# Full Length Research Paper

# Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* (fabaceae or mimosaceae) pods

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Antimicrobial activity of distilled water (DE), methanol (ME), ethanol (EE) and ethyl acetate (EAE) extracts of Samanea saman pods was investigated by well-diffusion method against five pathogenic organisms: Bacillus subtilis (B. subtilis), Staphylococsus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa) and Candida albicans (C. albicans). Only ME showed measurable inhibitory activity against both B. subtilis and S. aureus and at concentrations of 20 and 10 mg/ml whereas EAE inhibited only S. aureus at a concentration of 20 mg/ml. Qualitative phytochemical screening of the different solvent extracts of the S. saman pods indicated varied presence of the phytochemicals. However, acidic compounds, proteins and fats/oils were absent, but all the tested solvent extracts except DE showed moderate presence of reducing sugars. Furthermore, while ME and EAE indicated high presence of carbohydrates, DE and EE showed only moderate presence of carbohydrates. On comparison, ME has more of the secondary metabolites followed by EE and EAE and then, DE. The study apparently highlights the biochemical basis for possible use of the S. saman pods (especially the methanol extract) in ethno-medication.

Key words: Samanea saman, fabeaceae, phytochemical, antimicrobial, ethno-medication, narrow spectrum.

# INTRODUCTION

The multi-drug treatment protocol was apparently developed to fight resistant bacteria but, in a seeming challenge to mankind, bacterial strains that could resist the available antibiotics including multi-drug regime continue to emerge. The trend could, without doubt, pose a major hindrance to optimum health care delivery the world over. For instance, it could limit the efficacy of the available drugs which may lead to treatment failure, prolonged infections (Hancock, 2005) and ultimately death.

To counter the highlighted challenge, mankind ought to develop potent antimicrobials against the already emerged resistant bacteria strains and even the emerging ones since reported cases of resistance to new antibiotics seem to indicate the possible short life of such new antibiotic (Coates, 2002). Evidently, this is a global

problem that could be resolved through constant search for and development of new antibiotics and from new sources. Such search could be daunting hence requires the concerted effort of every stake holder, including pharmaceutical companies, academia and research institutes (Latha et al., 2006).

The search via synthetic drug option may not be viable for obvious reasons including the increasing cost and the side effects of synthetic drugs (Shariff, 2001). Fortunately, many higher plants possess phytochemicals (plant derived compounds with therapeutic potentials) that could act in concert to provide significant antimicrobial activity against varied human pathogenic organisms (Arora and Kaur, 1999; Prior and Cao, 2000). Indeed, phytochemicals are vital to human health and could play a significant role in disease prevention and control. They can achieve this through their potential to regulate cancer (prostate and testicular), quality of semen in men and breast cancer, cystic ovaries and endometriosis in women (Verger and Leblane, 2003). This

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can also be achieved through their capacity to induce the synthesis of hepatoprotective enzymes and to block possible damage to genetic materials (Okwu, 2004) through their possible antioxidant activities. These knowledge naturally heightened interest in the possible application of these phytochemicals in the development of new drugs for human disease management. In addition, the knowledge shifted the direction for new drug search towards plant sources thereby leading to the recent increased studies on different solvent extracts of plant species originally used in traditional medical practice (Rios et al., 2005).

S. saman or monkey pod tree is a large canopied tree with symmetrical crown. Although globally distributed, it is of tropical American origin and belongs to the family Leguminosae (pulse family). The tree is easily recognized by its characteristic umbrella-shaped canopy (Staples and Elevitch, 2006). Parts of the tree were used in the traditional medical practice for the mitigation of different diseases including cold, headache, intestinal ailments and stomach ache (Staples and Elevitch, 2006). In particular, the leaf infusion is used as a laxative and, in the West Indies; the seeds are chewed to prevent sore throat whereas in Colombia, the fruit decoction is used as a sedative. Also, the boiled bark of the plant is applied as a poultice to cure constipation. In the Philippines, a decoction of the inner bark and fresh leaves is used for diarrhea. In Venezuela, the roots are made into a hot bath for stomach cancer (Staples and Elevitch, 2006).

The mature pods of *S. saman* are black-brown, oblong, lumpy, 10 to 20 cm long, 15 to 19 mm wide, 6 mm thick, straight or slightly curved, not dehiscing but eventually cracking irregularly, and filled with a sticky, brownish pulp that is sweet and edible (Staples and Elevitch, 2006). The bottom line is that the pods of the S. saman tree are rarely used as a plant part source for herbs. Therefore, information on the phytochemical components and biological activity of the pods of S. saman tree is scanty. Knowledge of the phytochemical constituents of plant parts is required in understanding the basis for any therapeutic effect. For instance, the flavonoids, which are ubiquitous in higher plants and common part of human diet (Sathiamoorthy et al., 2007) could significantly inhibit microbes which are resistant to conventional antibiotics (linuma et al., 1994). Recently, isolated flavonoids were reported to exhibit antimicrobial activity (Abou-Donia et al., 2008; Redko et al., 2007; Rattanachaikunsopon et al., 2007). In addition, flavonoids through their free-radical scavenging activity have evoked multiple biological functions, including vasodilatory, anti-carcenogenic, antiinflamatory, anti-bactericidal, immune stimulatory, antiallergic and anti-viral functions (Kandaswami and Middleton, 1998; Middleton and Kandaswami, 1992; Waladkhani and Clemens, 2001; Okwu and Omodamiro, 2005).

Thus, the present study seeks to ascertain and compare the phytochemical and macronutrient components as well as the antimicrobial activity of the distilled water (DE), methanol (ME), ethanol (EE) and ethyl acetate (EAE) extracts of the *S. saman* pods. The results of the present study could contribute to knowledge on the biochemical basis for possible potential human uses of this plant part waste either as herbal medicine; natural remedies derived from herbs (Falodun, 2010), food or raw material for pharmaceutical and food industries.

#### **EXPERIMENTAL**

#### Solvent and chromatographic materials

The solvents used (methanol, ethanol and ethyl acetate) are products of Sigma-Aldrich® and are of analytical grade. Distilled water, standard grades of silica gel (70 to 230 mesh) and nutrient agar were used for the column chromatography. Other chemicals and reagents used were of certified grade and quality. This study was conducted between March 2009 and April 2010

#### **Test organisms**

The test organisms were obtained from Department of Microbiology, University of Nigeria Nsukka, Nigeria and include Bacillus subtilis (B. subtilis), Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa) and Candida albicans (C. albicans). A 24 h fresh culture of each was prepared in nutrient broth for use in the antimicrobial test.

#### Plant material: Collection and preparation

Fresh pods of *S. saman* (fabaceae or mimosaceae) were collected from the tree in front of Botanical Laboratory, University of Nigeria Nsukka, Enugu State in the south east of Nigeria (with the authorization of the Nigerian Government and in agreement with the United Nations Convention on Biodiversity). The pods were collected between May and October, 2009. The specimen was then identified by Mr. Ozioko of Bioresources Conservation and Development Programme (BCDP) Nsukka. Thereafter, the pods were rinsed with water, sun-dried for 10 days and ground into powdery form using an electric grinder.

#### **Extraction of plant material**

The powdery form of the plant pod was extracted using both polar and non-polar solvents.

#### Distilled water extraction

To 10 g of the resultant specimen, 500 ml of sterile distilled water (DW) was added, then shaken and allowed to stand overnight at 40 ℃. Then, the extract was filtered using Whatman filter paper and concentrated using water bath. Thereafter, the concentrated extract was re-suspended in water to make a concentration of 10, 25, 50 and 100 mg/ml that were used for antimicrobial study.

#### Methanol, ethanol and ethyl acetate extraction

To 10 g each of the powdered pods the different solvents were separately added and then extracted using Soxhlet extractor for 2 h. The ME, EE and EAE were separately concentrated using rotary evaporator and then preserved individually at 5°C in an air tight bottle until used for the phytochemical and antimicrobial screening.

#### Phytochemical screening

The phytochemical analysis of the DE, ME, EE and EAE extracts of *S. saman* pods was by standard methods as described in Evans (2000) and Harbone (1998). Specifically, the extracts were screened for phytochemicals (saponins, glycosides, proteins, steroids, reducing sugars, fats and oils, alkaloids, flavonoids, tannins, terpenoids, and resins) as thus explained.

#### Test for alkaloids

A quantity (0.2 g) of the dry extracts was boiled with 5 ml of 2% HCl on a steam bath for 10 min. The mixture was filtered and 1ml portion of the filtrate was measured into four test tubes. Each of the 1 ml filtrate was treated with 2 drops of the following reagents:

- Dragendorff's reagent: A red precipitate indicates the presence of alkaloids.
- 2. **Mayer's reagent:** A creamy-white colored precipitate indicates the presence of alkaloids.
- 3. Wagner's reagent: A reddish-brown precipitate indicates the presence of alkaloids.
- 4. **Picric acid (1%):** A yellow precipitate indicates the presence of alkaloids.

#### Test for flavonoids

A quantity (0.2 g) each of the dry extracts was heated with 10 ml of ethylacetate in boiling water for 3 min. The mixture was filtered consecutively and the filtrates used for the following tests:

- 1. **Ammonium test:** A quantity (4 ml) each of the filtrates was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed at the ammonia layer, which indicates the presence of flavonoids.
- 2. **Aluminum Chloride test:** A quantity (4 ml) each of the filtrates was shaken with 1ml of 1% aluminum chloride solution and observed for light yellow coloration. A yellow precipitate indicates the presence of flavonoids.

# Test for glycosides

Diluted sulphuric acid (5 ml) was added to 0.1 g each, of the extracts in a test tube and boiled for 15 min. in a water bath. It was then cooled and neutralized with 20% potassium hydroxide solution. A mixture, 10 ml of equal parts of Fehling's solutions A and B were added and boiled for 5 min. A dense red precipitate indicates the presence of glycoside.

#### Test for steroids and terpenoids

A quantity (9 ml) of ethanol was added to 1 g each of the extracts and refluxed for a few minutes and filtered. Each of the filtrates was concentrated to 2.5 ml in a boiling water bath. Distilled water, 5 ml was added to each of the concentrated solution, each of the mixtures was allowed to stand for 1 h and the waxy matter was filtered off.

Each of the filtrates was extracted with 2.5 ml of chloroform using a separating funnel. To 0.5 ml each of the chloroform extracts in a test tube was carefully added 1 ml of concentrated sulphuric acid to form a lower layer. A reddish-brown interface shows the presence of steroids. Another 0.5 ml each of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of

concentrated sulphuric acid for 10 min. on a water bath. A grey colour indicates the presence of terpenoids.

#### Test for saponins

A quantity (0.1 g) each of the extracts was boiled with 5 ml of distilled water for 5 min. The mixture was filtered while hot and the filtrates used for the following tests:

- 1. **Emulsion test:** To a quantity, (1 ml) each of the filtrates, was added two drops of olive oil. The mixture was shaken and observed for the formation of emulsion.
- 2. **Frothing test:** A quantity (1 ml) of the different filtrates was diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for a stable froth.

#### Test for tannins

A quantity (2 g) each, of the extracts was boiled with 5 ml of 45% ethanol for 5 min. Each of the mixtures was cooled and filtered. The different filtrates were subjected to the following tests:

- 1. **Lead Sub-acetate test:** To 1 ml of the different filtrates was added 3 drops of lead sub-acetate solution. A cream gelatinous precipitate indicates the presence of tannins.
- 2. **Ferric Chloride test:** A quantity (1 ml) each of the filtrates was diluted with distilled water and added 2 drops of ferric chloride. A transient greenish to black color indicates the presence of tannins.

#### Test for acidic compounds

A quantity (0.1 g) each of the extracts was placed in a clear dry test tube and sufficient water added. These were warmed differently in a hot water bath and cooled. A piece of water-wet litmus paper was dipped into the different filtrates and observed for color change. Acidic compounds turn blue litmus paper red.

#### Test for resins

Two tests were carried out to detect the presence of resins in the plant under investigation:

- 1. **Precipitate test:** A quantity (0.2 g) each of the extracts was treated with 15 ml of 96% ethanol. The alcoholic extract was then poured into 20 ml of distilled water in a beaker. A precipitate occurring indicates the presence of resins.
- 2. **Colour test:** A quantity (0.12 g) each of the extracts was treated with chloroform and the extracts concentrated to dryness. The residues were re-dissolved in 3 ml of acetone and 3 ml of concentrated hydrochloric acid added. The mixtures were now heated differently in a water bath for 30 min. Pink colour, which changes to magenta-red, indicates the presence of resins.

#### Macro-nutrients analyses

The tests extracts were screened for the presence of macronutrients as thus explained.

# Test for proteins (amino acid compounds)

A quantity (5 ml) of distilled water was added to 0.1 g each, of the extracts. This was left to stand for 3 h and then filtered. To 2 ml portion of the filtrate was added 0.1 ml Millon's reagent. It was

**Table 1.** Results of the phytochemical analyses on *Samanea* saman pods using the test solvent extracts.

	Tests for secondary metabolites	DE	ME	EE	EAE
1	Alkaloids				
	i. Dragendorff's reagent	++	++	++	++
	ii. Mayer's reagent	++	++	++	++
	iii. Wagner's reagent	++	++	++	++
	iv. Picric acid solution (1%)	++	++	++	++
2	Flavonoids				
	i. Ammonium test	_	_	_	++
	ii. Aluminum chloride test	_	-	_	++
3	Glycosides	_	++	++	+++
Ü	ally sociates				
4	Saponins				
	i. Emulsion test	++	++	+++	++
	ii. Frothing test	++	++	+++	++
5	Tannins				
	i. Lead sub-acetate test	_	++	_	_
	ii. Ferric chloride test	-	++	-	-
6	Steroids (conc. H <sub>2</sub> SO <sub>4</sub>	_	++	++	_
	test)				
7	Terpenoids (conc. H <sub>2</sub> SO <sub>4</sub>	_	+++	+	-
	test)				
8	Resins				
	i. Precipitate test	++	+++	++	+++
	ii. Colour test	++	+++	++	+++
9	Acidic compounds	_	_	_	_
	, totalo dompoundo				

Key: - (Absent), + (Low in abundance), ++ (Moderate in abundance), +++ (High in abundance).

shaken and kept for observation. A yellow precipitate indicates the presence of proteins.

#### Burette test

A quantity (2 ml) each of these extracts was put in a test-tube and 5 drops of 1% hydrated copper sulphate was added. A quantity, 2 ml of 40% sodium hydroxide was also added and the test-tube shaken vigorously to mix the contents. A purple coloration shows the presence of proteins (presence of two or more peptide bonds).

## Test for carbohydrates

A quantity of 0.1 g each of the extracts was shaken vigorously with water and then filtered. To the aqueous filtrate was added few drops of Molisch reagent, followed by vigorous shaking. Concentrated sulphuric acid, 1 ml, was carefully added to form a layer below the aqueous solution. A brown ring at the interface

indicates the presence of carbohydrate.

# Test for reducing sugars

A quantity of 0.1 g each of the extracts was shaken vigorously with 5 ml of distilled water and filtered. To each of the filtrates was added equal volumes of Fehling's solutions A and B and shaken vigorously. A brick red precipitate indicates the presence of reducing sugars.

#### Test for fats and oils

A quantity of 0.1 g each of the extracts was pressed between filter paper and the paper observed, warmed to 60 °C and observed again. A control was also prepared by placing 2 drops of olive oil on filter paper. Translucency of the filter paper which persists on warming indicates the presence of fats and oil. The disappearance of translucency on warming usually indicate the presence of essential oils

## Antimicrobial activity screening (antibiotic assay)

# The minimum inhibitory concentrations (MIC) agar dilution assay

The minimum inhibitory concentration was determined by Agar cup dilution method described by Adeniyi et al. (1996). The agar plates were incubated at  $37\,^{\circ}\mathrm{C}$  for 24 h (for the bacteria) and at  $25\,^{\circ}\mathrm{C}$  for 48 h (for the fungus). MIC was determined, according to Harbone (1998), as the lowest concentration of the extract/fractions or gentamicin sulphate that did not permit visible growth as compared to the negative control. The experiment was performed in quardliplicate for each of the concentrations. Inhibition zone diameters (IZD) were measured and the average IZDs were taken and the squared values obtained.

# The minimum biocidal concentrations (MBC) agar dilution assay

The minimum biocidal concentration (MBC) was essentially determined by the same procedure as the MIC. However, the MBC was taken as the minimum concentration of the extract/fractions or gentamicin in which no growth occurred in the agar plate after 72 h of incubation.

#### **RESULTS**

# Phytochemical screening

Generally, the different solvent extracts of the *S. saman* pods indicated low to high presence of alkaloids, saponins and resins but absence of acidic compounds. The results indicated that flavonoids were moderately present in EAE but absent in DE, ME and EE whereas glycosides were absent in DE but present in ME and EE in moderate abundance and in EAE in high abundance. Tannins were absent in all the tested extracts but present in ME in moderate abundance while steroids and terpenoids were absent in DE and EAE but present in ME and EE in varying abundance (Table 1).

Table 2.	. Results	of the	macronutrient	analyses	on of	Samanea	saman pods	using the test
solvent e	extracts.			-			-	_

	Tests for macronutrients	DE	ME	EE	EAE
1	Carbohydrates				
	Molisch test	++	+++	++	+++
2	Reducing sugars Fehlings' solutions A and B	_	++	++	++
3	Proteins (amino acid compounds) i. Millon's reagent ii. Burette's test	<u>-</u>	- -	- -	<u>-</u>
4	Fats and oils Translucency test	_	_	_	_

Key: – (Absent); + (Low in abundance); ++ (Moderate in abundance); +++ (High in abundance).

Furthermore, proteins (amino acid compounds) as well as fats and oils were not detected in any of the studied solvent extracts of the *S. saman* pods. On the other hand, all the tested solvent extracts except DE showed moderate presence of reducing sugars. Where ME and EAE indicated high presence of carbohydrates, DE and EE showed only moderate presence of carbohydrates (Table 2). On comparison, ME has more of the secondary metabolites followed by EE and EAE and lastly, DE.

## **Antimicrobial study**

Out of the studied solvent extracts (DE, ME, EE and EAE) of the plant pods only ME and EAE showed measurable inhibitory activity against some tested pathogens. ME inhibited both *B. subtilis and S. aureus*, at concentrations of 20 and 10 mg/ml whereas EAE inhibited only *S. aureus* at a concentration of 20 mg/ml (Table 3).

## **DISCUSSION AND CONCLUSION**

Phytochemical/macronutrient screening and antimicrobial activity of different solvent extracts of *S. saman* pods were studied in the present study. In comparison, ME has more of the secondary metabolites followed by EE and EAE and lastly, DE. The variable distribution of phytochemicals probably resulted from the difference in the volatility of the solvents used. The presence of flavonoids in the EAE (but not in the other solvent extracts of *S. saman* pods) may limit the therapeutic efficacy of the other extracts of *S. saman* pods and probably accounted for the potential of EAE to exhibit inhibitory effect against *S. aureus* inspite of its lesser phytochemical content as compared with ME.

The antifungal activity of saponins has been reported (Sodipo et al., 1991). And, although saponins were detected in all the solvent extracts of *S. saman* none elicited antifungal activity against the fungis, *C. albicans*. Probably, the tested concentrations of the solvent extracts were not lethal enough to elicit a measurable fungistatic effect.

Tannins are known antimicrobial agents that could inhibit the growth of microorganisms by precipitating out the microbial protein and thus depriving them of nutritional proteins needed for their growth and development (Sodipo et al., 1991). In the present study, tannins were detected in ME but were not present in the other solvent extracts of the *S. saman* pods. This could explain the comparatively better antimicrobial activity of ME on the tested pathogens. In addition, it seems to further highlight the limitations of DE, EE and EAE of *S. saman* pods in traditional management of (especially) those ailments, including nonspecific diarrhea, inflammations of the mouth and throat that were reportedly treated with tannins (Westendarp, 2006).

Steroids increase nitrogen level in the body, thereby producing proteins that help in the production of muscles. Steroids could enhance metabolism and thus inhibit the accumulation of fat correct such disorders like anemia to increase the production of red blood cells in the body and contribute to the treatment of arthritis, asthma, brain injury and some types of cancer. However, steroids could enhance the onset and progression of cardiovascular and liver diseases as well as acne (by stimulating the sebum to produce oil). In this study, steroids were absent in DE and EAE but were detected in ME and EE in moderate abundance suggesting that the DE and EAE of the pods of *S. saman* may not be effective against ailments that are treated with steroids but should be preferred in the management of cardiovascular and liver ailments.

Moderate quantity of alkaloids was found in all extracts of

Test	Tested	Gram	Concentrations (mg ml <sup>-1</sup> )				GS 10 µg ml <sup>-1</sup>
Samples Pathogens			100	50	25	10	GS 10 μg IIII
EE	B. subtilis	+	Nz	nz	nz	nz	18.34
EE	S. aureus	+	Nz	nz	nz	nz	20.10
EE	E. coli	_	Nz	nz	nz	nz	18.34
EE	P. aeruginosa	_	Nz	nz	nz	nz	20.10
EE	C. albicans	Fungus	Nz	nz	nz	nz	21.00
EAE	B. subtilis	+	Nz	nz	nz	nz	18.34
EAE	S. aureus	+	9.75	nz	nz	nz	20.10
EAE	E. coli	_	Nz	nz	nz	nz	18.34
EAE	P. aeruginosa	_	Nz	nz	nz	nz	20.10
EAE	C. albicans	Fungus	Nz	nz	nz	nz	21.00
DE	B. subtilis	+	Nz	nz	nz	nz	18.34
DE	S. aureus	+	Nz	nz	nz	nz	20.10
DE	E. coli	_	Nz	nz	nz	nz	18.34
DE	P. aeruginosa	_	Nz	nz	nz	nz	20.10
DE	C. albicans	Fungus	Nz	nz	nz	nz	21.00
ME	B. subtilis	+	11.25	9.75	nz	nz	18.34
ME	S. aureus	+	10.75	9.00	nz	nz	20.10
ME	E. coli	_	Nz	nz	nz	nz	18.34
ME	P. aeruginosa	_	Nz	nz	nz	nz	20.10
ME	C. albicans	Fungus	Nz	nz	nz	nz	21.00

GS- Gentamicin sulphate; nz (no inhibition zone); + (positive); - (negative).

S. saman pods. Thus, apart from having medicinal value, the pods of the plant may be toxic. Earlier, alkaloids were noted to be toxic by interfering with the digestive processes (Ihekoronye and Ngoddy, 1985) to inhibit the efficient utilization of nutrients (Enwere, 1998).

The presence of carbohydrates and reducing sugars in all the plant pods extracts (except DE that does not contain reducing sugars) seems to indicate the high energy content of the pods of *S. saman* that could be exploited as source for edible food or raw materials for industries that utilize carbohydrates/reducing sugars to produce food, drugs or biodiesel.

The antimicrobial activity results of the various extracts revealed that ME inhibited two bacteria species (*B. subtilis* and *S. aureus*) whereas EAE inhibited only *S. aureus*. Other extracts failed to elicit any measurable inhibition of any of the tested pathogens. Antimicrobial property is generally known to be conferred by the presence of phytochemicals, hence the higher number of different phytochemicals in ME probably explains its exhibition of better antimicrobial potential.

The study apparently highlighted the scientific basis for the possible use of *S. saman* pods in ethno-medication and the probable overriding efficacy (and, possibly, the reason for the preferred trado-medicinal use) of ME over the other plant extracts. Thus, among others, ME of *S. saman* pods appear a better source of natural but narrow spectrum antimicrobial. In conclusion, the phytochemical/

macronutrient components and antimicrobial activity results of the present study suggest that the otherwise waste *S. saman* pods could serve as a good source either for herbs (for herbal medicines), energy giving foods or raw materials for pharmaceutical, food and biodiesel industries. Also, the study suggests that the activity or therapeutic potency of *S. saman* may be dependent on the extraction solvent used and finally, that ME out of the other studied solvent extracts of the pods of *S. saman* should be preferred for use as herbal medicines. The extraction and characterization of the detected phytochemicals in the *S. saman* pods might result in the elucidation of its active therapeutic compound. Research in that direction is ongoing in our laboratory.

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