing in wasteland, roadsides, in lawns and forests areas. It is an annual herb or shrub, erect, branched, woody, attaining a height up to 1 meter, etc14.

*Sida cordifolia* Linn. also known as “Bala” and “Country mallow,” is a medicinal herb native to Northeastern Brazil and pantropical in nature. It grows in damp area and can with stand drought and heavy rainfall. Its various parts are used to treat fever, heart problems, rheumatism, Parkinson’s disease, facial paralysis, neurological ailments, and more15.It has received more attention after the finding of ephedrine and pseudoephedrine in it. It has weight reducing properties as it shows hypoglycemic effect on body weight 16. In India, *Sida cordifolia* Linn. is utilized as a diuretic agent, to strengthen the Central Nervous System, to cure neurological disorders, and many more 17. It is an upright, perennial and vertically erect shrub of the height of 1-1.5 metre tall. Its leaves are broad, heart shaped and serrate. It bears small and vibrantly yellow-colour flowers from August to December. Its fruiting occurs between October to January18.

**List 1 : TAXONOMIC HIERARCHY**

|  |  |
| --- | --- |
| Kingdom | Plantae |
| Order | Malvales |
| Family | Malvaceae |
| Genus | *Sida* |
| Species |  *acuta ,cordifolia* Linn. |

**MATERIALS AND METHODOLOGY**

**Collection of sample**

Plants were sourced from the Nahargarh area of Jaipur, Rajasthan, in the month of august 2024. Botanical identification was done in department of botany at IIS (Deemed to be UNIVERSITY), Jaipur, Rajasthan, India (26o58’48.3 N- 75o50’49.55 E).

**Selection of plants**

*Sida acuta* L. and *Sida cordifolia* Linn. were selected to evaluate the presence of phytochemical constituents and antioxidant activity in the plant extracts using Methanol and Ethyl acetate solvents.

**Preparation of plant extract**

Collected plants were washed with distilled water. Leaves and stem were segregated and subjected to shade drying for about 5 weeks to remove all moisture. Dried plant material was powdered using an electrical grinder and kept in air tight container. 20g of each powder was extracted with 200ml of different solvents, viz. Methanol and Ethyl acetate, using a Soxhlet extractor. Extracts were then concentrated and diluted with the mother solvent to perform phytochemical evaluation of the plants.

**Phytochemical analysis of plant extract**

The qualitative analysis was performed following standard methods as explained in Practical Pharmacognosy by Kokate C.K. and The Practical Evaluation of Phytopharmaceuticals” by Brain and Turner 19,20 .

The quantitative analysis of detected phytochemicals was done using standard methods.

**Extraction of Alkaloid (Harborne, 1995)**

To 5g of sample, 10% acetic acid was added and allowed to stand for 3-4 hours. The mixture was then filtered and evaporated to one-fourth of its initial volume on water bath. Alkaloids were precipitated by adding concentrated NH4OH to the filtrate drop wise until precipitation formation stopped. The collected precipitate was washed with ammonium solution and filtered. The precipitate, which contained alkaloid, was weighed after drying21.

**Lipids (Bligh and Dyer method, 1995)**

1g of plant material was homogenized with 10ml of distilled water and mixed with 30 ml of chloroform and methanol solution in a ratio of 2:1 (v/v). The prepared solution was allowed to stand for about 10-12 hours at room temperature. Later, chloroform and distilled water were added (each of 20ml), and the solution was shaken. This final solution was transferred into separating funnels for separation. Two separate layers were formed: the upper layer of water and the lower layer of chloroform containing lipids. The lower layer was collected and left for chloroform evaporation. The dried residue was lipid, which was later weighed22.

**Total Phenolic content (Singleton and Rossi, 1965)**

1micro liter of the sample was combined with 5micro liter of Folin –Ciocalteu reagent and left for 10minute. Later, 1.5 ml of 20% sodium carbonate was added, and the solution was made up to 10ml and incubated for 2 hours. Optical density was measured at 765 nm. The standard curve was formulated using Gallic acid (0.5-10 µl/mL). Values were expressed as Gallic acid equivalent (mg/g extract) 23.

Total phenolic content was calculated with the formula: (T= C.V/M)

Where,

T = Total phenolic concentration

C = Concentration of gallic acid from calibration curve (µl/mL)

V = Volume of extract (ml)

M = Wt. of methanol plant extract

**Estimation of Total flavonoids content (Chang et al., 2002)**

1ml of the sample was combined with 0.3ml of 5% of NaNO2 and 0.3ml of 10% AlCl3 solution and left for 6 minutes. Then, 2ml of 1N NaOH was added, and the volume was made up to 10ml using distilled water. The prepared solution was left for 15minutes, and the optical density was measured at 510nm. The standard curve was formulated using quercetin (0.2 to 1ml). The values were expressed as quercetin equivalent (mg/g extract) 24.

Total flavonoids content was calculated with the formula: (T= C.V/M)

Where,

T = Total flavonoids concentration

C = Concentration quercetin from calibration curve (µg/mL)

V = Volume of extract (ml)

M = Wt. of methanol plant extract

**DPPH radical scavenging activity (Chan et al., 2007)**

The antioxidant activities were calculated as the measures of radical scavenging using DPPH assay. 2ml of DPPH was mixed with 1ml of sample extract and left for 30min in the dark. The standard solution was formulated using L-ascorbic acid at different concentrations. The inhibition percentage of DPPH was calculated and results were expressed as % RSA (Radical scavenging Activity). Optical density was calculated at 517nm using methanol as a blank25.Percentage DPPH radical scavenging activity was calculated by the following equation:

% DPPH radical scavenging activity = ⁅ ( A0 – A1) / A0 ⁆ ×100

Where,

A0 = Absorbance of the control

 A1 =Absorbance of the sample

**RESULT AND DISCUSSION**

**Table 1: Qualitative analysis of *Sida acuta* L**

|  |  |  |
| --- | --- | --- |
| **Extracts** | **Methanol** | **Ethyl acetate** |
| **Plant metabolites** | **Leaf** | **Stem** | **Leaf** | **Stem** |
| **Flavonoids** | **+** | **+** | **\_** | **\_** |
| **Phenolic compounds** | **+** | **+** | **\_** | **\_** |
| **Tannins** | **+** | **\_** | **+** | **\_** |
| **Proteins** | **+** | **+** | **+** | **+** |
| **Phytosterol** | **\_** | **+** | \_ | **+** |
| **Reducing sugars** | **+** | **+** | **\_** | \_ |
| **Saponins** | **+** | **+** | **\_** | **\_** |
| **Lignin** | **+** | **+** | \_ | \_ |
| **Triterpinoids** | **+** | **+** | **\_** | **\_** |
| **Alkaloids** | **+** | **+** | **+** | \_ |
| **Steroids** | **+** | **+** | **\_** | **\_** |
| **Carboxylic acid** | **+** | **+** | \_ | \_ |
| **Glycosides** | **+** | **\_** | \_ | \_ |
| **Cardiac glycosides** | **+** | **\_** | **+** | \_ |
| **Coumarins** | **\_** | **+** | \_ | **+** |

The results of the qualitative phytochemicals analysis of *Sida acuta* L. are summarized in table 1. The phytochemical analysis of leaf extract in methanol showed the presence of alkaloids, flavonoids, phenolic compounds, tannins, reducing sugars, saponins, lignin, triterpenoids, steroids, carboxylic acids, glycosides, and cardiac glycosides. The Methanolic extract of stem showed the presence of flavonoids, phenolic compounds, proteins, phytosterol, reducing sugars, saponin, lignins, triterpenoids, steroids, carboxylic acids, and coumarins.

The ethyl acetate extract of the leaf and stem showed presence of tannin, proteins, alkaloids, cardiac glycosides, and proteins, phytosterol, coumarins, respectively. The Methanolic extract of the leaf and stem yielded more bioactive compounds compared to ethyl acetate extract. Previous studies on phytochemical analysis of *Sida acuta* L. are summarized in table 2. Studies conducted on different part of the plants using same or different solvents show the similar results.

**Table 2: Comparative studies (*Sida acuta* L.)**

|  |  |  |  |
| --- | --- | --- | --- |
|  **Plant parts**  |  **Solvents** |  **Phyto- constituents** |  **References** |
| **Leaf** |  **Aqueous** |  **Alkaloids, flavonoids, steroids, phenols, terpenoids, cardiac glycosides** | **26** |
| **Leaf, stem, root** |  **Methanol** |  **Alkaloids, steroids, tannins, flavonoids, saponin, cardiac glycosides, tannins** | **27** |
|  **Leaf** |  **Ethanol** | **Alkaloids, flavonoids, steroids, tannins, terpenoids, cardiac glycosides**  | **28** |
|  **Whole plant** | **Petroleum ether, chloroform, ethyl acetate, ethanol, water** | **Alkaloids, steroids, flavonoids, tannins, phenols, proteins, cardiac glycosides, saponin, terpenoids**  | **29** |

**Table 3: Qualitative analysis of *Sida cordifolia* Linn.**

|  |  |  |
| --- | --- | --- |
| **Extracts** | **Methanol** | **Ethyl acetate** |
| **Plant metabolites** | **Leaf** | **Stem** | **Leaf** | **Stem** |
| **Flavonoids** | **+** | **+** | **\_** | **\_** |
| **Phenolic compounds** | **+** | **+** | **\_** | **\_** |
| **Tannins** | **+** | **\_** | **\_** | **\_** |
| **Proteins** | **-** | **+** | **\_** | **\_** |
| **Phytosterols** | **+** | **+** | \_ | **\_** |
| **Reducing sugars** | **+** | **+** | **+** | + |
| **Saponins** | **+** | **\_** | **+** | **\_** |
| **Lignin** | **+** | **+** | \_ | \_ |
| **Triterpinoids** | **+** | **+** | **\_** | **\_** |
| **Alkaloids** | **+** |  **+** | **+** | \_ |
| **Steroids** | **+** | **+** | **\_** | **\_** |
| **Carboxylic acid** | **+** | **+** | + | \_ |
| **Glycosides** | **\_** | **+** | \_ | \_ |
| **Cardiac glycosides** | **+** | **\_** | **+** | \_ |
| **Coumarins** | **+** | **+** | \_ | **+** |

The Results of the qualitative phytochemicals analysis of *Sida cordifolia* Linn. are summarized in table 3.The qualitative analysis of the methanolic extract of the leaf showed the presence of flavonoids, phenols, tannins, phytosterol, reducing sugars, saponions, lignins, triterpinoids, alkaloids, steroids, carboxylic acid, cardiac glycoside, and Coumarins. Methanolic extracts of the stem showed the presence of flavonoids, glycosides, steroids, coumarins, phytosterol, reducing sugars, phenols lignins, triterpinoids, alkaloids, and carboxylic acid.

Meanwhile, the ethylacetate extract of leaf showed the presence of reducing sugars, saponins, alkaloids, carboxylic acid, and glycosides, while the stem extract showed the presence of reducing sugars and coumarins. Previous studies on the phytochemical analysis of *Sida cordifolia* Linn.are summarized on table 4. Studies conducted on different part of the plants using same or different solvents shows the similar results.

**Table 4: Comparative studies (*Sida cordifolia* Linn.)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Plant part**  | **Solvent** | **Phyto-constituents** | **References** |
|  **Roots** |  **Ethanol** |  **Reducing sugars, alkaloids, steroids, saponins** |  **30** |
|  **Leaves** | **Ethanol, Petroleum ether,**  | **Alkaloids, tannins, steroids, saponins, cardiac glycosides, flavonoids, anthraquinone** |  **31** |
| **Whole plant**  |  **Hexane, chloroform, methanol, aqueous** | **Carbohydrates, proteins, alkaloids, glycosides, flavonoids, tannins, steroids, saponins, phenols,**  |  **32** |

**Estimation of total phenolic content**

Phenolic compounds are a major chemical class of bioactive compounds manufactured in plants, provide an important criterion for quantitative evaluation, as they possess antioxidant, anti-inflammatory, and antimicrobial activities, which protects plants against biotic and a biotic stress33.

Total phenolic content of *sida acuta* L. and *sida cordifolia* Linn.was expressed in terms of Gallic acid equivalents using the standard curve equation as shown in figure 1 and the total phenolic content is presented in table 6. The total phenolic contents were ranging from 0.0489±0.007mg GAE/g to 0.0852±0.006mg GAE/g. The highest was found in *Sida cordifolia* Linn leaf while *sida acuta* L*.* stem possessed the lowest. The total phenolic content may be arranged from lowest as in *Sida acuta* stem L. < *Sida acuta* leaf L. < *Sida cordifolia* stem Linn. < *Sida cordifolia* Linn. leaf to the highest. Similar results were observed in the comparative experiments performed by M.D. Subramanya et al34, Cheruthazhakkat sulaiman et al35, and C. Beena36., where *Sida cordifolia* aerial parts were found to have more phenolic and flavonoid contents followed by *Sida cordifolia* roots and other Sida species.

 **Figure 1: Curve calibration of gallic acid standard.**

**Table 5: Total phenolic content**

|  |  |
| --- | --- |
| **Extract** | **GAE mg/ gm dry extract** |
| *Sida acuta* stem | 0.0489±0.007 |
| *Sida acuta* leaf | 0.0547±0.10 |
| *Sida cordifolia* stem | 0.0740±0.013 |
| *Sida cordifolia* leaf | 0.0852±0.006 |

**Estimation of total Flavonoid content**

Flavonoids are biologically active phytochemicals have been extensively used as anticancer, antimicrobial, antiviral, antioxidant, neuroprotective, and anti-proliferative agents. They have also been shown to prevent cardiac disorders and improve cognitive performance during aging.. Since these compounds have been found to have positive effects on human health, they provide an important criterion for quantitative analysis37.

The total flavonoid content of *sida acuta* L.and *sida cordifolia* Linn*.* was expressed in terms of quercetin equivalents using the standard curve equation as shown in figure 2 and the total flavonoids content are presented in table 6. The total flavonoids contents ranged from 0.0248±0.007mg QE/g to 0.1194±0.097mg QE/g. The lowest was found in *sida acuta* stem while *Sida cordifolia* Linn leaves had the highest. The total flavonoid content may be arranged from lowest as in *sida acuta* stem < *sida acuta leaf* < *Sida cordifolia stem* <*Sida cordifolia L* leaf to highest 34, 35, 36.

**Figure 2: Curve calibration of quercetin standard.**

**Table 6: Total flavonoids content**

|  |  |
| --- | --- |
| **Extract** | **Quercetin mg / gm of dry extract** |
| *Sida acuta* stem | 0.0248±0.007 |
| *Sida acuta* leaf | 0.0283±0.011 |
| *Sida cordifolia* stem | 0.0925±0.023 |
| *Sida cordifolia* leaf | 0.1194±0.097 |

**Estimation of alkaloids:** Alkaloids are nitrogen-containing compounds synthesized by plants from amino acids. They act as neurotransmitter and protect metabolic pathways. Alkaloids possess anti-carcinogenic, anti inflammatory, anti allergic properties 38.

The results for alkaloid contents were ranged from 1.102±0.004mg/g of dry weight to 1.257± 0.086mg/g. The lowest content was found in *Sida cordifolia* stem, while *Sida acuta* leaves possessed the highest. The alkaloid content may be arranged from lowest as in *Sida cordifolia* stem <*sida cordifolia* leaf < *Sida acuta* stem <*Sida acuta* L leaf to highest. The results are presented in table 7 and are expressed as mg per g of dry weight 34, 35, 36.

**Estimation of lipid:** Lipid contents were ranging from 1.019 ± 0.065 mg/g of dry weight to 1.66±0.016 mg/g. The lowest was found in *Sida acuta* Linn stem while *Sida cordifolia* leaves possessed the highest. The lipid content may be arranged from lowest as in *Sida acuta* stem < *Sida acuta* L leaf < *Sida cordifolia* stem <*Sida cordifolia* L leaf to highest. Sushama Rah, R.v et al also reported similar results where leaf of *Sida cordifolia* were found to have more lipid followed by root, stem and different parts of *sida acuta* 39. The results are presented in the table 7 and are expressed as mg per gram of dry weight.

**Table 7: Quantitative Estimation of Plant Metabolites (mg/gm dry weight)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Compound** | ***Sida acuta* L Stem** | ***Sida acuta* L Leaf** | ***Sida cordifolia* Linn Stem** | ***Sida cordifolia* Linn Leaf** |
| **Lipid** |  1.019 ± 0.065 |  1.171±0.013 |  1.333± 0.027 |  1.66±0.016 |
| **Alkaloids** |  1.134± 0.032 |  1.257± 0.086 |  1.102±0.004 |  1.110± 0.005 |

**Antioxidant activity**

The DPPH radical scavenging method was used to calculate antioxidant activity. This technique requires less time to measure the antioxidant potential of plant extracts and widely used. DPPH is an effective antioxidant removes free radicals by donating its hydrogen. Removal of free radicals is important, as they lead to various diseases like cancer, Alzheimer’s, etc40. The results of DPPH scavenging activity on methanolic extracts of different parts of plants and ascorbic acids are shown in tables and figure. The results show that the *Sida cordifolia* Linn. leaf extract exhibited the highest antioxidant activity, while *sida acuta* L. stem extract showed the lowest. The antioxidant activity may be arranged from lowest as in *sida acuta* L. stem < *sida acuta* L. *leaf* < *Sida cordifolia* stem Linn. stem <*Sida cordifolia* Linn. leaf to highest. The results for antioxidant activity were expressed as % RSA values and ranged between 53.39%±0.001to 70.59%±0.0017 at 1 mg/ml. The results were in accordance with observations made by C. Beena , M.D. Subramanya et al, and Cheruthazhakkat sulaiman et al., where *Sida cordifolia* Linn*.* leaves were found to have higher antioxidant activity compared to *Sida acuta* L34, 35, 36.

**Figure 3: Curve calibration of ascorbic acid standard.**

**Table 8: Antioxidant activity of *Sida acuta* L.**

|  |  |  |
| --- | --- | --- |
|  |  ***Sida acuta* L. Leaf** |  ***Sida acuta* L. Stem** |
| **Concentration(Mg/ml)** |  **Absorbance** |  **% RSA** |  **Absorbance** |  **% RSA** |
| 0.2 | 0.833 | 31.77%±0.006 | 0.920 | 24.65%±0.012 |
| 0.4 | 0.705 | 42.26%±0.019 | 0.798 | 34.64%±0.019 |
| 0.6 | 0.623 | 48.97%±0.021 | 0.758 | 37.51%±0.004 |
| 0.8 | 0.530 | 56.59%±0.009 | 0.681 | 44.22%±0.049 |
| 1 | 0.469 | 61.58%±0.004 | 0.569 | 53.39%±0.001 |

**Table 9: Antioxidant activity of *Sida cordifolia* Linn.**

|  |  |  |
| --- | --- | --- |
|  |  ***Sida cordifolia* Linn. Leaf** |  ***Sida cordifloia* Linn. Stem** |
| **Concentration(Mg/ml)** |  **Absorbance** | **% RSA** | **Absorbance** |  **% RSA** |
| 0.2 | 0.725 | 40.62%±0.007 | 0.755 | 38.16%±0.009 |
| 0.4 | 0.671 | 45.04%±0.014 | 0.675 | 44.71%±0.0120 |
| 0.6 | 0.542 | 55.6%±0.011 | 0.567 | 53.56%±0.014 |
| 0.8 | 0.416 | 65.92%±0.015 | 0.460 | 62.32%±0.005 |
| 1 | 0.359 | 70.59%±0.0017 | 0.409 | 66.50%±0.008 |

The results from our study show that both plants are rich source of phytochemicals; however, quantitative analysis revealed that the amount of phytochemicals present in each plant are different. We also observed that the methanolic extraction rate is significantly high (85%) compared to ethylacetae (23%) 41. From our study, where quantitative analysis is compared, the methanol extract showed that phenolic and flavonoids content are found higher in *Sida cordifolia* leaf compared to other extracts. It is also observed that extracts with higher concentrations of phenolic content and flavonoids have strong antioxidant effect. Similar results were stated in experiments performed by C. Beena, in *Sida cordifolia* Linn and Zainol et al. in *Centella asiatica,* which suggest that phenolic compounds are responsible for the antioxidant activities of the plant 42.

*Sida acuta* L. and *Sida cordifolia* Linn. are plants of ethanomedicinal importance and are well documented in Ayurveda. The study provides a comprehensive comparison of phytochemicals and antioxidant activity of both plants. Results show that both the plants are rich sources of phytochemicals and possess antioxidant potential. Methanol, being highly polar, is able to extract more phytochemicals compare to ethyl acetate. Based on the comparative studies, it can be concluded that *Sida cordifolia* Linn. leaf extract can be a good source of phytochemical and also exhibited the highest antioxidant activity among all the extracts.

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