International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182, Print ISSN: 2349-5979; Impact Factor: RJIF 5.72
Received: 08-04-2019; Accepted: 09-05-2019
www.allsubjectjournal.com
Volume 6; Issue 7; July 2019; Page No. 38-43

Antioxidant Activity of *Psidium guajava, Schoenanthus cymbopogon, Solenostemma argel, Hyphaene thebaica and Petroselinum crispum*

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Abstract
For centuries, plants have been used in traditional medicines and there has been recent interest in the chemo preventive properties of compounds derived from plants. In the present study, five samples of plants *Psidium guajava, Schoenanthus cymbopogone, Solenostemma argel, Hyphaene thebaica and Petroselinum crispum* are common plants widely used in many parts of Sudan for medicinal purposes or herbal medicine to treat various diseases, The collected plants were subjected to sequential extraction using petroleum ether, ethyl acetate, acetone and ethanol as solvents. The results clearly indicated variation of the total yields, the *P. guajava* extract gave the highest yield (7.001%) followed by *S. argel* (6.551%), *P. Crispum (*4.760%), *S. Cymbopogone (*4.633%) and *H. thebaica* (2.538%.The extract of plant samples were evaluated by employing *in vitro* anti-oxidant using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and 2,2´-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid(ABTS) free radical scavenging. Each sample under assay condition showed free radical scavenging effect of DPPH and ABTS. Petroleum ether extract for plant samples revealed a non-significant free radical scavenging activity with DPPH reagent while the highest scavenging ability was exhibited by ethanol extract (90%) that may be due to polarity of ethanol. Most extracts with ABTS reagent exhibited good antioxidant activities (69% – 94%) compared to DPPH, The highest scavenging ability was exhibited by ethanol extract from *P. guajava* (94%), while the lowest was determined in the acetone extract of *P. crispum* (69%). Among plant extracts, the *P. guajava* showed stronger IC_{50} values (0.477) than other plant extracts.

Keywords: Medicinal plants, Traditional uses, DPPH, ABTS, Antioxidant activity

1. Introduction
Natural products today are most likely going to continue to exist and grow to become even more valuable as sources of new drug leads. This is because the degree of chemical diversity found in the natural products is broader than from any other source, and the degree of novelty of molecular structure found in natural products is greater than that determined from any other source [1]. A wide range of medicinal plants part (leaves, seeds, roots, bark…etc) are used as raw drugs and they possess varied medicinal properties. The activity may reside in a variety of different components, including aldehyde and phenolic compounds, and other natural products research remain one of the main means of discovering bioactive compounds. Until recently, most natural products chemists have been more concerned with the isolation and structural elucidation of secondary metabolites than their biological activity. They realize that the, detection, isolation and structure determination of metabolites are only the first step toward answering much broader questions [2]. Plant-derived substances have traditionally played important roles in the treatment of human diseases. Today, about 80 % of the world population residing in third world countries still rely almost entirely on plant products for their primary health care. The remaining 20% of individuals living in the world use, in more than 25% of cases, pharmaceuticals which have been directly derived from plant products [3] [4]. Herbal medicine has provided the world's population with safe, effective and low cost natural substances (medicine) for centuries [5].

Many medicinal plants have been shown to contain large amounts of effective antioxidants, and the search for antioxidants and radical scavengers from natural sources is pursued worldwide, and is a key focus of our research group [6]. Free radicals may be defined as chemical species associated with an odd or unpaired electron. They are neutral, short lived, unstable and highly reactive to pair up the odd electron and finally achieve stable configuration. They are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to degenerative diseases of aging such as cancer, cardiovascular disease, immune system decline, liver diseases, diabetes mellitus, inflammation, renal failure, brain dysfunction and stress among others [7, 8].

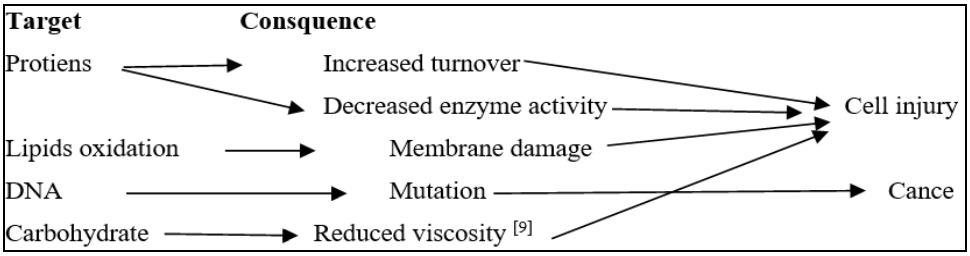

Fig 1

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system, that functions interactively and synergistically to neutralize free radicals. Thus, antioxidants are capable of stabilizing or deactivating free radicals before they attack cells [10]. An antioxidant is a substance that can efficiently reduce a pro-oxidant with concomitant formation of products having no or low toxicity. Indeed, a broader definition of antioxidant was suggested by [11]. In 2007, Halliwell [12] gave a more specific definition, stating that an antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule". Therefore, according to this definition, not all reductants involved in a chemical reaction are antioxidants; only those compounds which are capable of protecting the biological target meet these criteria. This protection may be based on several mechanisms of action, namely: inhibition of generation and scavenging capacity against ROS reducing capacity; metal chelating capacity; activity as ant oxidative enzyme; inhibition of oxidative enzymes[13] [14].Naturally there is a dynamic balance between the amount of free radicals produced in the body and antioxidants that protect the body against deleterious effects. Often times, amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases. Hence there has been an increased interest in the food industry and in preventive medicine in the development of "Natural antioxidants" from plant materials [15]. That is why plants with antioxidant properties are becoming more and more popular all over the world. In this context, some of the most commonly used methods for *in vitro* determination of antioxidant capacity is reviewed in the following sections, where the chemical principles, recent applications as well as the advantages and shortcomings are outlined.

The main aim of this study is to evaluate antioxidant capacity of *Psidium guajava, Schoenanthus cymbopogone, Solenostemma argel, Hyphaene thebaica and Petroselinum crispum*

2. Materials and methods

2.1 Plant materials

Collection and preparation

The plants samples leaves of the *Psidium guajava, Schoenanthus cymbopogone, Solenostemma argel, Petroselinum crispum* and *Hyphaene thebaica* fruits were randomly collected from different area of Sudan. The plant materials were dried under shade for 7 days except the fruits of *doom palm* which was found dry. They were ground into fine powder in preparation for extraction. The finely powdered materials were weighed separately and stored at room temperature.

2.2 Chemicals

chemicals solvents used in this study were petroleum ether, ethyl acetate, acetone, methanol and ethanol were from Sisco Research Lab (SRL), India, and the other chemical were used 2,2-diphenyl-1-picrylhydrazyl (DPPH),(2, 2´-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid) ABTS, propyl glate, ascorbic acid, dimethyl sulfuric(DMSO).

2.3 Instruments and apparatus

Spectrophotometer (BMG LABTECH, SPECTRO star Nano) Eppendorf, micro plate reader, test tube

2.4 Successive extraction

A 100 g of the finely ground material was soaked in petroleum ether a least polar solvent for 48 hours with occasional swirling to ensure thorough extraction. The soaked material were filtered and the crude extract collected in clean containers. The crude extract was dried at room temperature and weighed. The residue after extraction with petroleum ether was soaked in ethyl acetate solvent for 48 hours with occasional swirling to ensure thorough extraction. The soaked material were filtered and the crude extract collected in clean containers. The crude extract was dried at room temperature and weighed. The residue after extraction with ethyl acetate was soaked in acetone solvent for 48 hours with occasional swirling. This followed by filtering, drying and weighing. Finally the residue was soaked in ethanol and the above procedure was repeated.

2.5 Determination of antioxidant activity

2.5.1DPPH radical scavening assay

Antioxidant activity of the extracts was estimated using 2, 2-diphenyl-1-pycrilhydrazil hydrate (DPPH) *in vitro* according to the method of Villano [16]. The test samples were dissolved in DMSO while DPPH was prepared in ethanol. Assays were performed in 96-well, microtiter plates. The absorbance was measured spectrophotometric ally at 517 nm.Propyl galate was used as reference antioxidant compound. Every analysis was done in triplicates.

The ability of the extract to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) = 1- [(Abs_{sample} – Abs_{blank})]/ ($Abs_{control}$)] x 100

Where;

Abs_{sample} is the absorbance of DPPH radical + sample;

Abs_{blank} is the absorbance of sample + methanol;

$Abs_{control}$ is the absorbance of DPPH radical + methanol.

2.5.2 ABTS radical scavenging activity

The scavenging activity of the studied extracts on ABTS (2, 2´-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid) radical cation was estimated according to the method of Mandal *al*

[17]. Briefly, ABTS radical cation was freshly prepared by mixing 14 mm ABTS with an equal volume of 4.95 mm potassium persulphate and kept for 16 h in dark at room temperature. This ABTS radical cation solution was used for the assay after dilution in phosphate buffer saline (PBS) appropriately. To 50 µl of sample or standard, 150 µl of ABTS radical solution was added. After 6 min incubation at room temperature, the absorbance was measured at 734 nm. Methanol was used as blank solution, and ABTS solution wi5thout the sample served as control. Ascorbic acid was used as reference synthetic antioxidant compound. Reduction of ABTS radical in percent (R %) was calculated the same as described in DPPH radical assay

2.5.3 IC_{50} calculation

The IC_{50} (the concentration of test material, which possess 50% inhibition of free radicals) value was calculated from the linear regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity. The IC_{50} values obtained from the regression plots (Sigma Plots R 2001, SPSS Science) had a good coefficient of correlation, ($R^2 = 0.998$). The IC_{50} of all the extracts and their fractions was determined by monitoring the effect of different concentrations ranging from0.5 – 0.15 mg/ml. The IC_{50} of extracts and their fractions were calculated using EZ-Fit Enzyme Kinetic software Program (Perrela Scientific Inc, U S A.).

3. Results and discussion

3.1 Crude extract yield

The percentage yields of the extracted component from each plant using four different solvents (petroleum ether, ethyl acetate, acetone and ethanol) of varying polarities were calculated. The colour and texture of each extract was recorded. The obtained results are presented in Tables (1, 2, 3, 4 and 5).Successive extraction of *P. guajava, S. cymbopogon, S. argel, H. thebaica and P. crispum* results indicated variation in the percentage yields. The ethanol extract of studied materials, gave the highest yield in form of gummy or waxy extracts, while petroleum ether extracts recorded the lowest yield. Both ethyl acetate and acetone extracts of plants samples gave relatively moderate yield. P. uajava gave the highest yield with all solvents while H. thebaica was the lowest one. The variations in the extract yields from plants using different solvents might be explained by the polarity of extracted components and solvents applied [18].

Table 1: percentage yields (%) and physical characteristics of the extracted components from P. guajava

Solvent	Weight(g/100g)	Yield	Color	Texture
P. ether	0.01261	1.261	Dark green	Sticky
E. acetate	0.01345	1.345	Dark green	Powder
Acetone	0.01744	1.744	Dark green	Sticky
Ethanol	0.02651	2.651	Dark green	Waxy
Total yield	7.001%			

Table 2: percentage Yields% and physical characteristics of the extracted components from S. cymbopogon

Solvent	Weight(g/100g)	Yield (%)	Color	Texture
P. ether	0.00563	0.563	Brown	Waxy
E. acetate	0.00865	0.865	Brown	Waxy
Acetone	0.01324	1.324	Light brown	Waxy
Ethanol	0.01881	1.881	Dark brown	Waxy
Total yield	4.633%			

Table 3: percentage Yields% and physical characteristics of the extracted components from S. argel

Solvent	Weight(g/100g)	Yield (%)	Color	Texture
P. ether	0.00761	0.761	Dark green	Waxy
E. acetate	0.00945	0.945	Green	Waxy
Acetone	0.02344	2.344	Dark green	Sticky
Ethanol	0.0251	2.501	Brown	Waxy
Total yield	6.551%			

Table 4: percentage Yields% and physical characteristics of the extracted components from H. thebaica

Solvent	Weight(g/100g)	Yield (%)	Color	Texture
P, ether	0.00254	0.254	Yellow	Waxy
E. acetate	0.00721	0.721	Green	Waxy
Acetone	0.00331	0.331	Dark green	Sticky
Ethanol	0.01232	1.232	Brown	Waxy
Total yield	2.538%			

Table 5: percentage Yields% and physical characteristics of the extracted components from P. crispum

Solvent	Weight(g/100g)	Yield (%)	Color	Texture
P. ether	0.00501	0.501	Dark green	Waxy
E. acetate	0.00766	0.766	Dark green	Waxy
Acetone	0.00943	0.943	Dark green	Waxy
Ethanol	0.02550	2.550	Dark green	Waxy
Total yield	4.760%			

3.2 Antioxidant activity

The antioxidant activity of different plants from *P. guajava, S. cymbopogon, S. argel, H. thebaica and P. crispum* were determined using a solution of DPPH and ABTS reagents. They are molecules containing a stable free radical. The presence of antioxidant substances could be revealed by the decrease of the intensity the purple color typical of the free DPPH radical [19]. It is a direct and dependable method for determining the radical scavenging action. DPPH is very stable free radical. Unlike *in vitro* generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades when antioxidant molecule quench DPPH free radicals (i.e. by providing atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them into a colorless- /bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band [20]. Results of antioxidant activity of crude solvents extract were presented in Tables (2).

Table 6: antioxidant activity of petroleum ether, acetone, ethyl acetate and ethanol extracts of *P. guajava*, *S. cymbopogone, S. argel, H. thebaica* and *P. crispumusing*2, 2-diphenyl-1-pycrilhydrazil hydrate reagent

No. A	Sample	%RSA ±SD (DPPH)	IC_{50}±SD mg/ml (DPPH)
1	P. ether extract of *P. guajava*	29 ± 0.0.05	-
2	P. ether extract of *S. cymbopogone*	30 ± 0.04	-
3	P. ether extract of *S. argel*	Inactive	-
4	P. ether extract *H. thebaica*	Inactive	-
5	P. ether extract of *P. crispum*	16 ± 0.07	
6	E. acetate extract P. *P. guajava*	89 ± 0.01	0.038±0.03
7	E. acetate extract *S.* S. *cymbopogone*	43± 0.07	-
8	E. acetate extract *S. S. argel*	35± 0.08	-
9	E. acetate extract *H. H. thebaica*	23 ± 0.09	-
10	E. acetate extract. P. *P. crispum*	27± 0.08	-
11	Acetone extract of *P. guajava*	89.2 ± 0.02	0.029±0.01
12	acetone extract *S. S. cymbopogone*	21 ± 0.15	
13	acetone extract *S. S. argel*	32 ± 0.05	-
14	acetone extract of *H. thebaica*	47 ± 0.3	-
15	acetone extract P. *P. crispum*	30± 0.6	-
16	ethanol extract P. *P. guajava*	90 ± 0.02	0.477±0.02
17	ethanol extract *S S. cymbopogone*	45 ± 0.13	-
18	ethanol extract *S. S. argel*	18 ± 0.07	-
19	Ethanol extract *H. H. thebaica*	22 ± 0.06	-
20	ethanol extract P. *P. crispum*	07 ± 0.07	-
Standard	Propyl Gallate	93± 0.01	0.077µg/ml± 0.01

The obtained results were compared with Propyl gallate as a good antioxidant agent. For successive extraction, petroleum ether extract revealed a non-significant free radical scavenging activity with scavenging activity ranging between 0.16% for *P. crispum*, 29% for *P. guajava and*30% for *S. cymbopogon* extracts while *S. argel* and *H. thebaica* extracts showed no antioxidants effect. Concerning the antioxidant activity of ethyl acetate only *P. guajava* sample that exhibited high activity with scavenging rate 89%, the otherness samples their activity scavenging rates 23%, 27%, 35% and 43% for *H. thebaica*, *P. crispum*, *S. argel* and *S. cymbopogon* respectively. All acetone extracts showed no antioxidant effect except *P. guajava* that exhibited high activity which scavenging rate 89.2%, but their activities were relatively high than those of petroleum. All ethanol extract showed lowest effective free radical scavenging in the DPPH assay, while *P. guajava* extract exhibited a remarkable antioxidant effect which showed scavenging rate of 90%. The IC_{50} value of *P. guajava* was found to be (0.038, 0. 029, 0.477mg/ml) with ethyl acetate, acetone and ethanol extracts respectively.

Table 7: antioxidant activity of petroleum ether, ethyl acetate, acetone, and ethanol extracts of P. guajava, S. cymbopogone, S. argel, H. thebaica and P. crispumusing2, 2´-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid reagent

No.	Sample	%RSA ±SD (ABTS)	IC_{50}±SD mg/ml (ABTS)
1	P. ether extract of *P. guajava*	91.4±0.02	0.02±0.02
2	P. ether extract of *S. cymbopogone*	85.8±0.04	0.09±0.03
3	P. ether extract of S. *argel*	-10.5±0.03	-
4	P. ther extract of. H. *thebaica*	6.7±0.01	-
5	P. ether extract of *P. crispum*	43.1±0.1	-
6	E. acetate extract of *P. guajava*	89.9±0.02	0.15±0.01
7	E. acetate extract of *S.* S. *cymbopogone*	90.5±0.003	0.09±0.002
8	E. acetate extract of *S. S. argel*	59.9±0.08	0.11±0.004
9	E. acetate extract of *H. thebaica*	45.5±0.02	-
10	E. acetate extract. of *P. crispum*	34.4±0.05	-
11	Acetone extract of *P. guajava*	92.9±0.02	0.04±0.01
12	acetone extract of *S. cymbopogone*	88.9±0.02	0.1±0.02
13	acetone extract of *S. S. argel*	78.3±0.3	0.01±0.01
14	acetone extract of *H. thebaica*	91.4±0.01	0.12±0.003
15	acetone extract of *P. crispum*	69.5±0.1	0.240±0.02
16	ethanol extract of *P. guajava*	94.8±0.01	0. 4±0.008
17	ethanol extract of *S. cymbopogone*	92.4±0.01	0.09±0.02
18	Ethanol extract of. *S. argel*	47.3±0.08	
19	ethanol extract of *H. thebaica*	92.9±0.003	0.06±0.001
20	ethanol extract of *P. crispum*	88.5±0.02	0.17±0.004
Standard	Scorbic acid	95.2±0.01	0.0616 µg/ml ±0.01

The ability of the tested samples to scavenge ABTS radicals were compared to scorbic acid (vitamine c) standard. Most crude extracts exhibited good antioxidant activities (69% – 94%) compared to ascorbic acid (95.2%). The highest scavenging ability was exhibited by ethanol extract from *P. guajava* (94%), while the lowest was present in the acetone extract of *P. crispum* (69%). At petroleum ether extract two samples (*P. guajava*, *S. cymbopogon*) showed high

antioxidant effect rates 91.4%, 85% respectively while S. argel was inactive and *P. crispum, H.* Ethyl acetate three samples (*P. guajava, S. cymbopogon and S. argel*) that exhibited highest activity with scavenging rates 89.9%, 90.5% and 59.9% respectively except *H. thebaica and P. crispum* low antioxidant effect. All acetone extract showed a high effective free radical scavenging in the ABTS assay, it exhibited a remarkable antioxidant effect specially *P. guajava* extract which showed scavenging rate of 92.9%.Then Concerning the antioxidant activity of ethanol extract all samples showed a high effective free radical scavenging except *S. argel* which showed scavenging rate of 47%.The IC_{50} value ranging between (0.01 -0.4mg/ml) for S. argel and P. guajava respectively.

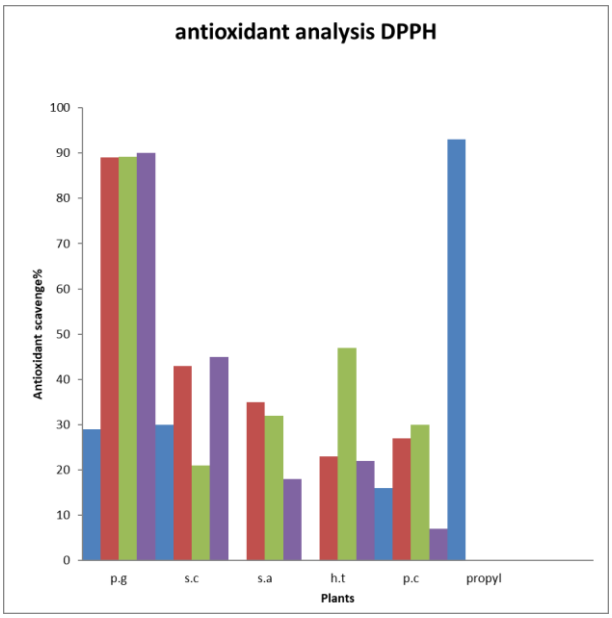

Fig 2: antioxidant activity of petroleum ether, ethyl acetate, acetone and ethanol extracts of *P. guajava, S. cymbopogone, S. argel, H. thebaica* and *P. crispum using* 2, 2-diphenyl-1-pycrilhydrazil hydrate reagent

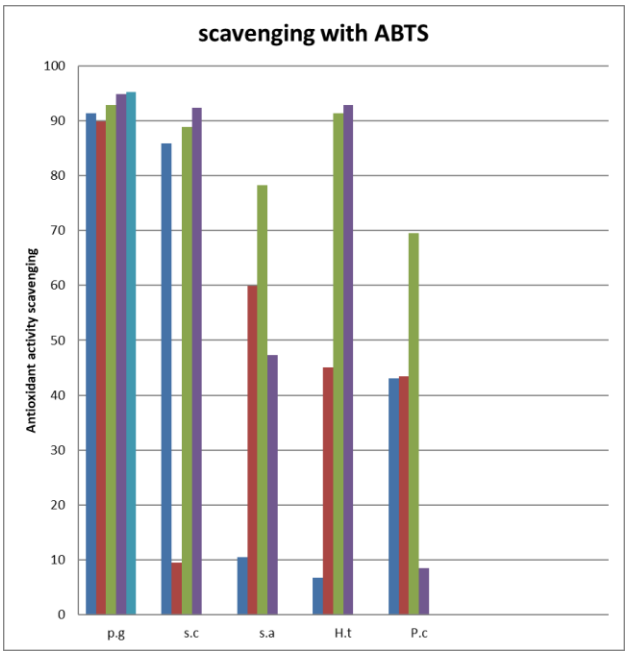

Fig 3: antioxidant activity of petroleum ether, ethyl acetate, acetone, and ethanol extracts of P. guajava, *S.* inobis (3-ethylbenzothiazoline- 6-sulfonic acid reagent

3.3 Conclusion

Phytochemicals that are present in plants have been shown to possess a range of bioactivity, including antioxidant properties. They are extensively researched by scientists for their health-promoting potential. Through the use of well-established scientific methods and research materials. General standard methods were adopted for extracting and analyzing the target compounds. The highest percentage yield of the extracted components was detected in ethanol extract. The antioxidant activities were evaluated based on the ability of the plant extracts to scavenge (DPPH) and (ABTS) free radical. Most crude extracts with ABTS reagent exhibited good antioxidant activities for percentage70% compared to DPPH reagent (15%). The highest scavenging ability was exhibited by ethanol extract that may be due to polarity of ethanol. P. guajava extract exhibited a remarkable antioxidant effect compared with other plant samples which showed scavenging rate of 94%.

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