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Phytochemical constituents of some medicinal plants used by the Nandis of South Nandi district, Kenya.

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1 SUMMARY

Alkaloids, saponins, anthraquinones, glycosides, phenolics, terpenoids and flavonoids distribution in ten medicinal plants belonging to different families were assessed and compared. The medicinal plants investigated were *Asparagus racemosus*, *Clutia abbysinica, Clerodendrum myricoides, Ehretia cymosia, Leucas calostachys, Toddalia asiatica, Rubia cordifolia, Spermacoce princeae,Carrisa edulis* and *Ajuga remota*. The leaves and roots of the plants were collected from their natural habitat in Aldai division South Nandi district. All the plant samples were identified at University of Nairobi and confirmed in national museums of Kenya. The Voucher specimens were deposited in the University Botanic garden Maseno herbarium. The harvested roots were washed with water and the barks peeled off while still fresh and cut into small portions. The materials were then air –dried under a tree shade at room temperature for one week when possible, but in the sun when the humidity was too high. Phytochemical screening was carried out at Centre for Traditional Medicine and Drug Research (CTMDR) KEMRI Nairobi according to Harborne, 1984 & 1973. All plants were found to contain alkaloids, terpernoids, saponins and flavonoids except for the absence of saponins in root extracts of *R. cordifolia* and *C.myricoides* and flavonoids in leave extracts of *L. calostachys* and *A. remota*.
The significance of the plants in traditional medicine and the importance of the distribution of these constituents were discussed with respect to the role of these plants in ethnomedicine in South Nandi district

2 INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al*, 2005). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolics compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu, 1999; 2001). This field of natural products research is

currently being carried out intensively though it remains far from exhaustion. An attempt to obtain bioactive agents from plants is a worthwhile exercise since only 10% of all plants have been investigated in detail (Harborne, 1973). The majority of these bioactive compounds are alkaloids, followed by sesquiterpenes, diterpenes, triterpene saponins, triterpene aglycones, flavonoids, sterols, coumarins, quinine's and monoterpenes. It is imperative that ethnobotanical researches and phytochemical tests lead to some patent-able and industrially exploitable compounds for drug development.

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3 MATERIALS AND METHODS

3.1 Collection and identification of plant materials: The leaves and roots of the plants were collected with acceptable bio-conservation methods from their natural habitat (uncultivated lands in Aldai division South Nandi district). All the plant samples were identified at University of Nairobi and confirmed in national museums of Kenya. The Voucher specimens were deposited in the University Botanic garden Maseno herbarium. The harvested roots were washed with water and the barks peeled off while still fresh and cut into small portions. The materials were then air –dried under a tree shade at room temperature for one week when possible, but in the sun when the humidity was too high. They were then packed in brown paper envelops for transportation to Kenya Medical Research Institute (KEMRI) Nairobi.

Phytochemical screening was carried out at Centre for Traditional Medicine and Drug Research (CTMDR) KEMRI Nairobi. The dry root barks were then ground into fine powder using an electric mill (Christy and Norriss Ltd. England) while the dried leaves were crushed using a porcelain mortar and pestle (trituration method) to increase the surface area for absorption of the solvents(Harborne, 1973). They were then stored in brown paper envelopes to prevent absorption of moisture (Harborne, 1984 & 1973).

Asparagus racemosus, Clutia abbysinica, Clerodendrum myricoides, Ehretia cymosia, Leucas calostachys, Toddalia asiatica, Rubia cordifolia, Spermacoce princeae, Carrisa edulis and *Ajuga remota* are extensively used in herbal medicine in South Nandi district, Kenya (Jeruto , 2008). Their various uses in traditional medicine are reviewed in Table 1 below. This study investigates the fundamental scientific bases for the use of medicinal plants by defining and quantifying the crude phytochemical constituents present in these plants

3.2 Plant extraction using organic solvents: Five (5) grams of the powdered plant material was weighed using a top loading balance (Salter) and transferred to a conical flask. Successive extraction was carried out using increasing polarity. Chloroform (separates lipids and terpenoids) then methanol (for more polar compounds) and sequentially water extraction .The appropriate solvent (chloroform) was added to cover the plant material under a fume hood and left to soak in the solvent at room temperature for 24 Hours with intermittent shaking**.** Extracts were filtered through No. 1 Whatman filter paper and the flask containing the filtrate closed with a stopper. The filtrate was then rotar vapoured to concentrate the extracts. The extracts were then examined by thin-layer chromatography (TLC) on silica gel using various solvents (Dragendoff, Vanillin, Ferric ferrocynide) systems and visualized using standard methods i.e. UV-254/364 nm and sprayed with suitable reagents (Harborne, 1973).

3.3 Plant water extraction (aqueous extract): Forty (40) grams of the plants sample was weighed using a top loading balance (Salter). Distilled water was added to cover the material (300ml) and then placed in a water bath for 2 hours at 70°C. The filtrate was filtered using Whatman filter paper No.1 into a plastic bottle and stored in a freezer to prevent

contamination and decomposition of the extract. The extract was then liquefied by placing the bottle in a warm water bath 70°C then transferred into a round-bottomed rotar evaporator flask. The extract was swirled in a crucible containing solid carbon dioxide and acetone until the extracts adsorbed on to the walls of the flask. The flask was fixed to the freeze dryer with a vacuum pressure of -1 and a temperature of between -40^{0}C to -50^{0}C for two days to give a concentrated extract. The extracts were then filtered, screened and tested for anti-malarial activity both *in-vivo* and *in vitro* (Harbone, 1973).

3.4 Sample storage: Empty vials were weighed using an analytical balance (Mettler) and their weights noted and labeled. The extracts from the distillation flask were then transferred to the vials using a spatula and then weighed again. The vials were labeled appropriately, stating the name of the extract, the part of the plant, the solvent used, and the weight stored. The vials were stored at room temperature (Harborne J.B 1984 & 1973). The quantity of each crude extract was calculated by:

Plant crude residue = (weight of vial + extract) - (weight of empty vial)

3.5 Preparations of thin layer chromatography plates (TLC): Plates were prepared in the laboratory according to the method of Stachl (1969). Coating 20cm x 20cm plates with silica gel. 45g of silica gel UV 254 nm was weighed in a weighing balance and using a filter funnel poured into 250ml of Mayer's flask, 90mls of distilled water was added and the mixture agitated thoroughly for 5minutes until uniform slurry was obtained, the slurry was quickly poured into the spreader and rapidly swept across the plates with 0.02mm thickness, the plates were spread out to dry at room temperature for about 6 Hours (Harborne, 1984 & 1973).

3.6 Preparation of the development tanks/mobile phases: Four (6x12x15) cm development tanks each lined with well-trimmed filter papers to facilitate the saturation of the tank with the developing solvent. Solution of each developing solvents were then poured into the tanks and covered to obtained equilibrium for a period of 15minutes. Development was allowed to proceed until the solvent front. The plate was then removed from the chamber and the solvent front immediately marked with a pointed object (Harborne, 1973). The plate was then allowed to dry in a fume cupboard. The position of the separated solutes was located by various methods. Colored substances can be seen directly when viewed against the stationery phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973).

3.7 Detection of alkaloids, phenolics and terpenoids using TLC: The chromatoplates were activated in an oven at 100^o c for about 15minutes. After complete cooling, they were spotted using chloroform extracts and, methanol extracts. Each sample was spotted 2 cm apart using a very thin capillary tube. An air blower was used to dry the samples spots so as to control the spot size, methanol in four beakers was used to clean the thin capillary tube before spotting the next sample. After the plates were developed, they were left to dry for about 10minutes, then viewed under U.V fluorescence light at wavelength 254 nm and 365 nm, and finally sprayed with the required detection reagent either dragendoff, ferrocynide, and Vanillin respectively) to determine the compounds present and the solvent system which gave the best observation and results is presented (Harborne, 1973).

3.8 Isolation and quantifying the plant extracts: Five (5) grams of the dried leaves were extracted in the chloroform and left to soak for 12 hours. The extract was filtered using filter paper and a filtering funnel. The extract was subsequently concentrated by evaporating off the solvent using the

Rotarvapour at a temperate have about 40-50^{0}C and weight of the crude extract determined (Harborne, 1973).

3.9 Sample spotting on the chromatoplates: The chromatoplates were allowed to develop till the solvent front was about 5/6 of the plate. The plates were developed in the following solvents; chloroform: hexane (75:25; 50:50; 20:80), chloroform: methanol: hexane (1: 1:2), MeoH: $CHCL_2$: EtOA (1:1:2), MeoH: EtoAC: H_2O: Acetic acid (10:30:1 drop: 5 Drops). They were then removed and allowed to dry in open air after marking the solvent front; the chromatoplates were then observed under UV light at a wavelength of 365 nm then sprayed with reagents. The best mobile phase giving best results was presented (Harborne, 1984 & 1973).

4 Preparations of spraying reagents

4.1 Dragendoff reagent for tests for alkaloids: The reagent was made of two portions

Reagent 1- 0.85g of bismuth substrate was dissolved in a solution of 10mls acetic acid and 40mls water.

Reagent 2-8g of potassium iodide (KI) was dissolved in 20mls of water (stock solution-mixture of equal parts of solution 1 and 2 (Harborne, 1973).The spray reagent was prepared by mixing 1ml of the stock solution with 2mls of fresh acetic acid and 10mls of water. Detection of alkaloids and other nitrogen compounds is by orange-brown spots on yellow background (Harborne, 1973).

4.2 Ferric ferrocynide reagent for phenolics test: Ten percent (10%) iron chloride ($FeCL_3$ (aq) was mixed with iron cyanide ($FECN_6$) (1g/100ml) or 0.1g/10ml) 0.1g of ferric chloride and 0.1g of potassium ferricynide ($K_3F_3CN_3$) was freshly prepared by dissolving in 10mls of distilled water. Equal portions of 1 and 2 was mixed, sprayed to the plates and heated at 110^{o}C. Change of colour to blue (instant) indicates the presence of phenolics (Harborne, 1984 & 1973).

4.3 Vanillin reagent for terpenoids test: Ten percent (10%) vanillin was dissolved in Ethanoic acid – concentrated sulphuric acid in ratio of 2:1 mixed and sprayed onto the plates and then they were put in the oven for 15mins. Presence of terpenoids was indicated by the separation into different colours; brown, dark green and purple colour (Harborne, 1984 & 1973).

4.4 Tests for flavonoids: TLC plate was exposed to ammonia. The presence of flavonoids is indicated by coloured spots e.g. yellow, pink, grey and brown spots (Harborne, 1973).

4.5 Tests for anthraquinones: TLC plate is sprayed with a solution of 10 ml CH_3OH and 10 g potassium hydroxide (KOH) . Change of the original yellow brown colour to purple shows a positive test (Harborne, 1973).

4.6 Tests for saponins: Persistent foam after shaking 0.5gms of the plant extract in a test tube for about 5 minutes shows a positive test (Harborne, 1984 & 1973).

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Table1. Review of the various medicinal uses of the study plants.

Species	Family	Traditional Use	Part Used.	Reference
Carissa edulis. (Forsk.) Vahl.	Apocynaceae	Venereal diseases, epilepsy, malaria, heartburns, arthritis, sorcery, cancer,	Roots, Decoction (internal)	Jeruto et al., 2008 Jeruto , 2008
Ehretia cymosa Thonn	Boraginaceae	Venereal diseases, pneumonia, epilepsy, dry cough, malaria, ECF, tonsils, mental problems, asthma, typhoid, wounds, aphrodisiac	Leaves, roots Infusion (internal)	Jeruto et al., 2008 Jeruto , 2008
Ajuga remota Benth.	Labiatae	Malaria, tonsil, antidiarrhoea, treat after birth pains, fever, toothache, dysentery, high blood pressure, tape worms	Leaves, roots Decoction (internal	Jeruto et al., 2008 Jeruto , 2008
Leucas calostachys Oliv.	Labiatae	Wounds, dry cough, amoeba, heartburns, muscle pull, waterborne diseases, cough, kidney problems, pneumonia, malaria, stomach-ache	Leaves, roots Decoction (internal)	Jeruto et al., 2008 Jeruto , 2008
Spermacoce princeae (K.Schum.)Verdc.	Rubiaceae	Chronic asthma, cancer, wounds, eye problems, mastitis in cows, venereal, skin diseases, pneumonia, typhoid, caterpillar bites, antidiarrhoea	Roots, leaves Decoction (internal) & paste (external)	Jeruto et al., 2008 Jeruto , 2008
Rubia cordifolia L.	Rubiaceae	Venereal, pneumonia, cough/cold, tonsils, uvula problems, asthma, purgative, nose bleeding, ulcers, arthritis, kidney, hypertension, diarrhea	Ash (internal) Roots, leaves	Jeruto et al., 2008 Jeruto , 2008
Toddalia asiatica (L.) Lam.	Rutaceae	Cancer, chest and urinary problems, chronic asthma, cough/cold, pneumonia	Roots, leaves Decoction (internal)	Jeruto et al., 2008 Jeruto , 2008
Clerodendrum myricoides (Hochst.) Vatke	Verbenaceae	Epilepsy, arthritis, malaria, diabetes, typhoid, cough/cold, eye problems, proper position of fetus, tonsillitis, rheumatism, gonorrhea, ECF	Roots Decoction (internal)	Jeruto et al., 2008 Jeruto , 2008
Clutia abyssinica Jaub. & Spach	Euphorbiaceae	Venereal and skin diseases, chest problems, cancer, fertility in both humans	Roots, leaves Decoction (internal	Jeruto et al., 2008 Jeruto , 2008
Asparagus racemosus Willd.	Liliaceae	Arthritis, venereal diseases, cancer, asthma, pneumonia, cough, sore throat, purgative, proper pregnancy, stomach up-sets, fertility in women	Roots Decoction (internal	Jeruto et al., 2008 Jeruto , 2008

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5 RESULTS

5.1 Quantitative analysis of the phytochemicals of the medicinal plant roots.

Table 2: Quantitative analysis of plant root crude extracts.

Plant species	Chloroform extracts (g)	Methanol extracts (g)	Water extracts (g)
Asparagus racemosus	0.0447	0.7885	1.2326
Clutia abbysinica	0.3220	0.3289	1.2535
Clerodendrum myricoides	0.2597	0.8991	1.1987
Ehretia cymosia	0.0763	1.3914	1.7295
Leucas calostachys	0.0704	0.7069	1.1788
Toddalia asiatica	6.8395	6.1727	1.2456
Rubia cordifolia	0.1150	1.7467	1.2521
Spermacoce princeae	0.0688	0.4458	1.1145
Carrisa edulis	6.1295	2.0776	1.2242
Ajuga remota	-	-	-

Table 3: Quantitative analysis of plant leaf crude extracts.

Plant species	Chloroform extracts (g)	Methanol extracts (g)	Water extracts (g)
Asparagus racemosus	0.1374	0.9638	3.5796
Clutia abbysinica	0.2564	1.1825	5.1498
Clerodendrum myricoides	0.1509	0.6285	3.3193
Ehretia cymosia	0.1614	0.3742	2.2245
Leucas calostachys	0.0863	0.4621	2.5643
Toddalia asiatica	0.1639	0.5166	2.1454
Rubia cordifolia	0.1325	1.0865	2.3123
Spermacoce princeae	0.1510	0.6692	2.4781
Carrisa edulis	-	-	-
Ajuga remota	6.2834	7.426	2.1456

Table 4. Qualitative analysis of the phytochemicals of the medicinal plant roots.

	(a)							(b)						
Plants	**Chloroform extracts**							**Methanol extracts**						
	Alkaloids	Saponins	Anthraquinones	Glycosides	Phenolics	Terpenoids	Flavonoids	Alkaloids	Saponins	Anthraquinones	Glycosides	Phenolics	Terpenoids	Flavonoids

A. racemosus	+	+	-	+	+	+	-	+	+	-	+	+	-	+
C. abbysinica	+	+	+	-	+	+	+	+	+	-	-	+	-	+
C. myricoides	+	-	-	+	-	+	-	-	-	-	+	+	-	-
E. cymosia	+	+	-	+	-	+	-	+	+	+	+	+	-	+
L. calostachys	+	+	-	+	+	+	-	-	+	-	-	+	+	+
T. asiatica	+	+	-	+	+	+	+	+	+	+	+	+	+	+
R. cordifolia	+	-	+	+	-	+	+	+	-	+	+	+	+	+
S. princeae	+	+	+	+	-	+	+	-	+	-	-	+	+	+
C. edulis	+	+	-	-	-	+	-	+	+	-	+	+	+	-
A. remota	+	+	-	-	-	+	-	+	+	-	+	-	+	-

Key: + = Presences of constituents - = Absence of constituents

Table 5. Qualitative analysis of the phytochemicals of the medicinal plant leaf.

	(a)							(b)						
Plants	**Chloroform extracts**							**Methanol extracts**						
	Alkaloids	Saponins	Anthraquinones	Glycosides	Phenolics	Terpenoids	Flavonoids	Alkaloids	Saponins	Anthraquinones	Glycosides	Phenolics	Terpenoids	Flavonoids
A. racemosus	-	+	+	+	+	+	-	+	+	+	+	+	+	+
C. abbysinica	-	+	+	-	+	+	+	+	+	+	-	+	+	+
C. myricoides	+	+	-	+	+	+	+	+	+	-	+	+	+	+
E. cymosia	+	-	+	+	+	+	+	+	-	+	+	+	+	+
L. calostachys	-	-	-	-	-	+	-	+	-	-	-	+	+	-
T. asiatica	+	-	-	-	+	+	+	+	-	-	-	+	+	+
R. cordifolia	-	-	-	+	+	+	+	+	-	+	+	+	+	+
S. princeae	+	-	-	+	+	+	+	+	-	-	+	+	+	+
A. remota	+	+	-	-	-	+	-	+	+	-	-	+	+	-

Key: + = Presences of constituents - = Absence of constituents

Toddalia asiatica and *Carissa edulis* contained the highest crude extract for both chloroform and methanol solvents, while *Clutia abbysinica* and *Spermacoce princeae* contained the lowest yield of the crude (Table 2). *Ajuga remota* showed the highest quantity of crude plant residue for both chloroform and methanol extracts (Table 3). *Leucas calostachys* contained the least crude yield. Water extracts for *Clutia abbysinica* and *Clerodendrum myricoides* showed that leave extracts contained the highest yield than the root extracts (Table 2; Table 3).

Alkaloids and terpenoids were present in all the chloroform plant root extracts. *Clutia abbysinicca* and *Spermacoce princeae* contained all the tested compounds except anthraquinones and phenolics respectively (Table 4). Most plants tested negative on anthraquinones (Table 4). For root methanol extracts, most plants contained phenolics except *Ajuga remota*. *Toddalia asiatica*, *Rubia cordifolia*, and *Ehretia cymosa* contained all the tested secondary metabolites. Saponins and terpenoids were absent in *Rubia cordifolia* and *E. cymosa* respectively. Only *E. cymosa*, *T. asiatica* and *R. cordifolia* showed the presence of anthraquinones (Table 4). In addition, saponin

tested positive in all the plants except in *R. cordifolia* and *C. Myricoides*.
Phenolics and terpenoids were present in all the methanol leaf extracts. Methanol extracts showed that Alkaloids, phenolics, terpenoids and flavonoids were present in all the plants. Flavonoids were absent in *L. calostachys* and *A. remota*. Only *A. racemosus*, *C. abbyssinica*, and *C. myricoides* showed the presence of saponins (Table 5)
Phenolics were absent in *L. calostachys* and *A. remota*. Only *C. myricoides*, *E. cymosa*, *T. asiatica*, *A. remota* and *S. princeae* showed the presence of alkaloids (Table 5).

6 DISCUSSION AND CONCLUSION.

Phytochemical screening showed that the leaves and roots were rich in chemical constituents. Alkaloids, saponins, anthraquinones, glycosides, phenolics, terpenoids and flavonoids have been documented in this study. These principles have been known for many years to exhibit biological activity, such as effects on the central nervous system, and antibacterial, antiturmour, and anthehelmintic activity (Harborne, 1973). Many alkaloids are known to have effect on the central nervous system and some act as anti-parasitic (such as morphine, a pain killer). For example, quinine was widely used against *Plasmodium falciparum*. In this respect, it is found from the phytochemical screening that most plants traditionally used to treat malaria contain alkaloids among other things. Analgesia is another property of many alkaloids –containing plants used in traditional medicine. Degenerative disorders, such as gouts and rheumatism, have also been traditionally treated with alkaloid-containing plants. Cochicine compounds are well known in treating gouts (Wakori *et al.*, 1996).
The presence of alkaloids and flavonoids in *Clutia abyssinica* concurs with the findings of Abdel-Fatta (1986), who maintains that certain classes of alkaloids occur in certain genera of the family Euphorbiaceae. He adds that the family is rich in flavonoids, particularly flavones and flavonols. Kokwaro (1993) and Harborne (1984) have reported oils, alkaloids and anthraquinones associated with plants to have medicinal value. Others are tritepenoids, which include: cardiac glycosides, sterols, saponins and tritepenes. Mode of action of compounds present in the extracts indicates that the extracts from these plants have the potential of solving the problem of multi-drug resistance. Therefore, the presence of these secondary compounds validates the use of the plants as herbal drugs in Nandi South District. Thus, further research is needed to work out the actual active compounds for commercialization.
On carrying out phytochemical analysis, plant extracts were shown to fluorescence under the UV at a wavelength of 254 – 365nm showing the presence of secondary metabolites. This is as a result of conjugation between the double bonds from the five oxygen atoms with the single bonds present in the structure allowing absorption of the UV light. The different colours of the fluorescence rings are due to different atoms present in the compound having different wavelengths. When atoms are excited to a higher energy level, they may fall back to their original position using the same or a different wavelength resulting to emission of different colours (Njunge *et al.*, 1975).
Steroidal compounds are known to behave like hormones. Structurally they are similar. From phytochemical screening it is found that *Toddalia asiatica, E. cymosia* and *Clerodendrum myricoides* contains steroids The extracts from these plants are traditionally used to improve lactation; possibly steroids do this by behaving like the hormones responsible for lactation (Harborne, 1973).
Phenolics compounds, such as anthraquinones have been used as purgatives (Harborne, 1973). Anthraquinones are the main constituents in *Clutia abyssinica* roots and leaves and the extracts are traditionally used to treat stomachache and constipation. It was also observed that the compounds in the crude extracts were higher due to synergistic effects of the different

compounds therein. Many compounds were found in the methanolic extracts than in the chloroform extracts. This is because methanol is much polar than chloroform hence extracting many of the active ingredients (Harborne, 1973).

The traditional medicine consisted of a topical and an oral preparation .The topical preparation had plants that contained phenolics, Antraquinones and glycosides which were identified.. It is interesting to note that the topical traditional –medicine preparation contained anthraquinones which are chemically related to anthralin. Anthralin has been employed clinically in topical preparations for psoriasis (Harris and Ferrington, 1971). This shows the medicinal value in the plants and therefore the plants in this study are validated as medicinal.

In this study, *Ajuga remota* contained terpenoids, glycosides and phenolics. This concurs with other researches. Kuria (1976) described the isolation of a cardio tonic principle from the plant. Some other compounds identified in this plant include iridoid glycosides (Ninhydrin-Cd), terpenoids glycosides ($SbCl_3$) and ajugarins (Kedde) (Njunge *et al*., 1975; Wakori *et al.,* 1996). The use of this plant to treat toothache has also been explained as an effect due to the antiseptic action of the phenolics compounds and the neuromuscular effects of the iridoids. Presence of these compounds in Ajuga *remota* validates its medicinal use by the Nandi practitioners.

7 CONCLUSION

The plants studied here can be seen as a potential source of useful drugs. Further studies are recommended on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. The antimalarial activities of these plants for the treatments of the diseases as claimed by traditional healers are also being investigated .

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