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Ethnobotanical, Physicochemical, Phytochemical and Antioxidant Activity of some Plants Used by Bwa Traditional Healers in the Management of Diabetes in Mali

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Abstract:
Background: *Diabetes represents a real problem of public health. It is a disease with strong progression around the world. The present work aimed to document scientifically medicinal plants used as antidiabetic by Bwa traditional healers (BTH) in the Municipality I of Bamako, Mali.*
Material and Methods: *A survey was conducted amongst the Bwa traditional healers using a semi-structured interview. The experimental investigation involved the plant material of five selected plants. The physicochemical analysis of samples was carried out using standard methods. The phytochemical screening was performed using colored and precipitated tube reactions associated to thin layer chromatography. The antiradical potential of polar extracts was determined using the scavenging reaction of the free-radical 1,1-Diphenyl-2-picryl-hydrazyl ($DPPH^+$).*
Results: *From the survey eleven plant species belonging to seven botanical families were recorded. The most cited families were Leguminosae (36.4%) followed by Anacardiaceae (18.2%). Decoction and oral administration were used at 100%. The samples of leaves of Lannea velutina and Ziziphus jujuba, the trunk bark of Parkia biglobosa, the root bark of Balanites aegyptiaca and the whole plant of Crotalaria retusa presented moisture contents less than 10% and low ash levels. Polyphenols (Tannins, flavonoids), saponins, sterols and terpenoids were main chemical compounds of the extracts from the five plants. The antioxidant activity of polar extracts was manifested by the presence of free radical scavenging substances.*
Conclusion: *The richness of the aqueous extracts of these plants in free radical scavenging substances with antidiabetic activities could justified their use in the traditional management of diabetes.*
Keywords: *Mali, Bô traditional medicine, phytochemistry, diabetes, antioxidant activity.*

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I. Introduction

According to world health organization (WHO), diabetes is a chronic and metabolic disease characterized by high levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves[1]. The most common is type 2 diabetes, usually in adults, which arises when the body becomes resistant to insulin or doesn't make enough insulin. In the past three decades the prevalence of type 2 diabetes has raised up dramatically in countries of all income levels. Type 1 diabetes, once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin by itself[1]. Some reactive oxygen species (ROS) are involved in the pathogenesis of diabetes and its complications[2]. For people living with diabetes, access to affordable treatment, including insulin, is critical to their survival. There is a globally agreed target to halt the rise in diabetes and obesity by 2025[1].

Diabetes represents a real public health problem around the world. Worldwide 422 million people have diabetes, the majority is living in low-and middle-income countries, and 1.5 million deaths are directly endorsed to diabetes each year. Both the number of cases and the prevalence of diabetes have been gradually increasing over the past few decades[1]. The prevalence and incidence of diabetes are also increasing in the African population. Number of diabetic patients in Africa is not precise, but in 2015 the International Diabetes Federation (IDF) estimated that 14.2 million adult patients with diabetes aged 20 to 79 resided in Africa. The same federation estimated that in 2040 the diabetic population will rise to 34.2 million in Africa[3]. And in Mali this disease represents about 5 % of the population[4].

Despite the presence of numerous anti-diabetic drugs, diabetes is amongst the top 10 causes of death, with a significant percentage increase of 70 % since 2000. This pathology causes the death of 1.6 million people per year worldwide[5]. To face this scourge, the treatment is mainly focused on the use of modern and conventional drugs. On the other hand the WHO is encouraging the development of herbal medicine, which is experiencing considerable growth due to the high cost of conventional drugs. Consequently the DMT's research led to the development of a phytomedicine (an improved traditional medicine) from *Sclerocarya birrea* leaves and called "Diabetisane 1" used against type 2 diabetes. This phytomedicine reduces post-prandial glucose levels considerably[6]. In addition, the use of antioxidant micronutrients is a promising option of research for complementary therapy in patients with diabetes[7].

Numerous studies conducted in DMT have already demonstrated the anti-diabetic and antioxidant properties of several medicinal plants[6]. However by contributing to the development of another improved traditional medicine used in the management of diabetes, we targeted to explore the Bô traditional medicine used by Bwa people of Mali. Hence out of the six Municipalities of Bamako, the Municipality I was chosen because it is where the Bwa people are staying in high number in Bamako. Therefore the current work aimed to investigate the medicinal plants used by Bwa traditional healers (BTH) in the traditional management of diabetes in Mali.

II. Material and Methods

A survey was conducted in the Municipality I, while experimental studies were carried out at the Department of Traditional Medicine (known as Département Médecine Traditionnelle (DMT) in french) of Bamako, Mali.

Survey

To select the investigated plants a preliminary study focused on a semi-structured interview survey was conducted from August to September, 2016, amongst Bwa traditional healers (BTH) in the Municipality I of Bamako. Any traditional healer belonging to the Bô tribe and consent for the study was enrolled. The questions addressed to the traditional practitioners related to the recipes used for the management of diabetes, the names of the plants, the plant parts, the methods of preparation and the methods of use.

Experimental studies

Five plants, less investigated in DMT were selected based on their use in the Bô traditional medicine as antidiabetic medicine.

Plant material

It involved leaves of *Lannea velutina* and *Ziziphus jujuba*, the trunk bark of *Parkia biglobosa*, the root bark of *Balanites aegyptiaca* and the whole plant of *Crotalaria retusa*. These plant samples were collected in October 2016, in areas surround Kati. And the five samples were identified and authenticated by a botanist at the Department of Traditional Medicine. Then plant samples were dried under shade in a contamination-free room at DMT and then pulverized using a grounder, FORPLEX type F1 N3139 having sieves with mill medium mesh. The obtained powders were used for extractions and phytochemical investigations.

Physicochemical analysis:

Moisture, total and hydrochloric acid ashes were determined for the five plant samples. The water content was determined by the gravimetric method (weight method). For total ashes, they resulted from the calcination of the non-volatile materials present in the plant powder, while the acid-insoluble ashes were generated by the attack of 10% hydrochloric acid (HCl) on the total ashes and then the calcination of the residue.

Extractions

We have prepared aqueous, hydroalcoholic and other extracts using solvents at increasing polarity.

Aqueous extracts:

10 % Maceration

To 100 g of each plant powder, authors added 1000 ml of distilled water, then macerate with magnetic stirring for 24 hours. The mixture was filtered on a compress; the volume of the measured filtrate was concentrated, then freeze-dried. With difficulties for the filtration of the macerated *Crotalaria retusa* and *Ziziphus jujuba* extracts, they were subjected to a centrifugation at 2000 revolutions / min for 5 minutes then we filtered on a compress.

10% Infusion

Authors projected 100 g of powder from each organ into 1000 ml of boiled distilled water. After cooling for 15 minutes the mixture was filtered through a compress. The filtrate was concentrated then frozen and lyophilized. Like the maceration, authors centrifuged before filtering the extracts from *Crotalaria retusa* and *Ziziphus jujuba*.

10% Decoction

To 100 g of the plant powder authors added 1000 ml of distilled water then they boiled the mixture on a hot plate for 15 minutes. After cooling, the mixture was filtered it on a compress, the extract obtained was concentrated and then frozen and lyophilized. The extracts from *Crotalaria retusa* and *Ziziphus jujuba* were centrifuged before their filtration.

Hydroethanolic (70% EtOH and 30% EtOH) extractions

Authors introduced 50 g of powder into a 1000 ml Erlenmeyer flask which was macerated with 500 ml of solvent with magnetic stirring for 24 hours. After filtration on a compress, the filtrate was concentrated in a rotary evaporator under vacuum at laboratory temperature. After concentration, the extract was taken up in distilled water, then frozen and lyophilized.

N.B: The powdered aqueous and hydroalcoholic extracts were stored in clean, sterile and hermetically sealed glass bottles before their phytochemical and biological analyses.

Extraction using solvents at increasing polarity

The following solvents were used: Petroleum ether, Dichloromethane (DCM), Methanol (MeOH), and water.

Operating mode:

The extraction using solvents at increasing polarity was carried out through maceration with magnetic stirring for 30 min using 50 ml of each above solvent, acting on 5 g of herbal drug. Authors successively used petroleum ether, DCM and MeOH. The residue from these extractions was extracted with 50 ml of distilled water at room temperature for 15 min to obtain an exhaustive maceration. The organic extracts (petroleum ether, DCM, and MeOH) were concentrated in a rotary evaporator and then dried in the open air, while the exhausted maceration extract from water was concentrated, and then lyophilized.

Phytochemical screening:

Chemical groups were determined by colored and precipitated tube reactions associated to thin layer chromatography.

Colored and precipitated reactions in tubes:

The main chemical constituents were characterized in extracts from samples according to their specific reactions in tubes used at DMT[8-10] but described by most recent scientists[11]. Conventional characterization reagents have enabled to highlight the following chemical groups: mucilages (reaction of 95% ethanol), alkaloids (Dragendorff and Mayer reagents), flavonoids (reaction of cyanidin), saponins (foam index), tannins (ferric chloride), steroids and triterpenes (Liebermann Buchard reaction).

Thin Layer Chromatography (TLC)

Materials and Operating Mode:

Aqueous and hydroalcoholic extracts of the five plants were analyzed. $G_{60}F_{254}$ silica plates with 0.25mm thick were used. Solutions to be analyzed have been prepared. A volume of 10 µl of each extract was placed on the starting line of the prepared plate using a capillary micropipette. For the development of extracts, the solvent system Butanol-Acetic acid-Water abbreviated as BAW (40-10-50) in TLC glass tank was used.

Observation and disclosure of spots:

After development, the plates were dried and observed with the naked eye and then with UV lamp at 254 and 366 nm before using Godin's reagents. The retention factor (Rf) was calculated for each spot detected.

Characterization of anti-radical constituents

The method described by earlier authors[12] and adapted by recent workers[13] was used. The activity was carried out through the scavenging of the free-radical 1-1 Diphényl 2 picryl-hydrazyl ($DPPH^+$).

Operating mode:

The activity was determined using the principle of free radical ($DPPH^+$) scavenging on TLC plate. Briefly the obtained chromatogram, resulted from the development of polar extracts (aqueous and hydroethanolic extracts) in BAW (60-15-25), was dried then sprayed using the methanolic solution of 1,1 Diphenyl-2-Picryl-Hydrazyl ($DPPH^+$), in the proportion 2 mg/10 ml. When present the anti-free radical constituents appear as yellow spots on a purple background.

Data analysis

Analysis of data from the ethnobotanical survey was carried out using GraphPad Prism 6.0 software.

III. Results & discussion

Survey data and selected plants

The respondents were seven men aged from 20 to 60 years and above. The age groups 30-39 and 40-59 were the most represented with 28.6 % each. Most of the respondents accessed to this practice by instruction (71.4 %) against 28.6 % for inheritance.

All of them recognized diabetes as sugar disease characterized mainly by polyuria associated to fatigue. In addition, the food habits were the causes mentioned by all respondents; however 42.9 % of them added inherited causes.

Nine antidiabetic recipes were recorded from which eleven plant species belonging to seven families were identified (Table 1). The most cited plant species belonged to the family Leguminosae (36.4 %) followed by Anacardiaceae (18.2 %) (Figure1). Stem bark and whole plants were most represented with 27.3% each (Figure 2). The herbal recipes were prepared in decoction at 100 % and also administered orally at 100 %. To our knowledge this the first time to investigate antidiabetic plants used by Bwa traditional healers in Mali. But a recent work conducted on medicinal plants against diabetes and its comorbidities in four local government areas of southwestern Nigeria revealed that the most frequently cited plant family was Leguminosae (12 %), followed by Euphorbiaceae (8%)[14].

Amongst these plants, those which matched the selection criteria were: *Balanites aegyptica* (L.) Delile (Zygophyllaceae), *Crotalaria retusa* L. (Leguminosae), *Lannea velutina* A. Rich. (Anacardiaceae), *Parkia biglobosa* (Jacq.) G. Don (Leguminosae) and *Ziziphus jujuba* Mill. (Rhamnaceae).

Table 1: List of recorded plants

Scientific name	Botanical Family	Local name	Part used
Arrachis hypogea L.	Leguminosae	Thimi	Shell
Balanites aegyptiaca (L.) Delile	Zygophyllaceae	Zèguènè	Root
*Entada africana*Guil. et Perr.	Leguminosae	Samanèrè	Root
Lannea velutina A.Rich.	Anacardiaceae	Viagninou	Trunk bark
Morinaga oleifera Lam.	Moringaceae	Bassiyirini	Leaf
Parkia biglobosa (Jacq.) G. Don.	Leguminosae	Nèrè	Trunk bark
Phyllanthus amarus Schumach.&Thonn.	Phyllanthaceae	Delakola	Whole plant
Sclerocarya birrea (A. Rich). Hochst.	Anacardiaceae	Ngounan	Trunk bark
Striga sp.	Orobanchaceae	sèguè	Whole plant
Ziziphus jujuba Mill.	Rhamnaceae	Tomonon	Leaf
Crotatalaria retusa L.	Leguminosae	Thio Thimi	Whole plant

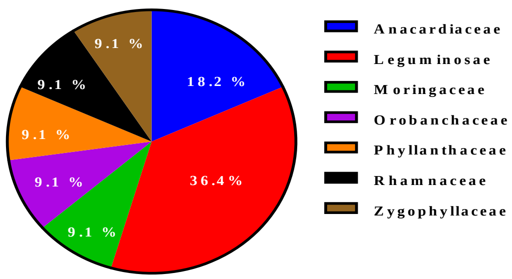


Figure 1: Distribution of plant families used by Bwa traditional healers in the management of diabetes

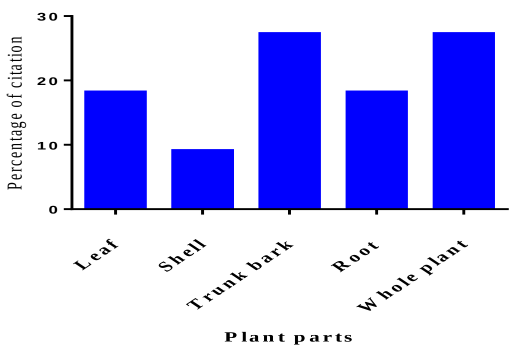


Figure 2: Distribution of plant parts used by Bwa traditional healers in the management of diabetes

Physicochemical data

All samples presented moisture content less than 10 %. The water content 10 % in the samples is favorable for their good storage[9]. Referring to extractive values, ethanol extracts better than water. *Balanites aegyptiac*a and *Parkia biglobosa* contained the highest total ash content with 8.4 % and 8.7 % respectively; this could be related to the plant organs used. *Crotalaria retusa* showed the highest water-soluble extractive value with a rate of 25 %. However *Ziziphus jujuba* gave the highest content of 70 % ethanol-soluble extractive value. The acid-insoluble ash content was less than 1% for all our samples (Table 2). The high total ash content explains the richness of the plant sample in mineral elements while the acid-insoluble ash indicates the richness in siliceous elements. Previous researchers reported that ash contents are playing an important role in determining the quality and purity of a given plant material especially in the powdered forms[15]. *Balanites aegyptiaca* and *Ziziphus jujuba* contained abundant saponins based on their foam indexes in Table 2. The team of Diallo found also 1000 as foam index value from the leaves of *Ziziphus mauritiana*, a synonym of *Ziziphus jujuba*[10].

Tableau 2: Contents of physicochemical parameters

Physicochemical parameters	Value (%)				
	Ba RB	**Cr WP**	**Lv Le**	**Pb TB**	**Zj Le**
Moisture content	**6.4**	**6.5**	**8.5**	**7.8**	**6.6**
Water-soluble extractive	14	**25**	13	7	13
70% EtOH-soluble extractive	15.8	7.8	16	11.4	**17.2**
Total ash	8.4	6.6	8.3	8.7	7.9
Acid-insoluble ash	**0.5**	0.4	0.2	0.4	0.1
Foam index	**1000**	250	166.7	166.7	**1000**

Key- Ba: *Balanites aegyptiaca*; Cr: *Crotalaria retusa*; Lv: *Lannea velutina*; Pb: *Parkia biglobosa*;
Zj: *Ziziphus jujuba*; TB: trunk bark; WP: whole plant; Le: leaves; RB: stem bark

Yields of extractions

Overall the best yields were obtained with the water decoction followed by maceration using 30 % EtOH. In addition, the root bark of *Balanites aegyptiaca* revealed itself to be more extractable while the trunk bark of *Parkia biglobosa* presented the lowest yields (Table 3). For *Lannea velutina* our finding was slightly higher than the results obtained by earlier work[16]. The decoction of the *Ziziphus jujuba* leaves gave a yield of 11.4 % which is very close to the finding of Diallo and collaborators in 2004, who worked on the leaves of *Ziziphus mauritiana* (synonym of *Ziziphus jujuba*)[10].

Tableau 3: Yields of extractions

Extracts	Yield (%)				
	Ba RB	**Cr WP**	**Lv Le**	**Pb TB**	**Zj Le**
10% Decoction	25.7	12.2	19.6	8.8	11.4
10% Infusion	20.8	8.0	17.5	7.3	10.0
10% Maceration	20.8	10.8	12.9	5.2	8.1
Exhausted Maceration	9.8	12.9	12.0	7.2	7.2
30% EtOH	25.7	13.5	17.0	9.6	9.2
70% EtOH	20.1	11.9	19.4	11.5	9.6

Key- Ba: *Balanites aegyptiaca*; Cr: *Crotalaria retusa*; Lv: *Lannea velutina*; Pb: *Parkia biglobosa*;
Zj: *Ziziphus jujuba*; SB: trunk bark; WP: whole plant; Le: leaves; RB: root bark

Data from the phytochemical screening

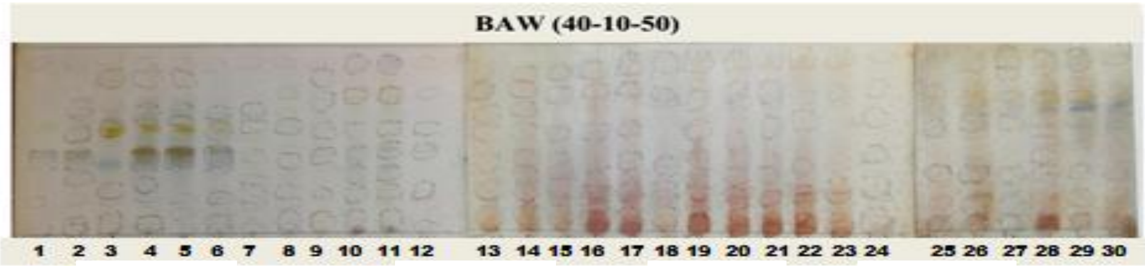
The chemical groups highlighted by the tube reactions and the thin layer chromatography are respectively shown in Table 4 and Figure 3. The samples contained mainly saponins, mucilages, polyphenols, sterols and triterpenes while alkaloids were absents. Previous investigators reported the presence of similar chemical groups[10,16–19]. This fact suggests that the matter of the locality did not affect significantly the phytochemical composition of our plant samples.

Tableau 4: Chemical groups highlighted or not in the five plant samples

Chemical groups	Results				
	Ba BR	**Cr WP**	**Lv Le**	**Pb TB**	**Zj Le**
Tannins	-	++	+++	+++	+++
Flavonoids	-	-	++	+++	+
Saponins	+++	++	++	+	+++
Steroids et triterpenes	++	++	+	+	++
Mucilages	++	+++	+++	+++	+++
Alkaloids	-	-	-	-	-

Key- Ba: *Balanites aegyptiaca*; Cr: *Crotalaria retusa*; Lv: *Lannea velutina*; Pb: *Parkia biglobosa*;
Zj: *Ziziphus jujuba*; SB: trunk bark; WP: whole plant; Le: leaves; RB: root bark

+++: Highly positive ++: Moderately positive +: Slightly positive -: Negative

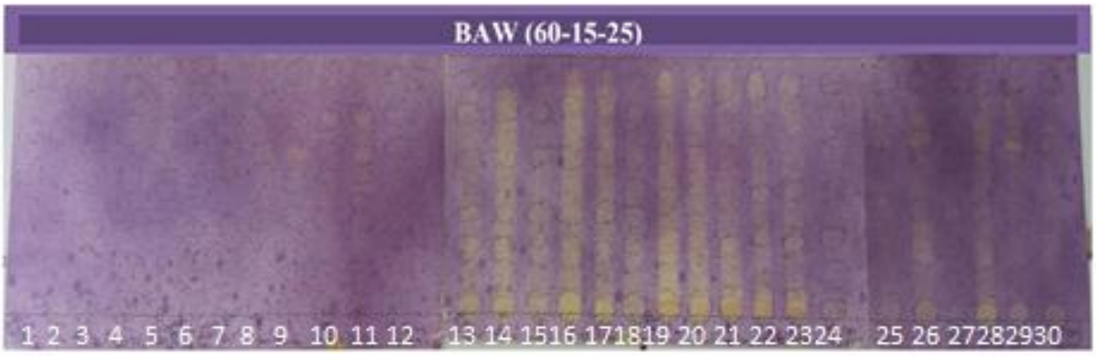


Flavonoids **Tannins** **Steroids** **Triterpenes**

Key- On the chromatogram polar extracts (10% Decoction, 10% Infusion, 10% Maceration, 30% EtOH, 70% EtOH, Exhausted Maceration) are arranged from left to right and respecting the alphabetical order of the five samples such as *B. aegyptiaca, C. retusa, L.velutina, P. biglobosa and Z. jujuba* where we have *B. aegyptiaca* (1-6), *C. retusa* (7-12), *L. velutina* (13-18), *P. biglobosa* (19-24) and *Z. jujuba* (25-30)
Figure 3: Chromatogram of polar extracts developed in BAW (40-10-50) solvent system and sprayed with Godin reagents.

Antioxidant activity

The appearance of several anti-free radical substances in our analyzed samples showed their antioxidant potential (Figure 4). Qualitatively *Lannea velutina* exhibited the highest free-radical scavenging effect. This activity could be due to the polyphenolic compounds. Flavonoids, tannins, saponins, sterols and triterpenes are responsible for the antidiabetic activity of some medicinal plants [20,21]. A recent literature review reported that flavonoids, triterpenoids and combretin B are the most compromising antidiabetic compounds from medicinal plants traditionally used for the treatment of diabetes in Africa [22]. In Mali very few antidiabetic activities have been done on *B. aegyptiaca, C. retusa, L.velutina, P. biglobosa*. While some *in vivo* pharmacological studies have been performed on *Ziziphus mauritiana* leaves and roots[19,23,24]. Some researchers revealed recently the richness of *Balanites aegyptiaca* in polyphenols, saponins, alkaloids, and phytosterols with biological activities such as antioxidant, antimicrobial, anticancer, anti-inflammatory, hepatoprotective and antidiabetic properties[25]. On the other hand Anim and co-workers established the health benefit of *Crotalaria retusa* through their investigation on the phytochemical, antioxidant and cytotoxicity of hydroethanolic extracts from different parts of this plant species[26]. Recently the team of Sut revealed that *Crotalaria retusa* leaves from Ivory Coast contained several flavonoids and their methanolic extract exhibited a significant antioxidant activity[27]. The antioxidant activity of the aqueous-methanolic stem bark of *Parkia biglobosa* was reported in a recent literature review[28]. The same authors reported the antidiabetic activity of this *Parkia biglobosa*[28]. This scientific evidence supports the use of the investigated samples in the management of diabetes by Bwa traditional healers.



Anti-free radical substances

Key- On the chromatogram polar extracts (10% Decoction, 10% Infusion, 10% Maceration, 30% EtOH, 70% EtOH, Exhausted Maceration) are arranged from left to right and respecting the alphabetical order of the five samples such as *B. aegyptiaca, C. retusa, L.velutina, P. biglobosa and Z. jujuba* where we have *B. aegyptiaca* (1-6), *C. retusa* (7-12), *L. velutina* (13-18), *P. biglobosa* (19-24) and *Z. jujuba* (25-30)
Figure 4: Chromatogram of polar extracts developed in BAW (40-10-50) solvent system and sprayed with DPPH.

IV. Conclusion

The richness of the five plants in anti-free radical substances responsible of anti-diabetic activities could justify their use in the traditional management of diabetes.
From these findings, pharmacological and clinical tests will be undertaken on the leaves of *Lannea velutina*, being a less studied plant and also endowed with the strong antioxidant activity.

Conflict of interest
Authors declare no conflict of interest directly or indirectly.

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