Phytochemical Analysis of Careya arborea Roxb. Root Extracts: A Qualitative Analytical Approach

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Abstract

The examination of phytochemicals and identifying the presence of bioactive substances within plants used for medicinal purposes; ultimately promotes the advancement of medication exploration. In this paper, a qualitative analysis was carried out on the root extracts of Careya arborea Roxb. with different solvents. The qualitative examination confirmed several phytocompounds in the extracts of C. arborea roots. The water extract had the maximum extraction yield of 18.97 g/100 g, while the petroleum ether extract exhibited the lowest extraction yield of 8.28 g/100 g. The presence of various bioactive constituents in C. arborea facilitates its use by conventional herbal healers and allows for its application in the treatment of numerous ailments. The research unveiled the distinct attributes of individual raw substances, rendering them appropriate for recognition and regulation in order to improve the market value of the plants.

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1. Introduction

The term “Medicinal Plant” pertains to plants containing natural bioactive constituents that exhibit pharmacological efficacy. Both the entire plant and certain portions of it can possess therapeutic properties.10 Herbal therapy refers to therapeutic treatments that consist of active compounds derived from herbal plants. The product has the potential to be derived from several components of the plant, including both the complete plant and its individual parts. Herbal medicine encompasses the application of byproducts of plants, including oily substances, gums, and other fluids, for medicinal purposes.11 The term 'phytochemical' describes the substances which are derived from plants with biological functionality. They are present in the foliage, stems, blossoms, fruits, seeds, and other plant components. It is widely assumed that these phytochemicals provide preventive properties against a range of physical disorders. Plants are the most ancient and paramount reservoir of medicinal substances.12 The safety, efficacy, cost, and availability of conventional drugs, which are frequently employed for the treatment of a multitude of disorders, may not always meet the needs of a significant portion of the global population.13 Despite the existence of multiple medicines derived from chemical resources, it remains a must to assess novel, affordable, and economically efficient pharmaceuticals in order to address the forthcoming difficulties associated with diseases. It is also well known that most of the synthetic drugs have some side effects. The application of herbal remedies as a form of therapy has made the conventional healthcare system widely recognized option for those seeking medical care. The WHO (World Health Organization) has noticed an ongoing increase in the consumption of natural remedies within the medical field.14 The application of therapeutic plants and their extracts has emerged as a significant avenue for the development of novel pharmaceuticals, yielding promising outcomes in the management of diverse medical conditions.15, 16 Therapeutic plants are obtained and subjected to extraction and processing methods in order to be deployed for intake directly as herbal remedies. The process of processing therapeutic plants for testing encompasses several essential steps, including the

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systematic and prompt acquisition of the plant material, verification of its authenticity by a qualified specialist, appropriate drying techniques, and subsequent crushing.[9] Subsequently, plant materials are subjected to removal, separation, and purification processes. Furthermore, it involves the assessment of both the quantity and quality of active substances. In current times, there has emerged an increasing global enthusiasm for using plants as a medicinal resource due to their natural world, easy accessibility within the local population, affordability, simplified handling, and potential reduced side effects. Additionally, natural remedies can serve as a beneficial alternative therapy in instances where there are multiple negative consequences and the growth of therapeutic susceptibility.[9] The extraction of therapeutic plants involves the separation of phytocomponents from plant materials by making use of a suitable solvent and a standardized extraction approach.[10] The selection of a successful extraction process is contingent upon several factors, including plant properties, sorting, pH value, temperature of solvent employed, and the proportion of solvents in the sample. The planned implementation of the final results is also an important consideration.[11] The first and essential stage in attaining superior study outcomes is preparing plants for testing. The process encompasses the extraction and assessment of both quality and purity elements prior to conducting the desired scientific experimentation. The process of obtaining high-quality active molecules involves several key steps, namely the careful choice of a suitable solvent, the implementation of effective extraction approaches, taking advantage of screening processes, and the application of separation and recognition processes.[12] The intricacies of these methodologies and the specific path pursued are contingent upon the chosen research design. Solvents frequently employed in the extraction of samples encompass polar solvents such as water and alcohols, intermediate polar solvents like acetone and DCM (dichloromethane), EA (ethyl acetate), and nonpolar solvents including n-hexane, pet ether (petroleum ether), and TCM (chloroform, or trichloromethane).[13]

Careya arborea Roxb generally called ‘Kumhi’, is a member of the Lecythidaceae family. It is of moderate height and deciduous nature, reaching a vertical stature of approximately 20 meters. The scientific description assigned to the plant is popularly referred to as ‘Padmaka’ in Ayurveda,[14] ‘Ka Li Yu Rui’ in Chinese medicine,[15] ‘Katabhi’ in Sanskrit,[16] and ‘Kumhi’ in Chhattisgarh. The use of this plant is prevalent in various regions, including Asia, Australia, and African countries. The distribution of this species encompasses various regions in India, including Kashmir, Laddakh territory, West Bengal, Madhya Pradesh, Chhattisgarh, and Tamil Nadu, Karnataka, as well as forested areas and meadows. The year 1819 marked the description of a particular genus of flowering plants belonging to the Lecythidaceae family, which was described as Careya. The taxonomic classification of the Lecythidaceae family places it inside the Ericales order, where it is regarded as a monophyletic group. The botanical term Lecythidaceae refers to a family of big arboreal species found in tropical regions, characterized by their ability to bear enormous fruits encased in a durable woody pericarp.[17] The Lecythidaceae family comprises over 20 distinct genera and encompasses a diverse assemblage of around 450 species, predominantly found in tropical regions. Careya arborea Roxb. is a visually appealing tree characterized by its wide-spreading crowns. The foliage has characteristics of simplicity, lacking hair or pubescence, and a pattern of arrangement that is alternate rather than opposite or whorled. The leaf shape is predominantly obovate or oblong, with a tendency for clumping towards the terminal ends of branches. The leaf structure is robust and exhibits a distinct margin. Additionally, throughout the winter season, the leaves undergo a transformation, acquiring a crimson hue.[18] The root exhibits a reddish-brown coloration, possesses a spicy flavour, lacks any discernible odour, and displays a crumpled texture on its outer layer. The uses of the root of Careya arborea Roxb. (C. arborea) in Ayurvedic medicine include its application in the treatment of TB (tuberculosis) and broken bones, as well as its inclusion in therapeutic regimens targeting ‘Vata-Kapha imbalances’[19] (‘Vata’ denotes psycho-neuro-musculo-skeletal reflexes or motion, ‘Kapha’ denotes bodily constituents or composition, as per the Ayurveda concept). C. arborea has a range of pharmacological activities, such as anti-fertility, antibacterial, hyperglycemic, anti diarrheal, and antifungal actions.[20,21] The purpose of the present investigation was to evaluate the roll of different solvents for the extraction active phytocomponents present in the C. arborea root.

2. Material and methods
2.1 Collection of plant materials
The root of C. arborea was obtained from Madanpur and Chandipara villages located in the Parghar region of Janjir-Champa District, Chhattisgarh, India, in February 2020. The plant samples were sent to the Botanical Survey of India (BSI) in Prayagraj, Uttar Pradesh, for official verification and received a confirmation letter.

2.2 Processing of Plant materials
The C. arborea root underwent a cleaning process, followed by rinsing with clean water and subsequent drying under shade until complete evaporation of water molecules occurred. The shade-dried root was then ground using an electronic grinder machine and made into a coarse powder. The powder samples were appropriately stored in a hygienic and hermetically sealed receptacle, along with appropriate labeling, in preparation for subsequent examination.

2.3 Preparation of plant extracts
The C. arborea root extract was made by multiple successive solvent extractions using a Soxhlet apparatus. Approximately 50 g of coarse powder derived from the roots of C. arborea

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were carefully placed into a thimble. Subsequently, the powder was subjected to individual extraction processes using 250 mL of different solvents. The solvents employed in the study included petroleum ether, DCM, TCM, EA, acetone, EtOH, MeOH, and water, chosen based on their respective polarity characteristics. The extraction procedure is typically conducted for 18–24 hours, or until the solvent within the syphon tube of the extractor achieves a colourless state. Subsequently, the extract was transferred into a beaker and placed into a water bath, where it was subjected to heating at a temperature range of 30–40 °C until complete evaporation of the solvent occurred. The dried extract was stored at a temperature of 4 °C in a freezer for subsequent usage in phytochemical studies. The extraction procedure was executed, and routes are given following Scheme 1.

2.4 Percentage of extraction yield
It is widely recognized that the percentage of solvent in the crude sample plays a significant role when estimating the amount of phytochemical that may be extracted from the sample. This makes it one of the most critical factors. Additionally, the polarity of the extractant is a significant factor in determining the extent to which active molecules and their components are recovered. Extraction of C. arborea coarsely powder was completed successive solvent extraction procedure. The coarse powder of C. arborea root was weighed, extracted with various solvents into the Soxhlet apparatus, and concentrated, respectively. The amount of extraction yield was determined using the following formula in Equation (1), and the results are displayed in Table 2.

\[
\text{Extractive yield} = \frac{W_1}{W_2} \times 100
\]

where, \( W_1 = \text{Crude extract weight} \), and \( W_2 = \text{Sample weight} \)

2.5 Qualitative phytochemical analysis
Evaluation of qualitative active molecules is a critically important step in locating novel routes to phytopotential medicinal value. These tests confirmed the presence or absence of a number of naturally active secondary metabolites. This raises the possibility that the therapeutic qualities of these chemicals are due to their composition. The qualitative phytochemical analysis of C. arborea root was conducted in accordance with our previously established techniques and standard guidelines.[8,9,22-26] The following section provides a description of the observations and inferences received from the phytochemical test.

2.5.1 Test for alkaloids
a) Dragendorff test
Several drops of Dragendorff reagent were added to the C. arborea root extract and stirred. A shift in extracts' colour from pale to brown indicates that there is an alkaloid.

b) Wagener test
Five drops of Wagner reagent were used to treat a tiny volume of C. arborea extracts. The extract is responsible for the rusty brown hue which indicates the existence of alkaloids.

2.5.2 Test for Flavonoids
a) Sinoda test
Pick a little quantity of C. arborea root powder, add some magnesium powder, and 1 mL of pure HCl. Observe the hue of the solution. If it has a pinkish-type red tint, this shows flavonoids are present.

b) Lead acetate test
Pick 1g of C. arborea root powder, and after adding several drops of an alcoholic lead acetate solutions, the golden precipitated forms occur. This shows flavonoids exist.

2.5.3 Test for Saponins
Foam test
A little quantity of C. arborea root powder was subsequently mixed with 5 mL of water in a beaker. The solution was vigorously agitated and thereafter monitored for the development of enduring foam, which serves as an indicator for the existence of saponins.

Scheme 1. Successive solvent extraction procedure.
2.5.4 Test for Phenol
Ferric chloride test
A tiny quantity of *C. arborea* root powder was treated with an alcoholic solution containing 5% FeCl₃. The extraction process yields a solution containing phenol that causes an end product with a deep blue or black hue.

2.5.5 Test for Carbohydrates
Molisch test
1 mL of Molisch reagent was poured into the *C. arborea* root powder. The reagent and sample weren’t mixed, resulting in the formation of the top layer. The appearance of a violet hue at the boundary between both layers serves as an indication of the existence of carbohydrates.

2.5.6 Test for Cardiac glycosides
Killer Killiani test
In a test tube, a small amount of *C. arborea* root powder was mixed with 1 mL of GAA (glacial acetic acid), and then a few drops of FeCl₃ solution were added. The experiment involved intentionally downplaying the concentration of H₂SO₄ to 1 mL. Earthy-coloured circles at the interface signified glycosides. In the acetic acid area, an emerald-hued circle indicates carbohydrates, while a purple circle sits beneath the center circle.

2.5.7 Test for Sterols
Liebermann-Burchard test
A tiny quantity of *C. arborea* powder was tested with 2 mL of acetic acid, 1 mL of TCM, and 1 mL of concentrated H₂SO₄. The resultant mixture was then inspected for the appearance of a bluish hue, which serves as an indication of the existence of sterols.

2.5.8 Test for Tannins
Braymer test
1 g of *C. arborea* root powder was subjected to boiling in 5 mL of alcoholic FeCl₃, with a concentration of ten percent. The resultant solution was then examined for the occurrence of a color change, namely the development of an aquamarine or emerald hue.

2.5.9 Test for Terpenoids
Salkowski test
A 2 mL of TCM was stirred into 1 g of *C. arborea* root powder, along with a 2 mL of pure H₂SO₄. The rapid development of a brownish-red precipitate is indicative of the existence of terpenoids.

2.5.10 Test for Amino acid and protein
a) Ninhydrin solution
1 mL of TCM and 1 mL of pure H₂SO₄ were added to 1 g of *C. arborea* root powder. The rapid formation of a reddish-brown precipitate serves as an indication of the existence of an amino acid, which is a type of protein.

b) Xanthoprotien test
Add 1 g of *C. arborea* root powder to a 5 mL concentrated H₂SO₄ solution. The inclusion of H₂SO₄ leads to the formation of a white precipitate, which serves as an indicator for the presence of protein.

2.5.11 Test for Steroids
a) Liebermann Buchard test
1 g of *C. arborea* root powder was combined with 1 mL of TCM, and afterwards, 1 mL of acetic anhydride was added. Next is the addition of pure H₂SO₄ from the sidewalls of the beaker. The observation of a chromatic transition from violet to a blue hue at the interface of the two liquid phases signifies the existence of steroids.

b) Salkowski test
1 g of *C. arborea* root powder was taken and mixed with 2 mL of TCM. 1 mL of sulfuric acid was then included, resulting in the formation of a yellow-coloured circle at the interface of the two fluids. Within a period of 1 minute, the yellow colour transformed into red, indicating the existence of sterols.

2.5.12 Test for Oxalate
A small quantity of the *C. arborea* root powder was treated with 5 mL of GAA (glacial acetic acid). The acquired blackish-green hue implies the existence of oxalates.

2.5.13 Test for Quinones
1 g of *C. arborea* root powder was tested with pure HCl, and subsequent observation was made for the occurrence of a yellowish precipitate. This particular outcome is indicative of the existence of quinones.

2.5.14 Test for Phlobatannins
Precipitate test
1 g of *C. arborea* root powder was subjected to boiling in 5 mL of diluted HCl. The observation of a red precipitate signifies the existence of Phlobatannins.

3. Results and discussions
The process of extracting, detecting, and identifying active chemicals present in plants have potential therapeutic applications is known as qualitative phytochemical investigation.

3.1 Qualitative phytochemical analysis
A comprehensive analysis has been done on a set of 14 phytochemicals, of which 11 were found to be abundant in various extracts, including Pet ether, DCM, TCM, EA, Ethanol, methanol, and water extracts of *C. arborea* root. The qualitative phytochemical study of the root of *C. arborea* confirmed the presence of flavonoids, terpenoids, tannins, saponins, and phenols in each of the extracts. However, the absence of quinones, phlobatannins, and oxalates was observed. Alkaloids, sterols, and cardiac glycosides have been identified in every one of the extracts except for the petroleum
ether. On the other hand, steroids are not detected in petroleum ether, ethyl acetate, and acetone extracts. The carbohydrate doesn't exist in the petroleum ether and TCM extracts, but the protein is only detected in the ethanol and water extracts of C. arborea root.

This observation suggests that C. arborea root possesses significant potential for active molecules. During the phytochemical procedure, diverse solvents were employed to assess the findings of different phytochemicals. The extracts include a variety of phytochemicals, like flavonoids, tannins, terpenoids, saponins, phenols, alkaloids, sterols, and cardiac glycosides. However, three phytochemicals, specifically steroids, carbohydrates, and proteins, are found in lower quantities in the extracts. The therapeutic importance of plants is attributed to their ability to stimulate specific biological responses in human organs, which can be attributed to the presence of specific phytocompounds. A diverse array of phytochemicals had been discovered, exhibiting a broad spectrum of functions that could potentially contribute to safeguarding against a multitude of illnesses. The antibacterial effect is attributed to the presence of flavonoids, sterols, terpenoids, and glycosides. Alkaloids have been found to exhibit protective effects against long-term illnesses. Saponin exhibits protective effects against elevated cholesterol levels and possesses antibacterial characteristics. Steroids and triterpenoids exhibit central nervous system (CNS) and pain relieves properties. Antifungal properties have been observed in the TCM extract of C. arborea root. The root of C. arborea possesses the ability to inhibit fertility. The findings of the qualitative phytochemical investigation done on the root of C. arborea are presented in Table 1, adopting established phytochemical tests.

### 3.2 Extraction yield

Plants have played a significant role as a valuable reservoir of natural bioactive chemicals in numerous medicine development initiatives, leading to the identification and isolation of multiple remarkable pharmaceuticals derived from plants. The extraction yield served as a quantitative measure to assess the efficacy of different extraction techniques. C. arborea root powder was extracted on a soxhlet apparatus using various solvents on the basis of their polarity characteristics and collected as crude extract. Obtained extraction yields are expressed as g/100 g. Water extract (18.97 g/100g) exhibited the highest extraction yield, while petroleum ether extract showed a lower extraction yield. An optimal concentration range for the extraction yield of crude drugs is typically between 22-25%. Findings on the extraction yield of C. arborea root are given in Table 2 and Fig. 1, respectively.

#### Table 1. Qualitative phytochemical analysis of C. arborea Root.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Solvent extracts → Phytochemical ↓</th>
<th>Pet. Ether</th>
<th>DCM</th>
<th>TCM</th>
<th>EA</th>
<th>Ace tone</th>
<th>MeOH</th>
<th>EtOH</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloid</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Terpenoid</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Steroid</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Saponin</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Phenol</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Sterol</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Cardiac glycoside</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Carbohydrate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>11</td>
<td>Protein</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Quinone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Phlobatannin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Oxalate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) = Present, (-) = Absent

![Fig. 1](image-url) Extraction yields of C. arborea Root with various solvent.
The extraction yields obtained from *C. arborea* root are also presented in Fig. 2, arranged in ascending order of their quantity in g/100g.

### Table 2. Extraction yield of *C. arborea* Root with various solvents.

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Solvent</th>
<th>Colour</th>
<th>Extraction yield (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum Ether</td>
<td>Light Yellow</td>
<td>8.48</td>
</tr>
<tr>
<td>2</td>
<td>DCM</td>
<td>Light Brown</td>
<td>11.74</td>
</tr>
<tr>
<td>3</td>
<td>TCM</td>
<td>Brown</td>
<td>12.8</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl Acetate</td>
<td>Rusty Brown</td>
<td>9.97</td>
</tr>
<tr>
<td>5</td>
<td>Acetone</td>
<td>Orange</td>
<td>9.5</td>
</tr>
<tr>
<td>6</td>
<td>Methanol</td>
<td>Red Brown</td>
<td>13.29</td>
</tr>
<tr>
<td>7</td>
<td>Ethanol</td>
<td>Rusty Red</td>
<td>14.63</td>
</tr>
<tr>
<td>8</td>
<td>Water</td>
<td>Dark Brown</td>
<td>18.97</td>
</tr>
</tbody>
</table>

The extraction yields obtained from *C. arborea* root are also presented in Fig. 2, arranged in ascending order of their quantity in g/100g.

### 5. Conclusion

Phytochemicals are bioactive plant compounds (may be nutritional or non-nutritional) found in fruits, vegetables, cereals, and other plant foods. For a prolonged time, plant medicines have served as a natural and useful reservoir of phytocomponents, contributing to the maintenance of optimal health for people. Researchers have established a relationship between the consumption of phytochemicals with health benefits for the prevention of various diseases. This has led to the popularization of phytochemicals. The health benefits of these phytochemicals depend on their structural stability and purity. The yield, structural stability and purity of extracted phytochemicals also depend on different conditions like - the method of extraction, the solvent used, experimental temperature, and the extraction time. Qualitative phytochemical analysis involves a thorough search for biologically active compounds and the use of the right solvents to extract them. Water found to be the best solvent for this extraction process with highest extraction yield (18.97 g/100g), trailing by ethanol and methanol with moderate value (14.63 and 13.29 g/100g respectively), whereas pet ether produced the lowest extraction yield (8.48 g/100g). The root extract of *C. arborea* produced ten major phytochemicals (Flavonoid, Terpenoid, Alkaloid, Steroid, Tannin, Saponin, Phenol, Sterol, Cardiac glycoside, Carbohydrate) suggesting its potency as a valuable natural healthcare product. Trace amount of Protein is found only in water and ethanol. Hence, from the current study we can conclude that; water is found to be the solvent and ethanol may be used in secondary alternative (methanol can be avoided for its toxicity on human health). Future studies should be focused on the comparison of different extraction techniques with the best fitted solvent (water and ethanol). Such studies will provide a richer and more evocative comparison of the techniques and solvents used for the extraction of various phytochemicals. We believe that, this study will help the Ayurveda practitioners and drug makers in their drug/food supplement preparation process.

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### Conflict of Interest

There is no conflict of interest.

### Supporting Information

Not applicable.

### References


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