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In Vitro Anti-Radical Study and Total Anti-oxidant Capacity of Chloroform Fruit Extract of *Ficus sycomorus L.* (Moraceae)

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Abstract
Research findings implicate free radicals particularly reactive oxygen species/reactive nitrogen species (ROS/RNS) in the etiology of human degenerative diseases such as arthritis, inflammations, atherosclerosis, diabetics, central nervous system injury, ageing, cancer and others. The objective of this study was to conduct quantitative phytochemistry, in vitro anti-radical, and antioxidant activities of chloroform fruit extract of Ficus sycomorus L sourced from Kano, north-western Nigeria. Using standard procedures, the extract was measured for phytochemicals quantitatively. The extract was investigated for antioxidant activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, reducing power assay, total antioxidant capacity (TAC) (phosphormolybdenum assay), and hydrogen peroxide (H_2O_2) scavenging assay. Results obtained indicate that alkaloids had the highest quantity, followed by phenols, tannins, and flavonoids. The result of in vitro DPPH radical scavenging activity of the Ficus sycomorus chloroform fruit extract showed increase in antioxidant activity with an increase in concentration with SC_{50} value of 71.94µg/mL. However, the radical scavenging property of the extract was comparably many folds lower than the standard ascorbic acid (SC_{50} 4.81µg/mL). Reducing power (IC_{50} 51.99 µg/mL), TAC (IC_{50} 44.94µg/mL), and hydrogen peroxide scavenging activity (IC_{50} 117.6µg/mL) showed an increase in percentage inhibition and radical scavenging in dose-dependent pattern. The fruit extract exhibited anti-radicals and antioxidant property due to the presence of flavonoids, polyphenols, alkaloids and other conjugated metabolites and it may be important in diseases involving oxidative stress such as age related cancers, stroke, inflammatory disorders, and cardiovascular diseases.

Keywords: Antioxidant activity, Conjugated phytochemicals, DPPH, *Ficus sycomorus*, SC_{50},

INTRODUCTION
Fig tree belongs to the *Moraceae* family and *Ficus* genus containing about 750 species, which usually grow up in warm region in the world, where *Ficus carica* a Mediterranean species of fig, is the most studied and reported (Abdelhakim *et al.,* 2016). The Asian-Australasian region

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of the world is most diverse and abundant area containing about 500 different *Ficus* species while Africa and the Neo-tropics have approximately 110 and 130 species of the plant respectively (Claudia *et al*., 2017). Most of the fig plant species constitute of numerous varieties, important genetic diversity, and huge pharmacological activities that are of remarkable medicinal and agronomic importance (Claudia *et al*., 2017). The plant traditionally has high commercial value, which attracts the attention of the researchers and industries worldwide for its biological properties, bioactive compounds such as primary and secondary metabolites, enzyme information, nutritional value and important genetic diversity which has a promising pharmacological properties (Claudia *et al*., 2017). It is locally called Sycamore fig (English), *Baure* (Hausa), *Tarmu* (Kanuri), among others (Hyde *et al.,* 2013). In northern Nigeria, the stem bark of *Ficus sycomorus* is used commonly to treat fungal infections, jaundice, diabetics and dysentery (Hassan *et al*., 2007; Aduom *et al*., 2012).

F. Sycomorus is also used for the treatment of tumours and diseases associated or characterized by inflammation and oxidative stress (Lansky *et al.,* 2008). The part used for medicinal or agronomic purposes include the fruits (in different stages of /maturity/ripening), fresh or dry, stem bark, leaves, twigs and young growing shoots, and also the latex from the tree bark, fruits and young branches (Lansky *et al.,* 2008). Free radicals are reactive chemical molecules generated during oxidation reactions in living system. It initiates chain reactions (peroxidation) resulting to cellular injury or damage (Valko *et al.,* 2007). The antioxidant agents particularly from plants and foods/diets have gained much attention recently, because oxidative damage or cellular injury is majorly related to many pathological conditions of many diseases (Valko *et al.,* 2007). Thus, the aim of this study was to estimate the phytochemicals as well as determine the *in vitro* anti-radical and antioxidant capacity of fig (*Ficus sycomorus*) fruit chloroform extract sourced from Kano, north-western Nigeria.

MATERIALS AND METHODS

Collection and Processing of Plant Material

Plant samples of *Ficus sycomorus* were collected from Kano, Northern Nigeria. The plants were identified at Faculty of Life Sciences, Bayero University Kano. The herbarium Accession number of specimen *BUKHAN 109* was given. It was chopped into small pieces and shade dried, then ground manually into powder using mortar and pestle.

Extraction of Plant Fruits

Powdered sample (250 g) was extracted using chloroform by soaking for 48 hours in 500 mL of chloroform using maceration method to allow the separation and extraction of wide range of components present in the sample with regular shaking of the extract at time interval of 2-3 hours. It was then extracted through Muslin clothes and Whatman filter paper and the extract was evaporated using water bath set at 50°C. The extract was weighed, and was transferred to a container until further analysis. The extract was dried in an oven as a means of preservation for about 60 minutes (Tiwari *et al.,* 2011).

Phytochemical Analysis
Quantitative Analysis

Determination of total tannins

The extract (500 mg) was weighed into a 50 mL plastic bottle which was then filled with 50 mL of distilled water and then shaken for 1 hour in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtered extract was

pipetted out into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferro-cyanide. The absorbance was measured at 120 nm within 10 minutes (Gracelin *et al.,* 2013).

Determination of total alkaloids

The sample (5 g) was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours at 28 ^oC. This was then filtered and the extract concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected, washed with dilute ammonium hydroxide and then filtered. The residue (alkaloid) was dried in an oven at 80 ^oC and weighed (Soladoye & Chukwuma, 2012; Gracelin *et al.,* 2013).

Determination of total flavonoids

The total flavonoid content was measured using a modified colorimetric method. The appropriate amount of extract was added to a test tube together with distilled water. Then 5% $NaNO_2$ was added to the mixture, after 5 minutes, 10% $AlCl_3$ and after another 5 minutes 1 M NaOH followed by the addition of distilled water. The absorbance was measured against a blank at 510 nm after 15 minutes. A standard curve was prepared using different concentrations of catechin. The flavonoid content was expressed as g catechin equivalents per 100 g of dry weight (g CE/100 g dw) (Ganapaty *et al.,* 2013).

Determination of total phenolic content (TPC)

The total phenolic content of the obtained extracts was spectrometrically analyzed by Folin–Ciocalteu method. This depends on the chemical reduction of Folin-Ciocalteau reagent by phenols to form mixture of blue oxides which absorb maximally at 760 nm. Gallic acid was used as standard for TPC estimation. Final volume of 5 mL was made by adding 500 µL of Folin–Ciocalteau reagent, 1.5 mL of 20% Na_2CO_3 and 2 mL of distilled water to 1 mL (1 mg/mL) of the extract. The mixture was incubated at room temperature for 30–40 minutes and the absorbance of the developed colour was recorded at 765 nm using spectrophotometer (Singleton *et al.,* 1999).

Antioxidant Activity of Chloroform Fruit Extract of *Ficus sycomorus*

DPPH radical scavenging activity

The free radical scavenging activity of the extract was measured using DPPH by the method of Blois (1958) with slight modifications. A 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of various concentrations (0.2 to 1.0 mg/mL) of sample dissolved in methanol to be tested. After 30 minutes, absorbance was measured at 517 nm. Ascorbic acid was used as a reference material. All tests were performed in triplicate. The scavenging activity was calculated as follows:

DPPH radical scavenging activity (%) = [(Absorbance of control – Absorbance of sample)/Absorbance of control] × 100 (Deepa *et al.,* 2013).

Total antioxidant capacity by phosphomolybdenum method

It is a quantitative determination of total antioxidant capacity (TAC) of natural plant and others spectrophotometrically. It involves the formation of phosphomolybdenum complex (based on the reduction of molybdenum, Mo (VI) to Mo (V), by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH which is measured at 695 nm (Prieto *et al.,* 1999).

Hydrogen peroxide scavenging (H_2O_2) assay

The ability of extract to scavenge hydrogen peroxide was estimated by following the method described by Ruth *et al.* (2016). The extract was dissolved in a phosphate buffer at a concentration of 1 mg/mL. A quantity of 1 mL of extract was added to 3.4 mL of phosphate buffer (50 mM, pH 7.4) followed by 60 µL of 400 mM H_2O_2. The solution was kept at room temperature for 40 minutes and absorption was measured at 230 nm. Ascorbic acid was used as standard control. The percentage of hydrogen peroxide scavenging was calculated as follows:

Scavenged H_2O_2 (%) = [Ai−At]/Ai×100

Where: ***Ai*** was the absorbance of control and ***At*** was the absorbance of test samples.

Reducing power assay

The reducing power of the extract was determined according to the method of Deepa *et al.* (2013). Briefly, 1.0 mL of different concentration sample (0.2 to 1.0 mg/mL) was mixed with 2.5 mL of a 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in a water bath at 50 °C for 20 minutes. Afterwards, 2.5 mL of a 10% (w/v) trichloroacetic acid solution was added, and the mixture was then centrifuged at 3000 rpm for 10 minutes. A 2.5 mL aliquot of the upper layer was combined with 2.5 mL of distilled water and 0.5 mL of a 0.1% (w/v) solution of ferric chloride, and absorbance was measured at 700 nm. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Statistical Analyses

Values were expressed as mean ± standard deviation (SD). The IC_{50} (concentrations of the extracts that inhibit 50% of the free radicals) values of the extract were calculated using Curve Fitting Method with Graphpad Prism Statistical Software.

RESULTS

Quantitative Phytochemicals of *F. sycomorus* chloroform Fruit Extract.

From the results, flavonoids was found to be 65.32 ± 0.45 mg/mL, tannins was measured to be 67.17 ± 0.36mg/mL, the phenolics was found to constitute 67.86 ± 0.06mg/mL and the alkaloid was 69.63 ± 0.03mg/mL. The quantitative phytochemicals analysis showed that the concentration of alkaloids was found to be the highest followed by phenols, tannins, and then flavonoids in *F. sycomorus* fruit chloroform extract (Figure 1).

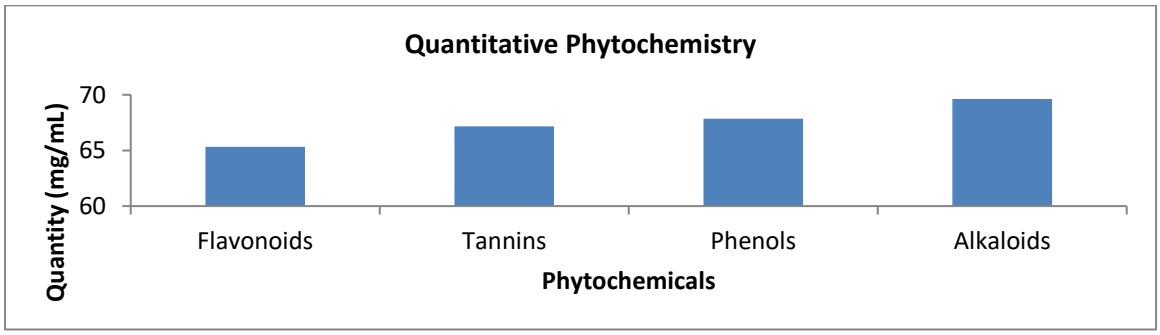

Figure 1: Quantitative phytochemicals of *F. sycomorus* chloroform fruit extract.

Antioxidant and Radical Scavenging Activity

DPPH radical scavenging activity

The result of *in vitro* DPPH radical scavenging activity of the *Ficus sycomorus* chloroform fruit extract showed increase in antioxidant activity with an increase in concentration of the extract. When the absorbance was taken at 25µg/mL to 50µg/mL it showed an increase in antioxidant % radical scavenging, also, the % scavenging was higher when the concentration was raised to 100µg/mL. There was a good association between the antioxidant activity and concentration of the *Ficus sycomorus* chloroform fruit extract (Figure 2).

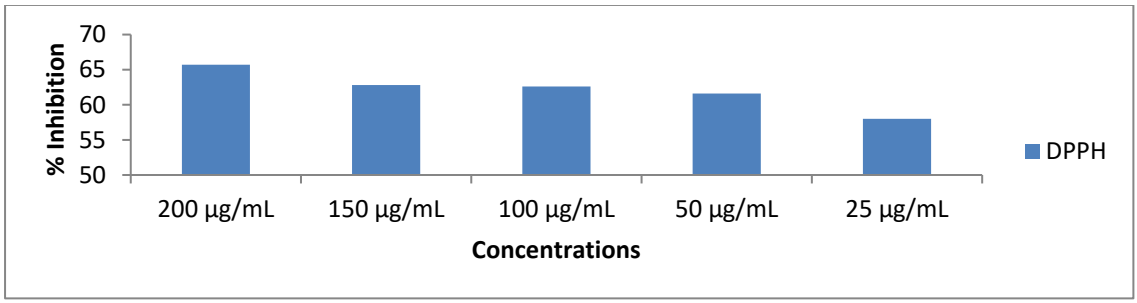

Figure 2: Free radical percentage inhibition against concentration (µg/mL) of the extract. Values are mean ± SD; n=3.

Phosphomolybdenum and reducing power assays

From the result obtained, there was an increase in percentage inhibition when the concentration was increased (Figure 3).

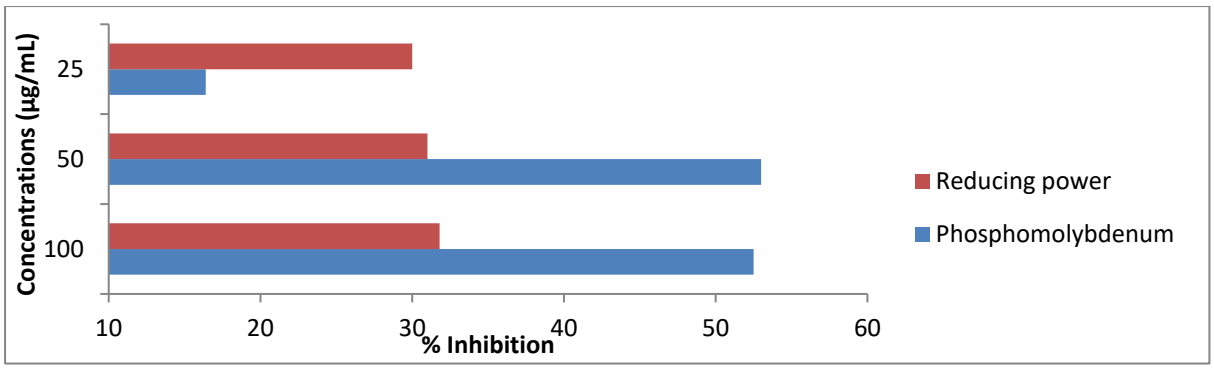

Figure 3; Phosphomolybdenum and reducing power assays. Values are means ± standard deviation; n=3.

High absorbance value in Phosphomolybdenum assay generally is indicative of strong antioxidant capacity (TAC) of the extract. This assay is a quantitative, because the TAC is expressed as the number of equivalent of a standard ascorbic acid.

Hydrogen peroxide scavenging activity (H_2O_2)

From the result, it can be seen that the absorbance of hydrogen peroxide scavenging activity increase with an increase in concentration of *Ficus sycomorus* chloroform fruit extract (Figure 4).

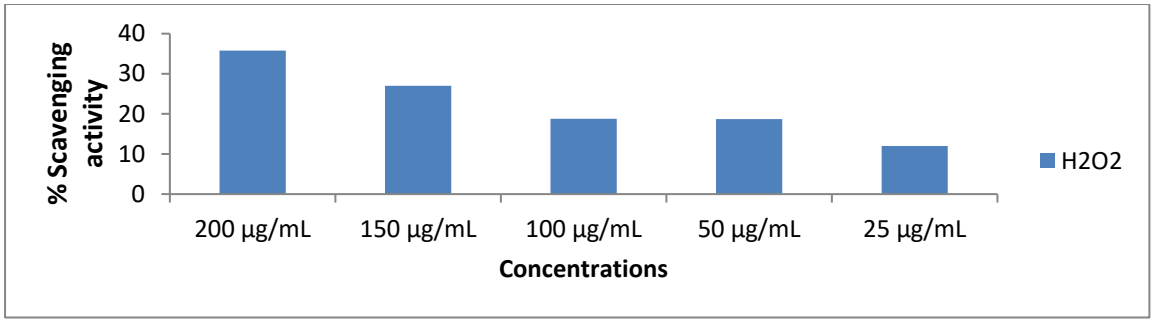

Figure 4. Hydrogen peroxide scavenging activity. Values are means ± standard deviation; n=3.

IC_{50}

Table 1: The IC50 of the extract and the standards (Ascorbic and Gallic acids)

Antioxidant and Radical scavenging parameters of CCEFS	IC_{50}(µg/mL)
DPPH Radical Scavenging Activity	71.94
Reducing Power	51.99
Total antioxidant capacity(TAC)	44.94
Hydrogen Peroxide Scavenging Activity	117.6
Standard Ascorbic acid	4.81
Standard Gallic acid	0.76

DISCUSSION

The reactive oxygen species (ROS/RNS) or oxidants are responsible for the etiology of many human diseases. Synthetic or natural antioxidants have the ability to neutralize the free radicals or reactive oxygen species and their actions. Many researches are in support of antioxidants supplementation in order to promote decrease in oxidative stress (a risk factor of diseases) as well as reducing the rate of disease development in an organism (Abdulqaiyum *et al.,* 2013). Plants naturally, serve as major sources of antioxidant bio-molecules. Several natural products are reported to contain high amount of antioxidant compounds apart from traditional vitamin C (ascorbic acid) and vitamin E (Javanmardi *et al.,* 2003).

Quantitative screening done in this study indicated that *F. sycomorus* had high level of alkaloids compared to other phytochemicals quantified. Alkaloids are one the largest group of plant metabolites found to be present in several plant species. It comprises of neuroactive biomolecules, such as caffeine, nicotine as well as others used in fighting different ailments (Helio & Arthur, 2015). The antioxidants properties of most plant extracts have been correlated to their phenolic contents (Ozgova *et al.,* 2003). They have very high antioxidant activity due to their redox properties and have been recommended as health promoting compounds in human diets. Also, phenols contribute to the flavour, colour, and sensory properties of fruits and vegetables. In the human body, they possess anti- radical property. They were found to be present in *F. sycomorus* fruit chloroform extract assayed in this study. Phenols are secondary metabolites produced in shikimic acid pathway of plant and pentose phosphate through phenylpronoid metabolism as it is found in different plant species that have several therapeutic significance and its scavenging activity was mainly due to the presence of hydroxyl groups (Veerabahu *et al.,* 2015).

The present study on antioxidant of the chloroform extract of *F. sycomorus* indicates the presences of antioxidants. The results obtained for DPPH (2, 2 Diphenyl-1-Picrylhydrazl) radical scavenging activity of fruit extract of *F. sycomorus* were concentration dependent. The highest DPPH scavenging activity (SC_{50} value of 71.94 µg/mL) was observed at the

concentration of 200 µg/mL. This shows good association of the antioxidant activity with the extract concentration. However, DPPH is a stable free radical which can accept hydrogen radical or electron to become a stable diamagnetic molecule (Bijiya & Bilkash, 2013). The SC_{50} of the extract in this study was the concentration required for 50 % DPPH radicals scavenging under the experimental conditions. The lower SC_{50} value indicates a larger scavenging property of the extract (Mohamed *et al.,* 2009; Tung *et al.,* 2009). Thus, the SC_{50} is inversely related to antioxidant activity of the extract. However, the radical scavenging property of the extract was comparably many folds lower than the standard ascorbic acid (SC_{50} 4.81µg/mL).

The radical scavenging assay results show that chloroform fruit extract of *F. sycomorus* (IC_{50} 71.94µg/mL) had a high anti-radical properties compared to crude extracts of *Tabernaemontana catharinensis* fruits (IC_{50} value of 1155.91 mg/mL). Piana *et al.* (2014) evaluated the antioxidant activity by the DPPH method in association with alkaloids content which showed an IC_{50} value of 1155.91 mg/mL and 202.17 mg/mL in fruits and branches crude extracts respectively. Also, Novelli *et al*. (2014) reported a significant correlation between the total alkaloids content of crude extracts of *F. benjamina* and 2,2-diphenyl-1-picrylhydrazyl assay (DPPH), suggesting that the level of antioxidant activity in this species is strongly correlated to the alkaloids content. These studies correspond to the findings of this study that revealed high content of alkaloids and the anti-radical property of the crude extract was dose dependent.

As recorded in this study, the reducing power of the extract is also an indicator of antioxidant property (Oliveira *et al.,* 2008). The extract showed an antioxidant activity having the ability to break free radicals by donating electrons. A similar study conducted by Thagriki *et al.* (2015) using *F. sycomorus* methanol leaf extract in which antioxidant property was evaluated using DPPH, reducing power and hydrogen peroxide corresponded with the findings of the present study in which the antioxidant activity of the plant could be attributed to the presences of phytochemicals. Also, similar findings were reported in a research conducted by El-sayed & Abdel hameed (2009) using eleven *Ficus* species including *F. sycomorus* subjected to 1,1 diphenylpicryhydrazly (DPPH) using different solvents such as chloroform, ethyl acetate, petroleum and butanol.

Total phenolic compounds had been reports as the major constituents of *Ficus spp.* in literatures which is in contrast with this study that pointed alkaloids as the major phytochemicals. In a study conducted by Liu *et al*. (2009), phenolics were the main antioxidant components, and their total concentrations were directly proportional to their anti-oxidant activity. However, in this study, we detected that antioxidant activity of plant crude extracts was not limited to phenolic compounds. Activity may also be due to the presence of other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins. Also, due to their chemical conjugated system, synthetic benzalacetone, chlorophyll, and their relatives have also been suggested as potent radical scavengers with potential antioxidant activities (Handayani & Arty, 2008). Thus, conjugated phytochemicals from this plant may also contribute to the observed radical scavenging and antioxidant activities of chloroform fruit extract of *F. sycomorus*.

The reported anti-inflammatory effect of this plant may be due the presence of these antioxidants. This is because inflammatory tissue injuries/damage is mediated by ROS/RNS from phygocytic cells such as neutrophils, monocytes, macrophages, and eosinophils. These cells invade the tissues and cause damage to essential cellular chemical components (Parfenov & Zaikov, 2000). Chemical agents that have scavenging activities toward ROS have been found to be beneficial in inflammatory diseases management (Koo *et al.*, 2006). The

antioxidant activity of the extract, detected in this study, may also support its traditional use for wound healing, jaundice, diabetics and cancers; because for example, in acute and chronic wounds and other injuries, oxidants cause cellular damage and eventually inhibits wound healing process as reported by Thang *et al*. (2001). In general, antioxidant compounds can delay or inhibit lipid oxidation *in vivo* by inhibiting the initiation or propagation of oxidizing chain reactions and are also involved in scavenging of free radicals via electron donation.

CONCLUSION

This study revealed the presence of phytochemicals which were confirmed using quantitative analysis. The scavenging property of the DPPH radical, H_2O_2 inhibition, and total phenolic contents are the most common methods for evaluating *in vitro* anti-radical potentials of extracts. The antioxidant assay of *F. sycomorus* showed a potent ability in scavenging the free radicals or oxidants due to the presence of phytochemicals particularly flavonoids, phenols, alkaloids and other conjugated compounds and it may be important in diseases involving oxidative stress as risk factor such as age related cancers, stroke, inflammatory disorders, and heart diseases. This result could serve as the basis for the claimed medical significance of the plant.

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