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PHYTOCHEMICAL SCREENING, PROXIMATE AND ELEMENTAL CONTENT ANALYSIS OF STEM BARK OF PILIOSTIGMA THONNINGII (SCHUMACH) MILNE- REDHEAD

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Abstract

The study was carried out to investigate the phytochemistry (such as phytochemical constituents, elemental contents and proximate compositions). The air-dried and pulverized plant sample was subjected to preliminary phytochemical screening and proximate analysis. Few portions of the sample was ashed, digested and analyzed for trace elemental contents using atomic absorption and emission spectroscopy (AAS and FES). The crude ethanol extract was subsequently subjected to partitioning/ fractionation using organic solvents; chloroform, ethyl acetate, n-butanol and water, and fractions obtained were 0.24%, 0.59%, 4.5% and 4.8% from each solvent respectively. The phytochemical studies of the stem bark of Piliotigma thonningii furnished some useful chemical compounds such as flavonoids, cardiac glycosides, tannins, saponins and terpenoids. The

KEYWORDS:
Phytochemical, Screening, Proximate, analysis , elemental , Stem bark, Ethanol

proximate analysis indicated dry matter having the highest percentage of 95.13 %, carbohydrate showed 89.03%, moisture content has 4.87 %, crude fibre showed 40.0 %, ash had 2.0 %, crude protein 2.10 % and ether or fat showed 2.0 %. The result of elemental analysis showed the concentration of Ca (38.30 ± 0.17 mg/L), Na (2.23 ± 0.03mg/L) while Mg (9.13 ± 0.02) and Potassium (3.72 ± 0.03) were within the recommended limit and Fe (4.87 ± 0.03), Mn (0.70 ±0.02), Cu (0.27 ± 0.01), Pb (0.22 ± 0.01), Zn (0.17 ± 0.01), Cr (0.16 ± 0.01) were also within the safety limit.

Introduction

From ancient time, about eighty percent of the world's populations depend on herbal based alternative systems of medicine. Some of these plants are sources of important chemical compounds with medicinal values (Sofowora, 2008). The pharmacological properties of African medicinal plants are immense: remedies made from plants play an important role in the health of millions of people especially in the rural areas (Grabley and Thiericke, 1999). Plants have evolved the ability to synthesize chemical compounds that help in fighting against a wide range of predators, such as insects, fungi and herbivorous mammals. By chance, some of these compounds whilst being toxic to plant predators turn out to have beneficial effects in treating human diseases.

Piliostigma thonningii (Schumach) Milne-Redhead is a leguminous plant belonging to the family, *Leguminaceae* sub family, *Caesalpiniaceae*. The tree is perennial in nature and its petals are whitish to pinkish colour produced between November and April. Locally, the tree is called "Abafe" or “Afemi” in the Yoruba land "Kalgo" (Hausa), "Kalur" (Kanuri) and "Okpoatu" (Ibo) (Nigeria). Other names include Monkey bread and Camel’s foot, (Akinniyi and Sultanbawa,1983). Maceration prepared from the stem bark of the tree is also used in the treatment of malaria, leprosy, sore throat, toothache and earache. (Aubreville,1950). According to Jimoh and Oladiji (2005) different parts of *Piliostigma thonningii* have been discovered to have medicinal value. Its roots and twigs have been used traditionally to treat fever, snake bites, wounds, respiratory ailments and skin infections.

INTERNATIONAL JOURNAL – MBP
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Phytochemical studies appear to be an important aspect of medicinal or herbal plant research, and it is used in evaluating the presence of the chemical constituents in plants. Hence, the need to carry out phytochemical investigations and as well evaluate the elemental content and proximate compositions of the stem barks extract of *Piliostigma thonningii.* In addition, the result of the investigation is expected to reveal the possibility of the plant as a potential raw material and drug.

EXPERIMENTAL

Sample Collection, Identification and Preparation.

Fresh sample of the *Piliostigma thonningii* stem bark was collected from Jumu'a village in Potiskum Local Government Area, of Yobe State in October, 2013. Jumu'a village is about 7km East of Potiskum Local Government Area (Appendix I). The plant material was transported in a polythene bag to the Department of Biological Sciences, University of Maiduguri. The plant was identified by a Plant Taxonomist from the same Department. The voucher specimen number 584B was deposited at the Postgraduate Research Laboratory, Department of Chemistry. Stem bark of *Piliostigma thonningii* (1kg) was air-dried for ten days, ground to powder (pulverized) and then kept in a clean laboratory apparatus (beaker) at room temperature until further use.

Plant extraction and partitioning of crude extract

Stem bark of *Piliostigma thonningii* (1kg) was air-dried for ten days, ground to powder and then extracted with ethanol by maceration (soaking the plant sample in ethanol) for three days. The extract obtained was then filtered and concentrated to dryness in the air at room temperature. The crude ethanol extract was weighed, labeled and kept in a desiccator until further use. The extract obtained was then filtered and concentrated to dryness in the air at room temperature.

The crude ethanol extract of *Piliostigma thonningii* stem bark was partitioned using chloroform, ethylacetate and n-butanol including water

using 200 ml of each of the solvents. The partitioned portion for each solvent was carried out in ten (10) different successions using 20mls for each round until total of 200 ml. 20 ml of the solvent were poured on the crude extract in the separating funnel apparatus, and was then shaken vigorously and allowed to stand for 5 minutes before separation. The extraction was done for ten (10) consecutive times to ensure effective extraction. The extracts obtained were then poured in a big tray and air-dried for 48 hours at room temperature. The fraction obtained with n-butanol involved dissolving the crude extract first before adding the solvent. The n-butanol fraction was found having the yield of 40.50 g, ethylacetate 5.9 g and chloroform having 2.4 g and 48.10 g was obtained in aqueous. Table 1 and scheme 1 show the extractions profile.

Phytochemical Screening

Phytochemical analysis of the plant sample, crude ethanol extract, aqueous, chloroform, ethylacetate and n-butanol fractions were subjected to qualitative chemical screening for the identification of the various classes of active chemical constituents according to the standard methods (Evans 2009, Harborne 1998, Sofowora 2008, Brain and Turner, 1975).

Elemental Content and Proximate Composition of the stem bark *Piliostigma thonningii*

Elemental Content of the stem bark of *Piliostigma thonningii*

Piliostigma thonningii stem bark plant sample was dried in an oven at 105°C for 24 hrs until they were brittle and crisp (APHA, 1992). A portion (1g) of dried plant samples was placed separately in 50 cm^3, Teflon beakers and then digested with 10 cm^3 of a mixture of HNO_3-$HClO_4$-HF (in the ratio of 1:1:1) to near dryness at 80 – 90°C on a hot plate. The digests were filtered into a 50 cm^3 volumetric flask using Whatman No. 42 filter paper and the volumes made up to the marks with water (USEPA, 1996; Radojevic and Bashkin,1999; Umoren and Onianwa, 2005).

INTERNATIONAL JOURNAL – MBP
VOL. 10 NO.3 DEC-2019 ISSN: 1999-5650

Levels of Ca, Na, Mg, Cu, Fe, K, Mn, Pb, Zn and Cr in the plant sample were determined using an SP 1900 Pye Unicam Atomic Absorption Spectrophotometer (AAS) equipped with an air – acetylene burner. These elements were determined at the appropriate lamp current for the specific elements. Concentrations of the metals in the sample was calculated using:

$$\text{Concentration } (\mu gg\text{-}1) = \frac{\text{concentration } (mgl^{-1}) \times V\ (1)}{M}$$

Where V = Final volume ($50cm^3$) of solutions after digestion, M = Initial weight (1g) of samples measured. The mean values of six determinations per sample were recorded.

Proximate Composition of the stem bark *Piliostigma thonningii*

The analysis was carried out at Animal Science Laboratory (University of Maiduguri) in accordance with analytical methods approved by AOAC (1990). The aim of the analysis was to determine ash value, total carbohydrate content, crude protein, fat, dry matter, fibre content.The following standard procedures were employed for each of the analysis and results obtained.

RESULTS AND DISCUSSION

The colour, form recovery extracts of stem bark of *Piliostigma thonningii* of the crude ethanol, chloroform, ethyl acetate, n-butanol and aqueous fractions are shown on Table 1 and Scheme 1. The highest recovery of 16.00 ($^{w}/_{w}$)% was from the ethanol extract, followed by aqueous extract with 4.81 ($^{w}/_{w}$)% while n-butanol and ethyl acetate had 4.50 ($^{w}/_{w}$)% and 0.59 ($^{w}/_{w}$)% respectively and chloroform had lowest recovery of 0.24 ($^{w}/_{w}$)%. The resulting stem bark Marc after the extraction was 66.50 ($^{w}/_{w}$)%.

The extract fractions (ethanol, chloroform, ethyl acetate, n-butanol and aqueous) were subjected to chromogenic (qualitative) phytochemical analysis and the result is shown in Table 2. The result revealed the presence of glycosides, cardiac glycosides, cardenolides, terpenes and sterols and

INTERNATIONAL JOURNAL – MBP
VOL. 10 NO.3 DEC-2019 ISSN: 1999-5650

terpenoids in all the extract; tannins, saponins, flavonoids and carbohydrate were present in crude ethanol, n-butanol and aqueous extracts, while phlobatannins, anthraquinones and alkaloids were absent in all the extract as shown in the Table 2.

The result of elemental analysis of the *Piliostigma thonningii* stem bark is shown in Table 3 and Figure 1. The stem bark showed high concentration of calcium(Ca) 38.30 ± 0.17 mg/L, sodium (Na) 22.34 ± 0.03 mg/L, magnesium (Mg) 9.13 ± 0.02 mg/L, iron (Fe) 4.87 ± 0.03 mg/L, potassium (K) 3.72 ± 0.03 mg/L,manganese (Mn) 0.70 ± 0.02 mg/L, copper (Cu) 0.27 ± 0.01 mg/L, lead (Pb) 0.22 ± 0.01 mg/L, zinc (Zn) 0.17 ± 0.01 mg/L and chromium (Cr) 0.16 ± 0.01 mg/L were detected in low concentrations compared to the standard values in Table 3

Figure 1 and Table 4 show the percentages of moisture content, ash value, total fibre, carbohydrate, fat, crude protein and dry matter contents of the stem bark of *P. thonningii.* The results of the proximate evaluation as shown in Table 4.2 indicate that the dry matter has the highest percentage of 95.13%, the moisture content is 4.87 %, carbohydrate has 89.03 %, while crude fibre has a percentage of 40.0 %, ash 2.0 %, crude protein 2.10 % and ether or fat 2.0 %.

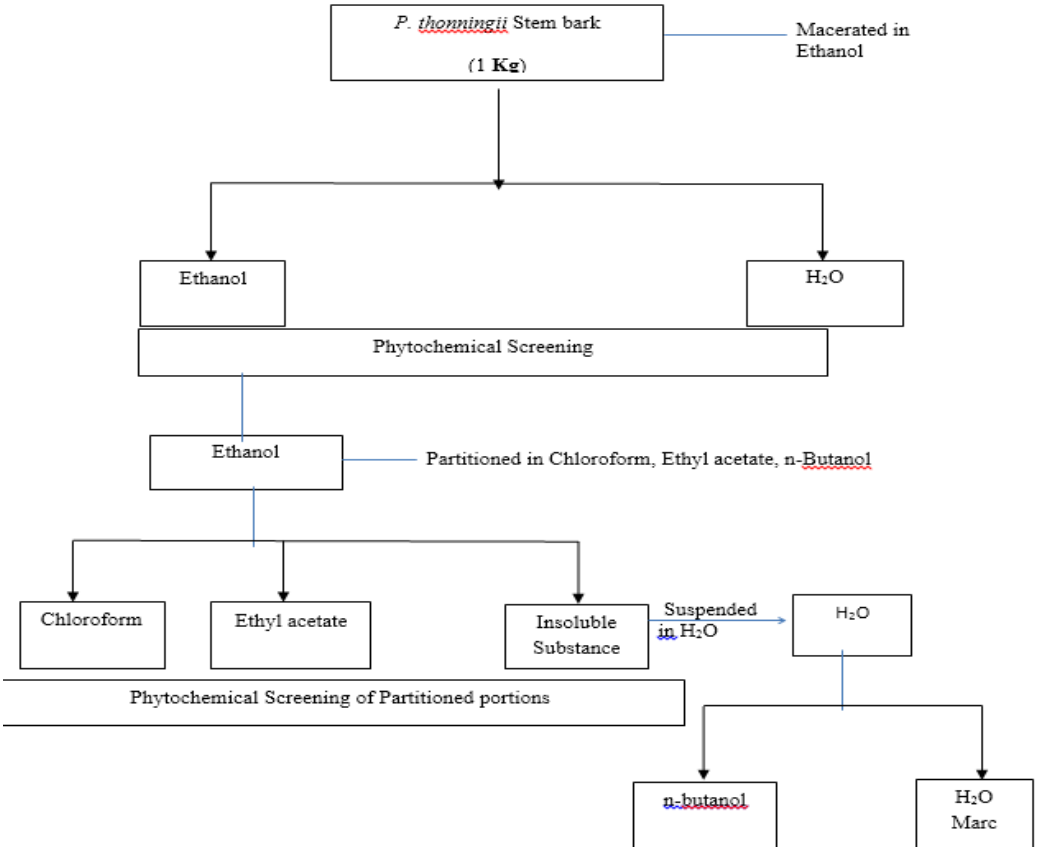
Table 1**:** The extraction profile of the crude ethanol and partition portions extract of *Piliostigma thonningii.*

S/N	Fractions	Mass (g)		% Recovery ($^{w}/_{w}$)	Colour	Texture
1	Ethanol	160.00		16.00	Reddish brown	Amorphous
2	Chloroform	2.40	0.24	Green		Gummy mass
3	Ethyl acetate	5.90	0.59	Brown		Gummy mass
4	n-butanol	40.50	4.50	Brown		Amorphous
5	Aqueous	48.10	4.81	Brown		Amorphous

Weight of Marc = 665g

INTERNATIONAL JOURNAL – MBP
VOL. 10 NO.3 DEC-2019 ISSN: 1999-5650

Extraction and partitioning profile chart



Scheme 1:Schematic Diagram of the Partitioning Profile of Ethanol Stem bark extract of *Piliostigma thonningiii*Various Organic solvents.

Table 2**:** Phytochemical screening of crude ethanol extract, chloroform, ethyl acetate, n-butanol and aqueous fractions of *Piliostigma thonningii.*
Results

S/N	Chemical Compounds	PTCE	PTCF	PTEA	PTNB	PTAQ
1	**Carbohydrate**					
	i. General test (Molisch's Test)	+	-	-	+	+
	ii. Test for Monosacharide(Barfoed's Test)	+	-	-	+	+
	iii.Test for free R.sugar(Fehling's solution)	+	-	-	+	+
	iv. Combined reducing sugar	+	-	-	+	+
	v.Standard Test for Ketoses(Salivanoff's Test)	+	-	-	+	+
	vi. Test for Pentoses	+	-	-	+	+

2	**Soluble starch**	+	-	-	+	+
3	**Tannins**					
i.	Ferric chloride test	+	-	-	+	+
	ii. Lead acetate test	+	-	-	+	+
	iii.Phlobatannins	-	-	-	+	+
4	**Glycosides**					
	i. Test for Free Anthraquinones	+	-	+	+	+
ii.	Test for Combined Anthraquinones	-	-	-	-	-
5	**Cardiac glycoside**					
	i. Salkwoski's test	+	+	+	+	+
	ii. Liebermann-Burchard's Test	+	+	+	+	+
6	**Terpenoids**	+	+	+	+	+
7	**Cardenolides**					
i.	Keller- Killiani Test	+	-	-	+	+
8	**Saponins Glycoside**					
i.	Frothing Test	+	-	-	+	+
ii.	Fehling's solution Test	+	-	-	+	+
9	**Flavonoids**					
i.	Shinoda Test	+	-	-	+	+
	ii. Ferric Chloride Test	+	-	-	+	+
iii.	Lead Ethanoate Test	+	-	-	+	+
	iv. Lead acetate Test	-	-	-	+	+
	v. Sodium Hydroxide Test	-	-	-	-	-
10	**Alkaloids**	-	-	-	-	-

Key: (-) = Absent, (+) = Present, PTCE = Crude ethanol extract, PTCF = Chloroform extract, PTEA = Ethyl acetate extract, PTNB = n-butanol extract, PTAQ = aqueous extract

Table 3**:** Elemental contents of the stem bark of *Piliostigma thonningii*

S/N	Elements	Concentration (mg/kg) Mean ± SD	**WHO Standard (WHO,1996) Conc. (mg/kg or ppm)**
1	Calcium	38.30 ± 0.17	**360-800**
2	Sodium	2.23 ± 0.03	**4-5**
3	Magnesium	9.13 ± 0.02	**100-200**

4	Iron	4.87 ± 0.03	**0.5-50**
5	Potassium	3.72 ± 0.03	**0.1-1.0**
6	Manganese	0.70 ± 0.02	**100-20,000**
7	Copper	0.27 ± 0.01	**1-3**
8	Lead 0.	22 ± 0.01	**0-2**
9	Zinc	0.17 ± 0.01	**15-20**
10	Chromium	0.16 ± 0.01	**0.03-14**

Table 4: Summary of the percentage proximate values of *Piliostigma thonningii* stem bark.

Parameters	**Values** (%)
Dried matter	95.12±0.02
Moisture content	4.86±0.01
Crude protein	2.09±0.01
Ether extract or fat	2.10±0.10
Crude fibre	40.10±0.10
Ash value	2.03±0.05
Carbohydrate	89.03±0.03

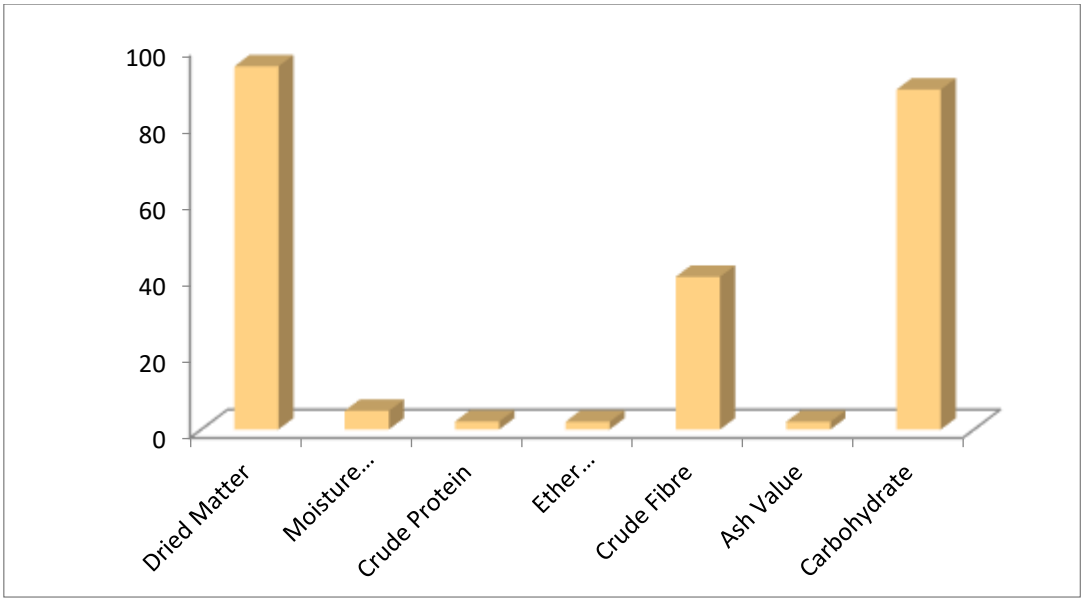


Figure 1: Chart showing the Percentage Proximate Composition of *Piliostigma thonningii* Stem Bark.

The results of the phytochemical screening on *Piliostigma thonningii* ethanol extract and partitioned portions revealed the presence of some secondary metabolites. These include flavonoids, steroids, alkaloids, saponins, cardiac glycosides, tannins and phlobatannins (Daniyan and Abalaka, 2012). These classes of chemical compounds are known to exert pharmacological and antagonistic effects and some are even capable of protecting the active ingredients in herbs from decomposition either chemically or physiologically (Abdulrahman and Onyeyili, 2001).

The chemical constituents present in this plant have many known therapeutic values. Flavonoids are large group of secondary metabolic compounds which are responsible for the colour and aroma of flowers.They also protect plants from different biotic and abiotic stresses and as antimicrobial defensive compound (Amalesh *et al.,* 2011). Flavonoids are found in several herbal remedies used in the treatment of breast cancer (Ren *et al.,* 2003) as such *P. thonningii* stem bark may probably be among such plants to be used for their antioxidant properties. Many biologically active compounds such as glycosides comprise several important classes of compounds that can improve pharmacokinetic parameters (Vladimir and Ludmila, 2001), *P. thonningii* was also found to contain saponin glycosides. Saponins are heterosides of plant origin, substances which contain one or more sugar molecules in their structure. Several researches have shown the defensive roles of saponins. In fact, these substances protect plants from phytopathogenic microorganisms, phytopagous mammals and insects (Haramatha, 2000). Saponin glycosides are used as pesticides and as natural surface active compounds such as soaps, shampoos, creams, lotion and shaving products and they significantly improved learning ability and cognitive functions in brain-damaged rats, in dose dependent manner and enhances the strategic performance on normal manner (George *et al.,* 2002). *Piliostigma thonningii* being in possession of such constituents may probably have these properties.

Plants act as vehicle for transferring pollutants into the food chain at level not harmful to plant but may pose significant threat to animal and humans that ingest it (Moses *et al.,* 2002). Many trace/heavy elements are known to

influence various functions due to their direct or indirect action in physiological or toxic concentration (Abdulrahman *et al.,* 2002). In addition, these elements are used extensively in both chemotherapy and radiography and elements such as Na, Mg and Fe play essential roles in human health and diseases (Moses *et al.,* 2002).

The presence of elements in the stem bark of *P. thonningii* could be an indication of the types of mineral present in the soil (Clarke and Clarke, 1975). Mineral elements are essential in nutrition (Hakeem, 1987). The concentration of some elements such as iron (Fe) and sodium (Na) in the stem bark of *P. thonningii* from elemental analysis in this study are within safety limit and recommended level when compared with the report by WHO (1996) and Alloway (1995) as shown in table 3. Trace elements play very important roles in health and diseases, for example, moderate intake of magnesium (Mg) is known to regulate calcium (Ca) transport and therefore can play important roles in bone metabolism (Sojka and Weaver, 1995). These macro nutrients (Na and Ca) regulate the fluid balance of the body and thereby influence the cardiac output and changes in their level result in hypertension. Manganese (Mn) is essential for normal functioning of central nervous system and is good anti-oxidant (Jimoh and Oladiji, 2005). Adequate zinc (Zn) nutrition is essential for human health, its deficiency affects children's physical growth, hence the risk severity of a variety of infections. Since these elements occur within safety limits and are essential for growth and metabolic functions of the body, this plant (*P. thonningii* stem bark) when ingested may probably play the same role. Some of these elements, especially lead is toxic, and is found in this study to be in negligible quantity, implying that the stem bark of *Piliostigma thonningii* extract may be less or non-toxic for these elements.

Proximate evaluation of the stem bark of *P. thonningii* indicates that dry matter has higher percentage of 95.13%, followed by carbohydrate which is 89.03% and crude fibre is 40.10%, moisture content is 4.63%, crude protein is 1.4%, ash has a percentage of 2.0% and fat has the lowest percentage of 10%. The overall data obtained in this study suggest that *P. thonningii* stem bark may protect against accumulation of cholesterol and triglycerides in the

blood since it has been reported that fibre helps in the maintenance of human health and has been known to reduce the cholesterol level of the body (Bello *et al.,* 2008). This may be useful in the treatment or management of atherosclerosis and coronary heart disorders. However, it is strongly recommended that the use of the plant extract should be within the limits that are non-toxic to the body cell and tissues, preferably between 200-250 mg/kg of body weight (Jimoh and Oladiji, 2005).

CONCLUSION

In conclusion, the phytochemical study reveals the presence of saponins, cardiac glycoside, tannins, flavonoids, terpenoids and carbohydrates in the stem bark extracts of *Piliostigma thonningii*. Heavy trace elements were present in low concentrations in the stem bark of the plant sample while essential elements like sodium and potassium, magnessium occurred at concentrations within safety limits as compared to WHO acceptable standard level. Proximate evaluation of the stem bark of the plant sample indicates its numerous human health benefits.

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CONFLICT OF INTEREST

None declared

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