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Phytochemical Profile of *Telfairia occidentalis* Leaf Grown in Soilless and Soil Media using HPLC

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Abstract

The phytochemical components of *Telfairia occidentalis* Hook F. leaf grown in soil (HS) and soilless (NM) media were assessed using High Performance Liquid Chromatography (HPLC). The media used for the soil medium and soilless medium were humus soil and hydroponic solution, respectively. White-sand from the river was used to raise the seedlings before transferring into hydroponic solution. The plants were allowed to grow for a month for both HS and NM. The phytochemicals assessed were hydrogen cyanide, phytate, saponin, tannin, trypsin inhibitor, flavonoids and alkaloids. Standard procedures were followed in the analyses. The assessed phytochemicals for *T. occidentalis* revealed the following: phytate (9.774 ppm; 8.857 ppm), tannin (2.546 ppm; 2.127 ppm), oxalate (5.897 ppm; 5.927 ppm), saponin (6.829 ppm; 7.253 ppm), trypsin-inhibitor (1.290 ppm; 2.584 ppm), total flavonoids (10.019 g/100g; 8.064 g/100g), and total alkaloids (11.614 g/100g; 9.082 g/100g) for NM and HS media, respectively. Hydrogen cyanide content of *T. occidentalis* (0.001 ppm) was the same in both NM and HS. The phytochemical profile of *T. occidentalis* leaf varied in both media. Hydroponically grown *T. occidentalis* leaf had higher concentration of phytochemicals when compared to orthodox use of soil for cultivation of vegetable.

Keywords: Soilless, soil, growth, phytochemicals, *Telfairia occidentalis*

1. Introduction

Many chemical components of natural food products have been identified as toxicants and some of these include cyanogenic glycoside, hemagglutinin, saponin, gossypol, goitrogen, trypsin-inhibitor, oxalates, phytates and antivitamins. Amino acids serve as building blocks in the production of alkaloids, a secondary metabolites, which offers chemical resistance in plants that makes it biologically useful for consumption (Moran-Palacio *et al.*, 2014).

Alkaloids as secondary metabolites exit in the form of salts with organic acids. They are elementary natural products occurring mainly in plants. Cytochrome oxidase inhibitor such as cyanide actively impedes the breathing system of organisms making cyanogenic glycosides important component of food. Tannins have the ability to change the colour of natural food and processed products. Also, numerous effects are associated with products when tannins attach to proteins and carbohydrates. Enzyme inhibitors remain inherent natural chemical components found in both plant and animal tissues which tends to hamper the activities of certain enzymes from performing their function of breaking down complex molecules into simpler molecules. Studies have shown that anti-nutritional component of food impedes growth with consequent complications in experimental organisms (Chunmei *et al*., 2010). Flavonoids which are polyhydroxylated compounds are known as antioxidants and are also involved in the management of cardiovascular disorders (Onyeka *et al*., 2010). Phytates have been reported to occur naturally in food as salts of potassium, magnesium and calcium (Zhou and Erdman, 1995). Important minerals in food substances such as calcium, iron, magnesium and zinc react with phytic acid to form insoluble salts thereby making its absorption into the blood stream inaccessible. Phenols are secondary metabolites synthesized by the plants. These are potentially effective antioxidants which have the ability to prevent free radicals from harming organisms (Velioglu *et al*., 1998; Chu *et al*., 2002; Kaur and Kapoor, 2002; Materska and Perucka, 2005; Nadeem *et al*., 2011).

Kajihausa *et al.* (2010) reported that food intake from natural sources is on the increase because of its importance to the well-being of humans. In some parts of Nigeria, the leaves of *Telfairia occidentalis* are extracted and used to cure and control illnesses such as convulsion, respiratory diseases, fever and headaches (Iwu *et al*., 1999; Ayandele and Adebiyi, 2007). This leafy vegetable is useful and serve several possesses in the nutritional formulations for Africans, mostly Nigerians.

The choice of *T. occidentalis* leaves as source of medicine and part of everyday meal of many people led to the study. It is aimed at assessing and quantifying of phytochemicals inherent in *T. occidentalis* grown in soilless and soil media using high performance liquid chromatography (HPLC).

2. Materials and Methods

2.1 Source of Materials

The seeds of T. occidentalis were sourced from a farm in Choba. The seeds were divided into two batches and planted in white sand from the Choba River and top humus soil (0-25 cm) from a garden in University of Port Harcourt respectively as a medium for germination. The two weeks old seedlings from the white sand were transferred into a non-circulating hydroponic nutrient system. The nutrient solution used for the hydroponic was bicfarmsconcept formulation. The plants in both soilless medium (NM) and soil medium (HS) were allowed to stand for a month, when it can be harvested to prepare food.

2.2 Determination of Various Compounds

The matured leaves were harvested and used to determine the phytochemicals, flavonoids,

alkaloids, organic acids, glycosides and phenolic compounds of *T. occidentalis* grown in both geoponic and hydroponic medium.

2.2.1 Cyanogenic Glycosides

Sample (5 g) was ground into a paste. The paste was transferred into a corked conical containing 50 mL distilled water and left overnight to facilitate cyanide extraction. The extract was filtered and the filtrate used for cyanide determination. To 1 mL of the filtrate, 4ml alkaline picrate was added and incubated in a water bath for 5 minutes. After colour development (reddish brown colour), the absorbance of the corked test tube read in spectrophotometer at 490 nm. Also, the absorbance of the blank containing only 1mL distilled water and 4 mL alkaline picrate solution. Then, the cyanide content was extrapolated from a cyanide standard curve.

2.2.2 Phytate

The rapid determination of phytate was according to the method of Oberlease *et al*. (1962).

The plant material was extracted with 0.2 N HCl such that we have 3-30 µg/mL phytate solution. The extract (0.5 ml) was pipetted into a test tube fitted with a ground-glass stopper and 1 mL of ferric solution was added. The test tube was heated in a boiling water bath for 30 minutes. Sample was cooled in ice water for 15 minutes and allowed to adjust to room temperature. The content of the tube was mixed and centrifuged for 30 minutes at 3000 rpm. The supernatant (1 mL) was transferred to another and 1.5 mL of 2, 2-Bipyridine solution. Absorbance of the solution measured at 519 nm against distilled water. The method had to be calibrated with the reference solutions as a substitute for the sample solution with each set of analyses. Preparations of the calibration curve was carried out by plotting the concentrations of the reference solutions against their corresponding absorbance. Then the absorbance of the test sample was used to obtain the concentration from the calibration curve.

2.2.3 Tannin

The Folin-Denis spectrophotometric method was used to determine tannin. The method was described by Pearson (1976). One (1) gram of the sample was dispersed in 10 mL distilled water and agitated. This was left to stand for 30min at room temperature, being shaken every 5 minutes. At the end of the 30 minutes, it was centrifuged and the extract gotten. 2.5 mL of the supernatant (extract) was dispersed into a 50 ml volumetric flask. Similarly, 2.5 mL of standard tannic acid solution was dispersed into a separate 50ml flask. A 1.0 mL Folin-Denis reagent was measured into each flask, followed by 2.5 mL of saturated $NaCO_3$ solution. The mixture was diluted to mark in the flask (50 mL), and incubated for 90min at room temperature. The absorbance was measured at 250 nm wavelength in a Genway model 6000 electronic spectrophotometer. Readings were taken with the reagent blanket zero. The tannin content was given as follows.

% Tannin-An/As x C x 100/w x Five

Where, An = absorbance of test sample; As = absorbance of standard solution; C = concentration of standard solution; W= weight of sample used; Vf= total volume of extract;

Va= volume of extract analysed

2.2.4 Enzyme Inhibitors

The trypsin inhibitor activity (TIA) assay via spectrophotometric method described by Arntfield *et al*., (1985).

Extraction of sample: Test sample weight of 1.0 g was dispersed in 50ml of 0.5 M NaCl solution, the mixture was stirred for 30 minutes at room temperature and centrifuged. The supernatant was filtered through filter paper (Whatman No. 41) and the filtrate used for the assay. To 10 mL of the substrate in a test tube, 2ml of the standard trypsin solution was added and a blank of 10 mL of the same substrate in a test tube with no extract added. The content of the test tubes were allowed to stand for at least 5 minutes and then measured at wavelength of 410 nm. One trypsin unit inhibited is given by an increase of 0.01 absorbance units at 410 nm, given a 100 mL of the mixture. That is one trypsin unit inhibited (TUI) is equal to an increase of 0.01 in absorbance unit at 410 nm. The trypsin inhibitor activity is expressed as the number of trypsin units inhibited (TUI) per unit weight (g) of the sample analysed.

$$\text{TUI/mg} = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \text{ X } 0.01F$$

$$\text{TUI/mg} = \frac{b-a}{0.01} \text{ X F}$$

Where b = absorbance of the test sample solution; a = absorbance of the blank (control);

F =experimental factor, given by

$$F = \frac{1}{w} \text{ X } \frac{Vf}{Va} \text{ X D}$$

Where w = weight of the sample; Vf = total volume of extract; V_a = volume of extract used in the assay; D = dilution factor (if any)

2.2.5 Oxalates

This determination involves three major steps digestion, oxalate precipitation and permanganate titration.

Digestion: Sample (2 g) was suspended in 190 mL of distilled water in a 250 mL volumetric flask. To this, 10 mL of 6 M HCl was added and the suspension digested at 100˚C for 1 hour, cooled and made up to 250 mL mark before filtration.

Oxalate Precipitation: Duplicate portions of 125 mL of the filtrate were measured into beakers and four drops of methyl red indicator added. This was followed by the addition of conc. NH_4OH solution (drop wise) until the test solution changed from salmon pink colour to a faint yellow colour (pH 4 – 4.5). Each portion was then heated to 90˚C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90˚C and 10 mL of 5% $CaCl_2$ solution added while being stirred constantly. After heating, it was cooled and left overnight at 5˚C. The solution is then centrifuged at 2500 rpm for 5 minutes. The

supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4 solution.

Permanganate Titration: The total filtrate resulting from digestion of 2 g of test sample was made up to 300 mL. Aliquots of 125 mL of the filtrate was heated until near-boiling and then titrated against 0.05 M standardized $KMnO_4$ solution to a faint pink colour which persists for 30 seconds. The calcium oxalate content was calculated using the formula.

$$\text{Oxalate } (\frac{\text{mg}}{100\text{g}}) = \frac{\text{T x (Vme)(Df)x } 10^5}{\text{(ME) x (Mf)}}$$

Where T is the titre of $KMnO_4$ (ml), Vme is the volume – mass equivalent (1cm^3 of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid), Df is the dilution of factor V_T/A (2.4 where V_T is the total volume of titrate (300 mL) and A is the aliquot used (125 mL), ME is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and Mf is the mass of the test sample used.

2.2.6 Flavonoids (Extraction and Analysis)

Plant sample (1.5g) was weighed into a set of extraction tube(s) and 20ml of boiled ultra-pure water dispensed into each extraction tubes. The setup was allowed to stand for 1.5 hours and voltexed for 5 minutes. The solution was transferred to a set of centrifuge tubes, shaked for 15 minutes and centrifuged for 5 minutes at 3000 rpm. Thereafter, a set of vials were used to collect the supernatants for determination on water 616/626 HPLC. The conditions for the analysis of flavonoids were as follows: (i) An autosampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room. (v) Detector by fluorescence (vi) Carrier gas: Nitrogen gas at flow rate of 60 ml/min. (vii) Temperature: Detector- 147^{o}C; Injector port- 166^{o}C and Column: 115^{o}C (viii) Computer facilities for storing data. (ix) Printer for results reporting

2.2.7 Alkaloids (Extraction and Analysis)

Ten gram (10g) of plant sample was de-fated, out of which 5g was weighed into a flask and 100 mL of 12% alcohol added, shaked, filtered and washed with industrial alcohol. The extracted residue was washed into a flask with 50ml of ammonia water (ultrapure water) and heated in boiling water for 20 minutes and allowed to cool. Then, 0.1g of diastase (+ water) added and maintain at 50-55^{o}C for 2 hrs. It was cooled and made up to 250 mL with ultrapure water, swirled and filtered. The filtrate (200 mL) was mixed with 20 mL hydrochloric acid (sp.g. 1.125) and heated in boiling water for 3 hours. Thereafter, it was allowed to cool, neutralized with sodium hydroxide solution and made up to 250 mL. The sample was shaked, centrifuged and supernatant decanted for determination using water 616/626 HPLC. The conditions of HPLC (Water 616/626) for the analysis of alkaloids were as follows: (i) An autosampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room. (v)

Detector by fluorescence (vi) Carrier gas: Nitrogen gas at flow rate of 40 mL/mins. (vii) Temperature: Detector- 170^{o}C; Injector port- 190^{o}C and Column- 125^{o}C (viii) Computer facilities for storing data. (ix) Printer for results reporting

3. Results and Discussion

The presence and concentration of phytochemicals in *T. occidentalis* grown in NM and HS are presented in Table I. Tannin, saponin and phytate contents of *T. occidentalis* were (1.290 ppm, 6.829 ppm and 9.774 ppm) and (2.584 ppm, 7.253 ppm and 8.857 ppm) for NM and HS, respectively. The hydrogen cyanide remained constant in both media while the trypsin-inhibitor were 1.290 ppm and 2.584 ppm for NM and HS, respectively. Kuku *et al.* (2014) reported that trypsin-inhibitors remain sensitive to heat and gradually or totally loses shape due to high temperature. According to Tsao and Akhtar (2005), some phytochemicals have the potential to act as antioxidant. Also, Young *et al.* (2005) suggested that phytochemicals have capacity to act as a chemo-preventive measure to human cancer by controlling the cell cycle, spread and initiation of apoptosis. Vegetables and fruits are the greatest sources of phytochemicals (Schippers, 2000; Liu, 2004). Jack and Nna (2015) reported 18.716 ppm, 4.95 ppm and 0.2243 ppm for tannin, saponin and phytate content on *T. occidentalis* in that order while oxalate was not detected. However, this study detected oxalate in *T. occidentalis* grown in hydroponic medium (5.897 ppm) and geoponic medium (5.927 ppm). The presence of saponin is well documented in some underutilized plants in Nigeria (Akundu, 1984; Edeoga *et al.*, 2006; Belewu, *et al.*, 2009). Studies have shown the existence of considerable variation in tannin concentrations from one plant to another (Gonzalez-Hernandez *et al.*, 2003) and tannin content of a species is dependent on soil pH (Nicolai, 1988; Northup *et al*., 1995) and soil fertility (Keinanen *et al*., 1999). However, it has been reported that leaves containing tannins can be used for the treatment of intestinal disorder (Akindahunsi and Salawu, 2005). Also, tannins are said to be the source of aroma in tea and its astringent properties makes it suitable for the management of skin epidemic and other therapeutic purposes (Dutta, 2003).

Table I. Phytochemicals present in *T. occidentalis* grown in soilless and soil medium.

Phytochemicals (ppm)	Growth medium	
	NM	HS
Hydrogen cyanide (HCN)	0.001	0.001

Oxalate	5.897	5.927
Phytate	9.774	8.857
Saponin	6.829	7.253
Tannin	2.546	2.127
Trypsin-inhibitor	1.290	2.584

HS represents Soil medium; NM represents Soilless medium

Flavonoids: The presence of 36 flavonoids (8 flavones, 3 isoflavones, 12 flavanones, 8 flavan-3-ols, 4 flavonols, and 1 anthocyanin) were detected in the leaves of *T. occidentalis* (Table II). The most abundant flavonoids in *T. occidentalis* was epigallocatechin followed by daidzein and catechin for NM while the sequence for HS was daidzein, catechin and genistein. Maeda *et al.* (2003) reported that the presence of catechins in tea helps to prevent the spread of the smooth muscle cells in the arterial wall thereby reducing the development of the atheromatous injury. Catechin has been reported to be the dominant scavenger among subgroups of flavonoids, comprises of two benzene rings structurally and frequently used as reference point in the determination of flavonoids contents in leaves (Hue *et al*., 2012). Consumption of flavonoids could be related to decline of having tumour, heart and inflammatory disorder in living organisms. Flavonoids can be derived from food containing vegetables (Haytowitz *et al*., 2003). Studies have shown that flavonoids (daidzein, genistein or their glycosides) supplemented meal have the capacity to stop bone damage and trabecular size triggered by ovarietomy (Nakajima *et al.*, 2001). Smith *et al.* (2004) reported that genistein enhances the lung of patients suffering from asthma. According to Kumar *et al.* (2014), these flavonoids possess resistant to micro-organisms and significant antioxidative activity. They also reported that flavones and flavonols are abundant amongst the subgroups of flavonoids. However, the study showed more of flavanones when compared to others in *T. occidentalis*. The total flavonoids (10.019 g/100g NM, 8.064 g/100g HS) obtained in *T. occidentalis* using HPLC were higher compared to the works of Chibueze and Akubugwo (2011), Aminu *et al.* (2012) and Otitoju *et al.* (2014), with values of 6.67 g/100g, 0.84 g/100g and 0.07 g/100g, respectively in same plant. Stefova *et al*. (2003) reported that flavonoids play crucial roles in biochemistry and physiology of plants. It is responsible for the biological effects of plants and their extracts as well as preparations on humans. Studies have suggested that flavonoids could confer defence against disorder like injury, cancer, aging,

atherosclerosis, inflammation and neurodegenration by boosting antioxidant of the body immune system (Pal *et al.,* 2012; Dilipkumar and Preeti, 2013). The anthocyanin content (0.082 g/100g) was higher than the result obtained by Onyeka and Nwambekwe (0.02 g/100g). However, the low anthocyanin content was expected among vegetables because anthocyanin is usually more in vegetables and fruits without green pigmentation (Dewanto *et al.,* 2002). According to Scalbert *et al.* (2005), the formation of tumour, diabetes, heart and neurodegenerative disorder can be prevented by anthocyanin intake. Also, flavonoids have several health benefits (Brodowska, 2017). Flavonoids are indeed essential to human system. Studies have shown that flavonoids play a similar role in the human body as vitamins (Ostrowska and Skrzydlewska, 2005; Mitek and Gasik, 2009).

Table II. Flavonoids composition of *T. occidentalis* grown in soilless and soil medium.

Subgroups of flavonoids	Flavonoids (g/100g)	Growth medium	
		NM	HS
Flavones	Apigenin	0.003	0.000
	Luteolin	0.096	0.007
	Nobiletin	0.001	0.001
	Tangeretin	0.005	0.005
	Diosmin	0.003	0.002
	Rhoifolin	0.015	0.020
	Neodiosmin	0.013	0.019

	Acacetin	0.017	0.009
	Sinensetin	0.017	0.018
Isofavones	Daidzein	2.546	2.308
	Genistein	1.076	1.222
	Glycetin	0.246	0.201
Flavanones	Eriodictyol	0.026	0.053
	Hesperetin	0.009	0.009
	Hesperidin	0.012	0.006
	Nanirutin	0.015	0.007
	Naringin	0.021	0.022
	Naringenin	0.002	0.003
	Raxifolin	0.004	0.009

	Taxifolin	0.002	0.004
	Didymin	0.019	0.008
	Eriocitrin	0.003	0.003
	Neoeriocitin	0.022	0.011
	Poncirin	0.009	0.009
Flavan-3-ols	Catechin	1.718	1.555
	Epicatechin	0.965	1.200
	Epicatechin gallate	0.015	0.009
	Epigallocatechin	2.620	0.991
	Epigallocatechin gallate	0.016	0.011
	Theaflavins	0.140	0.090
	Thearubigins	0.049	0.047
	Proanthocyanidins	0.023	0.015

Flavonols	Isorharmnetin	0.170	0.075
	Kaempferol	0.002	0.000
	Myricetin	0.003	0.002
	Quercetin	0.032	0.026
Anthocyanin	Anthocyanin	0.082	0.083
Total Flavonoids		10.019	8.064

HS represents Soil medium; NM represents Soilless medium

Alkaloids: Forty five alkaloids were identified by HPLC (Table III). Quinoline (4.221 g/100g NM; 3.610 g/100g HS) and emetine (2.721 g/100g NM; 2.014 g/100g HS) were determined as the most abundant alkaloids in the leaves of *T. occidentalis* while apoatropine, ephedrine, eserine, narcotine, psychotrine and theobromine were the least (0.001 g/100g). All the alkaloids in *T. occidentalis* varied in the different growth medium. The total alkaloids (11.614 g/100g NM, 9.082 g/100g HS) obtained in *T. occidentalis* using HPLC was higher compared to other works (Onyeka and Nwambekwe, 2007; Otitoju *et al.*, 2014; Odufuwa *et al.*, 2013), which reported 0.81 g/100g, 0.04 g/100g and 0.039 g/100g, respectively for total alkaloids in *T. occidentalis*. Also, Oduse *et al.* (2012) reported 13.83 mg/kg for *Cnidoscolus aconitifloina* and 10.13 mg/kg for *Vernonia amygdalina.* However, the results showed that *T. occidentalis* leaf in NM contained a high amount of alkaloids than in HS. Alkaloids are involved in prevention of bacterial species from causing harm to a system (Nuhu *et al.*, 2000; Tor-Anyiin, 2009). Alkaloids such as caffeine affects the central nervous system particularly on the gaps

between nerve cells in the conduits of the nervous system. Alkaloids are generally soluble in lipids and this account for its relevance (Lewis and Elvin-Lewis, 1998).

Table III. Alkaloids composition of *T. occidentalis* grown in soilless and soil medium.

Group of alkaloids	Alkaloids (g/100g)	Growth medium	
		NM	HS
Tropane alkaloids	Apoatropine	0.001	0.005
	Atropine	0.015	0.015
	Hyoscine	0.020	0.022
	Cocaine	0.013	0.012
Acridine alkaloids	Acridine	0.011	0.009
Quinoline alkaloids	Cinchonidine	0.002	0.003
	Cinchonine	0.012	0.016
	Quinidine	1.220	0.877

	Quinine	0.001	0.001
	Quinoline	4.221	3.610
Indole/benzypyrole alkaloids	β-carboline	0.009	0.008
	Ergotamine	0.019	0.017
	Eserine	0.002	0.001
	Rauwolfia	0.009	0.009
	Reserpine	1.821	0.950
	Strychnine	0.036	0.074
Vinca alkaloids	Vinblastine	0.766	0.426
	Vincristine	0.109	0.054
Isoquinoline alkaloids	Berberine	0.005	0.004

	Codeine	0.009	0.005
	Cephaline	0.012	0.007
	Heroin	0.080	0.082
	Emetine	2.721	2.014
	Morphine	0.010	0.014
	Narcotine	0.001	0.001
	Papaverine	0.015	0.015
	Psychotrine	0.001	0.001
	Tubocurarine	0.004	0.085
	Apomorphine	0.003	0.003
Purine (Pseudo) alkaloids	Caffeine	0.008	0.009
	Theobromine	0.001	0.001
	Theophylline	0.007	0.005

β-Phenylethylamine alkaloids	Ephedrine	0.001	0.003
	Norpseudoephedrine	0.009	0.009
	Phenylethylamine	0.013	0.023
Colchicine alkaloids	Colchicine	0.008	0.011
Piperidine alkaloids	Coniine	0.013	0.029
	Lobeline	0.070	0.059
	Piperine	0.027	0.022
Pyridine alkaloids	Nornicotine	0.021	0.012
	Pyridine	0.050	0.048
	Ricinine	0.002	0.005

	Nicotine	0.144	0.293
	Pelletierine	0.095	0.211
Imidazole alkaloids	Pilocarpine	0.001	0.003
	Total alkaloids	11.614	9.082

HS represents Soil medium; NM represents Soilless medium

4. Conclusion

The phytochemical profile of *T. occidentalis* grown in soil and soilless media revealed the inherent potentials and the comparative advantage of soilless medium over soil medium. Phytochemical content of *T. occidentalis* leaf varied in soilless and soil media. Soilless medium provides an alternative in improving the bioactive components and growing leafy vegetables. However, the hydrogen cyanide was the same in hydroponic and geoponic systems.

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