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Original Research Article

Phytochemical Assessment, Anti-inflammatory and Antimalarial Activities of *Beta vulgaris* (*Chenopodiaceae*) Root Extract

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ABSRTACT

The study evaluated the phytochemicals, acute toxicity, antioxidant, anti-inflammatory and antimalarial activities of *B. vulgaris* extract. The antioxidant potential was examined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and the formalin-induced inflammation technique was used to evaluate the anti-inflammatory activity. Also, the in vivo antimalarial activity was evaluated against *Plasmodium berghei* parasites and the required doses were given according to the weight of the animal two hours after inoculation of parasites on D_1, then once daily for D_2-D_4. Phytochemical analysis showed that *Beta vulgaris* extract contained alkaloids, terpenoids, tannins, saponins, phenolics, anthraquinones, and flavonoids. The oral administration of crude ethanol extract of *B. vulgaris* to Swiss mice was not toxic even up to a dose of 5000 mg/kg. The root extract had the highest percentage inhibition of 74.46 ± 0.98 and for ascorbic acid 98.66 ± 0.16 at 1000 µg/mL extract in the antioxidant evaluation. *Beta vulgaris* had significant anti-inflammatory activity at 50 mg/kg at 1hr being the most effective. There was a dose-dependent increase in percentage chemo-suppression of the parasites by the different groups with maximum effect at 800 mg/kg (59.68-35.09%). This study validates the phytomedicinal use of beet root extract for the management of inflammation, malaria and oxidative stress-related infections.

Keywords: *Beta vulgaris*, Antimalarial activity, Anti-inflammatory activity, Antioxidant activity, Acute toxicity.

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Introduction

Therapeutic or medicinal plants can also be termed as medicinal herbs. They have been known and applied traditionally in folk medicine practices since primordial times.[1] The use of plants as drugs to meet health needs have increased globally in modern times.[2] According to world health organization, 65-80% of the world's population depends on traditional medicine which are being used to treat diverse diseases.[3] Hence, there is now an improved attention to traditional medicine and high demand for more drugs sourced from plants due to the recent general conviction that "green medicine" is relatively safe and more reliable compared to the expensive synthetic drugs, several of which are known to exhibit adverse side effects. Medicinal plants used as traditional medicines exhibit great potential for the discovery of anti-inflammatory, antioxidant, antimicrobial, anti-mutagenic, anti-carcinogenic and antimalarial drugs.[3] They are also a pool of new and fresh antioxidants principally those with high phenolic contents. Hence, there is an ever-increasing concern in natural antioxidants from food, predominantly vegetables and fruits.[4]

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Inflammation is defined as local response of living-tissue to injury due to any agent. Inflammation expresses usually in form of painful swelling associated with some changes in skin covering the site. It can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.[5]

Plate 1: Red beet root

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In the face of considerable progress made in the treatment of parasitic diseases, malaria remains a major public health challenge in prevalent areas of the world, predominantly because of the widespread resistance of malaria parasites to currently existing anti-malarial agents, the resistance of the mosquito vectors to currently obtainable insecticides, the limited success in the development of malarial vaccines and the devastating and undesirable reactions of conventional anti-malarial drugs.[6] Malaria is responsible for the deaths of over a million people each year and about three billion people are at risk of getting malaria infection by *Plasmodium falciparum* and *Plasmodium vivax*.[7] Therefore, developments and the intricacy of producing efficient vaccines underscore the imperative need for new antimalarials. In developing countries, especially in Africa, accessible treatments against malaria are mainly based on the use of traditional herbal medicine. Indeed, local plants play a significant role in the treatment of many infectious diseases and a good number of them rely on herbal remedies.[6] Chenopodiaceae family includes approximately 1400 species divided into 105 genera. Members of this family are dicotyledonous. Beet root, scientifically known as *Beta vulgaris* is one of the well-known plants belonging to this family. It is an erect annual herb with tuberous root stocks. There are basically four varieties of Beetroot known as Detroit dark red, Crimson Globe are commonly grown in India and another two varieties are Crosby Egyptian. It has numerous cultivated varieties of which, the most well-known is the root vegetable known as the beetroot or garden beet. Other cultivated varieties include the leaf vegetable chard; the sugar beet, used to produce table sugar; and mangel-wurzel, which is a fodder crop.[8] *Beta vulgaris* is used in Indian traditional system of medicine, specifically to enhance the activity of sex hormones. It makes an excellent dietary supplement being not only rich in minerals, nutrients and vitamins but also has unique phytoconstituents, which have several medicinal properties. Several parts of this plant are used as anti-oxidant, anti-depressant, anti-microbial, anti-fungal, anti-inflammatory, diuretic, expectorant and carminative.[9]

It is one of the natural foods which boosts the energy in athletes as it has one of the highest nitrates and sugar content plant. Beet root contains Betaine (Betacyanin pigment responsible for its red colour) which is used as natural food colour in dairy and meat products. It can be taken as salad during pregnancy because it is helpful in the growth of foetus.[9,10] There are some reports indicating the potential hepatoprotective, antioxidant, anti-inflammatory and antiplasmodial activities of *Beta vulgaris.*[5,11] Nevertheless, this study stands to update the various works and information on the scientific investigation and assessment of *Beta vulgaris* for the cure and management of various ailments as established by folklore. Hence, the purpose of this research was to evaluate the phytochemicals, antioxidant, acute toxicity, anti-inflammatory and antimalarial activities of ethanol extract of *Beta vulgaris*.

Materials and Methods

Collection of plant samples

Fresh *Beta vulgaris* were procured in January 2020 from a local market in Benin City, Edo State, Nigeria. They were identified and authenticated by Dr. Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria.

Sample preparation and extraction

The beetroots were thoroughly washed with clean water to eliminate dirt and air-dried for three weeks and thereafter they were pulverized using the British milling machine. The pulverized roots (300 g) were macerated in 750 mL of ethanol with continuous stirring and shaking for 72 hours. The extract was filtered using Whatman paper no. 1 and the filtrate was concentrated to dryness in the vacuum at 50°C using a rotary evaporator. The weight of the extract was taken and then stored in the fridge until used.

$$\% \text{ Extraction yield (w/w)} = \frac{\text{Weight of dried extract}}{\text{Weight of the Sample used for extraction}} \times 100 \qquad (1)$$

Preliminary phytochemical screening

Phytochemical screening to detect the presence of alkaloids, steroids, tannins, saponins, flavonoids, terpenoids, phenols and anthraquinones were carried out using standard methods. [12-14]

Antioxidant activity assessment

Methanol was used to prepare 2,2-diphenyl-1-picrylhydrazyyl (DPPH) of a concentration of 0.1 mM solution. A solution of *Beta vulgaris* root extract was also prepared in methanol. 3.0 mL of the extract was mixed along DPPH (1.0 mL). A micropipette was used to add equal volumes of the resulting mixture to different concentrations of the root extract (10-200 mg/mL) in different test tubes. The reaction mixture was vigorously shaken and left for 30 minutes to stand in the dark. The absorbance was measured at 517 nm.[15] The radical scavenging activity was calculated by using the following equation:

$$\% \text{ DPPH scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of test sample}}{\text{Absorbance of blank}} \times 100 \qquad (2)$$

Experimental animals

Swiss mice (18 –24 g) and Wistar rats (152-187 g) of either sex obtained from the University of Benin, Edo State, Nigeria were used for the study. The animals were kept in plastic cages at room temperature and under naturally illuminated environment of 12:12 hour dark/light cycle. They were fed with standard diet and had water *ad libitum* according to the National Institutes of Health (NIH) Guide for the care and use of laboratory Animals.

Acute toxicity studies

The acute toxicity study was performed by a method described by Igbe *et al*,[16] as modified by Ogbeide *et al*.[6] The mice were divided into four separate groups of five mice each labeled 1-4. Groups 2-4 were the test groups, while group 1 was the control. Groups 2-4 received 100, 1000, 5000 mg/kg of the ethanol crude extract suspended in gum acacia respectively by oro-gastric syringe, while group 1 (control) received 10% gum acacia solution by oral route. The animals were observed for common symptoms of toxicity and mortality within 24 hours, and the animals that survived after 24 hours were observed for any signs of delayed toxicity for 14 days. The median lethal dose (LD_{50}) was calculated using equation 3:

$$LD_{50} = \sqrt{D_a \times D_b} \qquad (3)$$

Where;
a = lowest lethal dose (where death of mice occurred)
b = highest non-lethal dose (where no death of mice occurred).

Anti-inflammatory study

Formalin-induced rat hind paw oedema

The effect of ethanol extract of *B. vulgaris* on formalin-induced inflammation in rat paw was investigated by following the method described by Shibata *et al*,[17] as modified by Ogbeide *et al.*[18] Rats were randomly divided into five groups, each consisting of five animals (n = 5). Group I serving as a negative control was given only distilled water (dose). Group II, III and IV were given 50, 100 and 200 mg/kg of the root extract respectively and Group V, which served as a positive control was given aspirin (100 mg/kg). The animals were fasted 12 hours prior to the experiment. All drugs were administered orally. Thirty (30) minutes after oral administration of the extracts, 0.1 mL 1% formalin suspension was injected subcutaneously in the left hind paw of each animal, leading to the formation of oedema (localized inflammation) *in situ*. The volume of paw oedema was measured hourly for five hours using a vernier calliper after administration of formalin. The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula:

$$\% \text{ Inhibition of paw edema} = 1 - \frac{Vt}{Vc} \text{x } 100 \qquad (4)$$

Where V_c and V_t represent average paw volume of control and treated animals respectively.

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Antimalarial studies
Parasite inoculation
Plasmodium berghei was obtained from the National Institute of Medical Research (NIMR), Lagos, Nigeria. Parasitaemia of the donor's blood was first determined. These mice were then sacrificed by cervical dislocation, and blood was collected in a Petri dish with an anticoagulant (0.5% trisodium citrate) by severing the jugular vein. The blood was then diluted with physiological saline (0.9%) based on parasitaemia of the donor mice and the red blood cells (RBC) count of normal mice in such a way that 1 mL blood contained $5x10^7$*P. berghei*– parasitized erythrocytes. Each mouse used in the experiment was infected intraperitoneally with 0.1 mL of infected blood containing about 1 x 10^7*P. berghei*– parasitized erythrocytes.[19]

Preparation of giemsa solution
Giemsa powder (3.5 g) was dissolved in a mixture of 250 mL of glycerol and 250 mL of methanol. The procedure was done in a dark room. The solution was poured in a dark reagent bottle and kept in a dark cupboard for a week.[4]

Preparation of phosphate buffer saline (pbs)
Sodium dihydrogen phosphate (10.9 g) and 3.2 g of disodium hydrogen phosphate were dissolved in distilled water and the solution was made up to 1000 mL using distilled water. The pH of the solution was adjusted to 7.2 using dilute solution of NaOH.[4]

Evaluation of suppressive activity of extract on early infection (4-day test)
This test was used to evaluate the antiplasmodial activity of the root extract and chloroquine against early *P. berghei* infection in mice. On the first day (D_1), twenty-five Swiss mice were inoculated (intraperitoneally) with *P. berghei* parasitized erythrocyte (0.1 mL of infected blood containing about 1 x 10^7*P. berghei*-parasitized erythrocytes) and randomly divided into five groups (Group A, B, C, D and E) of five animals each and treated for the next four consecutive days ($D_1 – D_4$). Groups A, B and C received daily doses of the extract (200, 400 and 800 mg/kg body weight of animal respectively) by oral route, group D received no treatment, while group E received 5 mg/kg of chloroquine daily by oral route. The required dose was given according to the weight of the animal two hours after inoculation of parasites on D_1, then once daily for three more days ($D_2 – D_4$).[20]

Evaluation of parasitaemia
On day five (D_5) of the study, thick and thin films were prepared with blood collected from the tail of each mouse. The thin film was fixed with methanol and both films were stained with giemsa and the thin blood films of infected and treated mice were examined for parasitaemia level under the microscope from day five through day eight ($D_5 – D_8$). The stained slides were mounted in oil immersion. Red blood cells were counted in 10 fields and the parasitized also noted. The percentage parasitaemia (Pp) in a group was calculated as illustrated below.
Pp = the average percentage suppression for each dose of each extract was calculated in comparison to control as follows:

$$\text{Average \% suppression} = \frac{X-Y}{X} \times 100 \qquad (5)$$

Where, X= Average % parasitaemia negative control
Y= Aaverage % parasitaemia treated groups.[4]

Statistical Analysis
Data was analyzed using windows SPSS version 20.0. Results obtained from the study are expressed as mean ± standard errors of the mean (SEM). They were analysed with one-way analysis of variance (ANOVA), followed by Bonferroni t-test or student-Newman-Keulsposthoc tests. $P < 0.05$ was considered significant.

Results and Discussion

The percentage yield from crude extract of *Beta vulgaris* root obtained was 2.14% (Table 1). The phytochemical analysis of the ethanol and aqueous crude extracts of the root of *B. vulgaris* revealed the presence of alkaloids, saponins, phenols, flavonoids, terpenoids, tannins and anthraquinones (Table 2). Phytochemicals are compounds that act as free radical scavengers to help eradicate the highly charged oxygen molecules that are by-products of metabolized oxygen[21] and are believed to provide several health benefits.[21] Saponins are known to exhibit anti-inflammatory activity and erythrocyte haemolysis.[23,24] Flavonoids commonly found in fruits and vegetable have been linked to decreased risk of mortality from coronary heart diseases as well as many others.[25] Alkaloids, Phenols and Tannins are also known for their antimicrobial, antidiarrhoeal and anthelmintic properties. Also, terpenes are known to possess cytotoxic properties. Therefore, the presence of terpenoids in the root of *B. vulgaris* suggests that the extract could be used in the preparation of anti-tumor and anti-viral drugs and remedies. Furthermore, anthraquinones stand out for their remarkable biological activities: anticancer, anti-inflammatory, diuretic, antiarthrittic, antifungal, antibacterial and antimalarial.[26]
The discolouration of DPPH radical has remained the most commonly applied approach in assessing the antioxidant potential of various extracts from herbs.[27] DPPH free radical scavenging action of *B. vulgaris* root extract showed an appreciable and dose-dependent increase in scavenging effect for the standard (ascorbic acid) and crude extract (Table 3).

Table 1: Percentage yield of crude extract of *B. vulgaris* root

Powdered Plant Sample (g)	Solvent	Mass of Crude Extract (g)	% Yield
300	Ethanol	6.42	2.14

Table 2: Phytochemical analysis of the crude extract of *B. vulgaris* root

Phytochemicals	Aqueous	Ethanol
Alkaloids	+	+
Steroid	-	-
Terpenoids	+	+
Saponins	+	+
Phenols	+	+
Tannins	+	+
Flavonoids	+	+
Anthraquinones	+	+

(+) = Present and (-) =Absent

Table 3: DPPH-scavenging potential of crude extract of *B. vulgaris* root

Concentration (µg/ml)	Ethanol (%)	Ascorbic acid (%)
1000	74.46 ± 0.98^b	98.66 ± 0.16^a
500	56.84 ± 0.97^b	91.69 ± 0.28^a
250	53.38 ± 1.55^b	89.10 ± 1.26^a
125	46.34 ± 1.96^b	78.03 ± 1.17^a
62.5	37.13 ± 1.23^b	74.08 ± 1.80^a
31.25	33.94 ± 0.37^b	65.17 ± 1.03^a
15.625	0.00 ± 0.00^b	55.04 ± 0.97^a

Values are mean ± standard error of the mean of triplicate analysis. Different alphabets across the same row indicates significant difference at $p<0.05$

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At the highest concentration (1000 µg/mL), the percentage inhibition of the crude pod extract obtained was 74.46 ± 0.98% while the percentage inhibition of ascorbic acid (standard) obtained was 98.66 ± 0.16%. Thus, the appreciable antioxidant property displayed by the extract may be attributed to the phytochemicals detected.

The oral administration of crude root extract of *B. vulgaris* at graded doses of 100, 1000 and 5000 mg/kg body weight showed no indication of acute toxicity (Table 4), because there was no record of mortality of the animals even up to 72 hours of cautious watching from the least to the maximum dose. Thus, there was no indication of any toxicity and variation in the pattern of behaviour and physiological responses detected such as raised tails, salivation or paw licking. According to Hodge and Sterner,[28] an experimental drug which is orally administered can be classified into six categories based on the LD_{50}. If the LD_{50} is equal to or less than 1 mg/kg, it is regarded as 'extremely toxic' and when the LD_{50} is between 1 and 50 mg/kg, then it is said to be 'highly toxic'. Similarly, when the LD_{50} is between 50 and 500 mg/kg, it is termed to be 'moderately toxic' and when the LD_{50} is between 500 and 5000 mg/kg, it is only 'slightly toxic'. Furthermore, when the LD_{50} is between 5000 and 15000 mg/kg, it is practically or basically 'non-toxic' and finally, an experimental drug is declared relatively 'harmless' at LD_{50} equal to or greater than 15000 mg/kg. Hence, *Beta vulgaris* root extract is practically non-toxic up to the dose of 5000 mg/kg.

Inflammation induced by formalin resulted in the formation of the rat paw oedema, which is, swelling of the paw.

In the reference and the test groups, there was however, marked increase in the paw volume within the hour from Table 5, due to the action of antioxidants present in them. The result of this study indicates dose-dependent activity of the plant extract in inhibiting inflammation with the highest % inhibition of 100.00 at 50 mg/kg (least dosage at 1hr) and lowest % inhibition of 41.13 at 200 mg/kg (highest dosage) after 5 hours (Table 6). The highest % of inhibition with reference to the drug, aspirin, was 100.00 at 1hr, hence, the plant showed anti-inflammatory activity comparable to the standard drug at 50 mg/kg (least dosage at 1hr). Another study reported on the anti-inflammatory activity of aqueous extract of *B. vulgaris* leaves which showed percentage of inhibition of 44.82 comparable to the reference drug; Indomethacin which is 51.72 at 1000 mg/kg after 4 hours.[29] This result further ratifies *B. vulgaris* as an anti-inflammatory drug.

Table 4: Oral acute toxicity results of the crude extract of *B. vulgaris* root in mice

Group	Doses (mg/kg)	Number of deaths	Percentage mortality
Control	DW	0/3	0
Root extract	100	0/3	0
Root extract	200	0/3	0
Root extract	500	0/3	0

Table 5: Effect of *B. vulgaris* root extract on rat paw oedema at each time interval

Groups	Dose (mg/kg)	1 hr	2 hrs	3 hrs	4 hrs	5 hrs
Control	DW	9.67 ± 2.33	11.00 ± 2.65	15.33 ± 2.03	14.67 ± 2.91	11.33 ± 1.20
Aspirin	100	0.00 ± 0.00***	0.00 ± 0.00***	0.33 ± 0.10***	0.33 ± 0.10***	0.00 ± 0.00***
B. vulgaris	50	0.00 ± 0.00***	3.00 ± 1.73**	4.00 ± 1.53**	3.67 ± 1.45**	5.33 ± 2.33*
B. vulgaris	100	1.00 ± 0.00**	3.67 ± 1.86**	5.00 ± 0.58*	5.67 ± 1.67*	6.67 ± 1.20
B. vulgaris	200	1.67 ± 0.88**	4.33 ± 0.88**	5.67 ± 0.67*	5.33 ± 1.20*	6.67 ± 0.88

Values represent mean ± standard error of mean* P<0.05 (significant), **P< 0.01(highly significant), ***P< 0.001(very highly significant)

Table 6: The percentage of inhibition of *B. vulgaris root* extract on rat paw oedema at each time interval

Groups	Dose (mg/kg)	% inhibition 1 hr	% inhibition 2 hr	% inhibition 3 hr	% inhibition 4 hr	% inhibition 5 hr
Control	DW	0.00	0.00	0.00	0.00	0.00
Aspirin	100	100.00	100.00	97.85	97.75	100.00
B. vulgaris	50	100.00	72.73	73.91	74.98	52.96
B. vulgaris	100	89.66	66.64	67.38	61.35	41.13
B. vulgaris	200	82.73	60.64	63.01	63.67	41.13

Table 7: Effect of *B. vulgaris* extract and standard drug (Chloroquine) on *Plasmodium berghei NK65*

Treatment	Dosage (mg/kg)	% Parasitaemia			
		D_5 (% ± SD)	D_6 (% ± SD)	D_7 (% ± SD)	D_8 (% ± SD)
No treatment	-	5.01 ± 0.23	7.33 ± 0.41	10.11 ± 0.20	13.45 ± 0.27
Extract	200	2.83 ± 0.21	3.93 ± 0.12	6.12 ± 0.14	9.32 ± 0.41
Extract	400	2.43 ± 0.12	3.77 ± 0.06	5.97 ± 0.13	9.01 ± 0.09
Extract	800	2.02 ± 0.06	3.12 ± 0.09	5.42 ± 0.28	8.73 ± 0.12
Chloroquine	5	0.23 ± 0.02	0.69 ± 0.11	1.01 ± 0.13	1.88 ± 0.12

Not treated group = Negative control, Extract = *Beta vulgaris* root extract, Chloroquine = positive control, SD = Standard deviation

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Table 8: Mean survival time and %Chemo-suppression of *Plasmodium berghei berghei* by the *B. vulgaris* root extract and Standard drug (chloroquine)

Treatment	Dosage (mg/kg)	Average %chemo-suppression				Mean Survival time (M ± SD) days
		D_5 (%)	D_6 (%)	D_7 (%)	D_8 (%)	
No treatment		-	-	-	-	15 ± 3.12
Extract	200	43.51	46.38	39.47	30.71	27 ± 2.69
Extract	400	51.50	48.57	40.95	33.01	24 ± 2.62
Extract	800	59.68	57.44	46.39	35.09	21 ± 2.59
Chloroquine	5	95.41	90.59	90.01	86.02	28 ± 0.45

Not treated group = Negative control, Extract = *Beta vulgaris* root extract, Chloroquine = Positive control.

The extract caused a significant ($p < 0.05$) suppression activity when compared to the untreated group (Table 8). In this regard, the extract had the least and highest suppression activity at doses of 200 and 800 mg/kg respectively thus, all the treatment groups survived significantly compared to the untreated group. The chemo-suppressive activity of the extract shows that the extract might have attacked the young *Sporozoites* cells, thereby inhibiting their growth or killing them like the standard drug (Chloroquine) and/or the extract helped in boosting the immune system of the mice against microbial attack. The suppressive activity of the extract increased with increase in the dosage of the extract administered through D_5 to D_8 suggesting that the extract suppressed the growth of the parasite in a dose-dependent manner. However, there was a reduction in the activity of the extract from D_5 to D_8 with a corresponding increase in percentage parasitaemia as shown in Table 7. This could be as a result of the growth of some of the young parasite which escaped the period of treatment and grew to maturity and were subsequently released into the blood stream.[6,30,31]

Conclusion

This research has clearly illustrated that the ethanol extract of red beet root (*Beta vulgaris*) is relatively non-toxic. The aqueous and ethanol extracts contain phytochemicals which could be responsible for its antioxidant activity, dose-dependent anti-inflammatory activity as well as its antimalarial activity which supports these bioactivities as claimed by folklore and other authors.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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