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Original Article

GC-MS Analysis and Antiproliferative Effect of Ethanolic Extract of *Apium graveolens* L. seeds on MCF-7 Human Breast Cancer Cell Lines

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ABSTRACT

Objectives: The aim of this study was to identify main phytocompounds and to evaluate the antiproliferative effect of the ethanolic extract of *Apium graveolens* seeds. **Materials and Methods:** *in vitro* antiproliferative study of *A. graveolens* ethanolic extract was investigated against MCF-7 cells breast cancer cell lines using the MTT colorimetric assay. Further, the main bioactive components were elucidated by Gas Chromatography–Mass Spectrometry (GC–MS). Commercial mass spectral library was used for the depiction of individual phytocomponents. **Results:** Terpenes were the main compounds found in the ethanolic extract of seeds of *A. graveolens*. In antiproliferative assay, the ethanolic extract in analysis showed significant activity in the proposed conditions. About six components were identified through GC-MS. This study showed the presence of different phytocomponents like Oleanane-3, 16-diol, 13, 28-epoxy-, diacetate, (3.beta., 16.alpha.) [Triterpenes]; Bacchotricuneatin C [Diterpene Lactone]; Cyclopropa[5,6]cholestane, 3-ethoxy-3',6-dihydro-, (3.alpha.,5.alpha.,6.beta.) [Phytosterol]; Fumaric acid, decyl 3-pentyl ester; Anthra[2,3-d]-1,3-dioxole-5,10-dione, 3a,4,11,11a-tetrahydro -9-hydroxy-7-methoxy-2,2,3a-trimethyl-, cis- [Glycoside]; Olean-12-en-28-oic acid, 3.beta.-hydroxy-21-oxo-, methyl ester [Triterpenes]. **Conclusion:** *In-vitro* cytotoxic activity of the ethanolic extract of the plant seeds has been evaluated and showed significant anti-proliferative effect against MCF-7 breast cancer cell lines. The study represents the first report of these compounds from *Apium graveolens* seeds ethanolic extract.

Keywords: *Apium graveolens*, GC-MS, Structural Elucidation, Phytocomponents, MCF-7, Antiproliferative.

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INTRODUCTION

Traditionally, herbs are being used since ancient times. Secondary metabolites are the plant components that are usually synthesized by different plants for performing different functions.[1] *Apium graveolens* L. belongs to Apiaceae family and is commonly known as 'celery' in English and 'Ajmoda' in Hindi. It is hardy, biennial, annual and a herbaceous plant having a height of 60-120 cm. Usually, essential oil is obtained from its seed and its leaf is commonly used as vegetable. Fixed oil is also a component of seeds which is especially used as a flavouring agent in food like sauces, pickle etc. It is also used as a condiment.[2,3] Ajmoda is an oval shaped schizocarpic seed like fruit having brown colour. Seeds are consumed for its nervine stimulant and carminative properties. The leaves are used as vegetable especially in salad. Stems are branched and green in color, roots are succulent having oblong shaped pinnate leaves with a size of 7-18 cm. The sessile umbel flowers are white in color. The plant has fruit bearing seeds. Seeds are small, oval, greenish brown in colour and are about 1-2 mm in length. Celery seeds have a crisp texture with a subtle flavor and bitter taste.[4,5] Celery crops are cultivated mainly in Punjab, Haryana and Uttar Pradesh.

The phytoconstituents of celery includes phenolic compounds like furanocoumarins, flavones etc., phytosterols, glycosides; the leaves are rich source of Vitamins A and C, calcium, phosphorus, iron. The seeds are also used as spice or condiment as flavouring agent. Celery seeds are carminative, effective in rheumatic disorders and used as a nervine tonic in traditional system of medicine. Seed oil is used in industries related to perfumery and pharmaceuticals. Research studies showed that *Apium graveolens* exhibit antispasmodic,[6,7] anticonvulsant, tranquilizing, hypolipidemic,[8] hepatoprotective,[9] antioxidant,[10]

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anticancer and anti bacterial properties. Seeds are also used in different pathological conditions like bronchitis, asthma, spleen and liver disorders.[11] The research work was aimed to assess *in vitro* anti-proliferative activity of the ethanolic extract of the plant seeds against breast cancer (MCF-7) cell lines. The structural elucidation of phytocomponents of has been established on the basis of Gas Chromatography/Mass Spectrometry (GC/MS) analysis. The study represents the first report of these compounds from ethanolic extract of seeds of *Apium graveolens*.

MATERIALS AND METHODS

Plant Material

The seeds of *Apium graveolens* seeds procured and authenticated from Vindhya Herbals, MFP-PARC, Bhopal, M.P. (Voucher specimen no. is VHTRL11122001ER).

Extraction

The coarse powdered drug was extracted in Soxhlet assembly with 90% ethanol to get 55.45 g (29.49% yield) of dark green brown extract. Qualitative phytochemical test were performed to assess the presence of various phytoconstituents. The preliminary screening revealed the presence of alkaloids, sterols, alkaloids, phenolic compounds, flavonoids, glycosides, fats and oil in ethanolic extract.

GC/MS analysis

The phytocompounds of the ethanolic extract were isolated and identified by the gas chromatography and mass spectrometry respectively using Shimadzu's GC–MS equipment (GCMS-QP2020). Samples were analyzed on the capillary column. The initial temperature of GC–MS system was 60°C for 1 min were as follows: the initial oven temperature was detained at 60°C for 1 min and gradually increased at 4°C/min rising upto 250°C. Helium, at a flow rate of 1 mL/min was used as a carrier gas; 0.1 µL of the sample was injected manually at the split/splitless injector at the maintaining temperature of 260°C with a split ratio of 1:50. 270°C was set as transfer line temperature for GC–MS study. The scan range was 30–450 *m/z* for obtaining mass spectra 70 eV (EI). GC–MS data processing was done using AMDIS computer program version 2.62 with NIST library version 2.0. Retention indices and obtained spectra were compared with available data present in the literature.

Antiproliferative Assay

Ethanolic extract from seeds of *A. graveolens* were subjected to *in vitro* antiproliferative study against MCF-7 breast cancer cell lines using MTT colorimetric method. In this study, different concentrations of the sample were subjected for anti-proliferative study. Doxorubicin was taken as positive control in this study. MTT [(3-(4,5- dimethylthiazol-2-yl)- 2,5-diphenyl tetrazolium bromide)] is cleaved by living cells from a pale yellow substrate to a dark blue formazan product. Dead cells are unable to cleave significant amount of MTT as this process needs the availability of active mitochondria. Therefore, the number of viable cells is directly proportional to the amount of cleaved MTT that is further quantified by colorimetry. The samples were dissolved in DMSO and diluted serially with medium to get a range of test concentration. The concentration of DMSO was kept < 0.1% in all samples. Appropriately maintained MCF-7 cells were seeded in 96 well plates, treated with different test concentrations and then incubated for 96 hr in 5% CO_2 incubator at 37°C. MTT was then added to the wells and incubated for 4 hr. The dark blue formazan product so formed by the cells was allowed to dissolve in DMSO and readings were taken at 550 nm. IC_{50} values were determined by calculating percentage inhibitions and plotting them with the concentrations.[12]

Statistical Analysis

Statistical analysis of differences was carried out by Analysis of Variance (ANOVA). A value of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The powdered drug was extracted with 90% ethanol to get 55.45 g (29.49% yield) of dark green brown extract. List of phytocompounds from *Apium graveolens* seeds extract obtained from GC/MS analysis is demonstrated in Table 1. According to the Table 1, about six components were identified after the characterization of individual components using mass spectral library. This study showed the presence of different phytocomponents like Oleanane-3, 16-diol, 13, 28-epoxy-, diacetate, (3. beta., 16.alpha.) [Triterpenes/ S1]; Bacchotricuneatin C [Diterpene Lactone/ S2]; Cyclopropa[5,6]cholestane, 3-ethoxy-3',6-dihydro-, (3.alpha.,5.alpha.,6.beta.) [Phytosterol/ S3]; Fumaric acid, decyl 3-pentyl ester [S4]; Anthra[2,3-d]-1,3-dioxole-5,10-dione, 3a,4,11,11a-tetrahydro-9-hydroxy-7-methoxy-2,2,3a-trimethyl-, cis- [Glycoside/ S5]; Olean-12-en-28-oic acid, 3.beta.-hydroxy-21-oxo-, methyl ester [Triterpenes/ S6]; [Figures 1-6] were also elucidated through GC-MS analysis and represents the first report of these compounds from *Apium graveolens* ethanolic extract. Essential oils bearing plants exhibits potent anticancer properties.[13] A preliminary study showed that the *A. graveolens* ethanolic extract exhibited antiproliferative activity against MCF-7 breast cancer cell lines [Table 2]. Our data demonstrated that in a concentration dependent matter, *A. graveolens* L. ethanolic extract inhibited the viability of MCF-7 cell lines. Anti-proliferative effect as percent inhibition were observed at the concentrations ranging from 10, 1, 0.1, 0.01, 0.001 µg/mL as 61.24%, 51.36%, 12.62%, 8.54%, 2.65% for MCF-7 cell lines. The range of concentrations assayed was 0.001 to 10 µg/mL. Doxorubicin (500 nM) was used as a positive control and the extract exhibited an IC_{50} value of 1 µg/mL for the three treatment

Table 1: **Phytocomponents of *Apium graveolens* seeds ethanolic extract.**

Sl. No.	Retention Time	Retention Index	Area	Mol. Weight	Percent %	Mol. Formula	Name of the Phytocomponent
1.	5.030	3425	7612	542	1.88	$C_{34}H_{54}O_5$	Oleanane-3, 16-diol, 13, 28-epoxy-, diacetate, (3. beta., 16.alpha.)
2.	5.067	2486	5398	342	1.33	$C_{20}H_{22}O_5$	Bacchotricuneatin C
3.	9.225	2620	5316	400	1.31	$C_{28}H_{48}O$	Cyclopropa[7,8]cholestan-3-ol, 3',7-dihydro-, (3.beta.,5.alpha.,7.beta.,8. alpha.)
4.	9.585	2188	8370	326	2.07	$C_{19}H_{34}O_4$	Fumaric acid, decyl 3-pentyl ester
5.	10.184	2665	3633	344	0.90	$C_{19}H_{20}O_6$	Anthra[2,3-d]-1,3-dioxole-5,10-dione, 3a,4 ,11,11a-tetrahydro-9-hydroxy-7-methoxy-2,2,3a-trimethyl-, cis
6.	17.193	3324	9358	484	2.31	$C_{31}H_{48}O_4$	Olean-12-en-28-oic acid, 3.beta.-hydroxy-21-oxo-, methyl ester

Table 2: **Percent inhibition and IC_{50} value of *Apium graveolens* seeds ethanolic extract at different concentrations.**

Sample	Concentration	Inhibition (%) ± SD	IC_{50} (µg/mL)
Apium graveolens seed ethanolic extract	10(g/mL)	61.24 ± 0.58[b]	1 µg/mL
	1(g/mL)	51.36 ± 0.70[b]	
	0.1(g/mL)	12.62 ± 0.34[a]	
	0.01(g/mL)	8.54 ± 1.02[a]	
	0.001(g/mL)	2.65 ± 0.80[a]	
Positive Control	Doxorubicin-500 (nM)	0.2 ± 0.04	

Data expressed as mean ± standard deviation (S.D.) from triplicate determinations.[a]$p<0.01$; [b]$p<0.05$

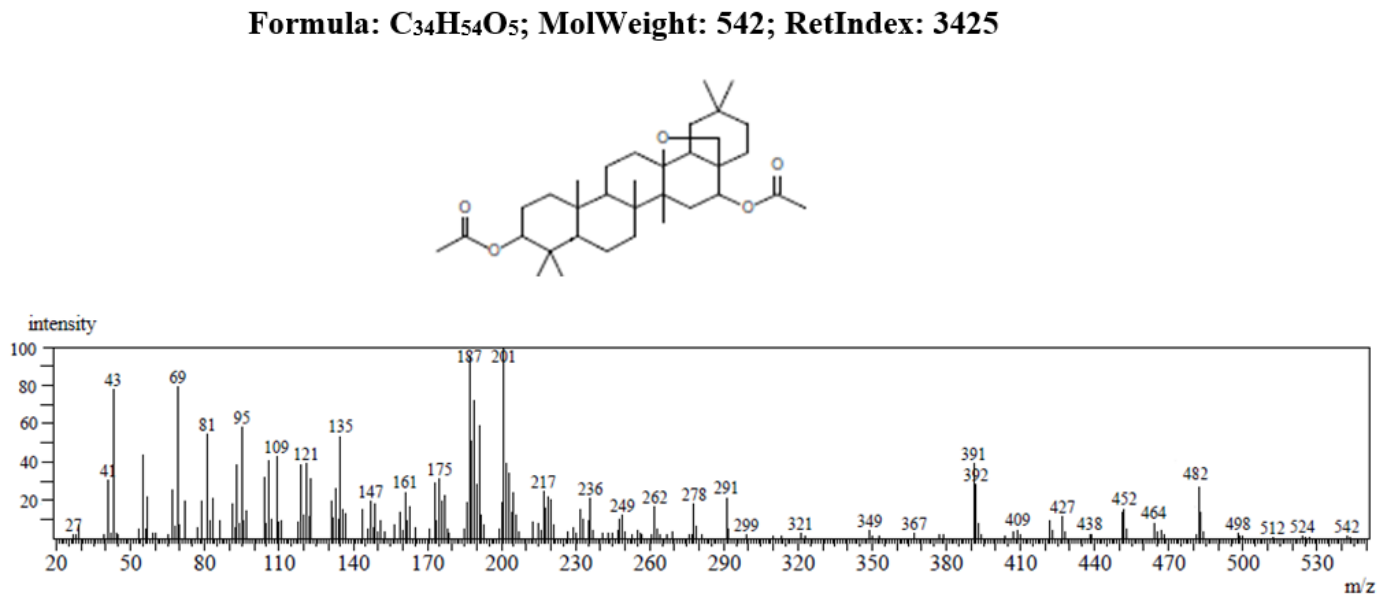

Figure 1: Oleanane-3, 16-diol, 13, 28-epoxy-, diacetate, (3. beta., 16.alpha.).

Formula: $C_{20}H_{22}O_5$; MolWeight: 342; RetIndex: 2486

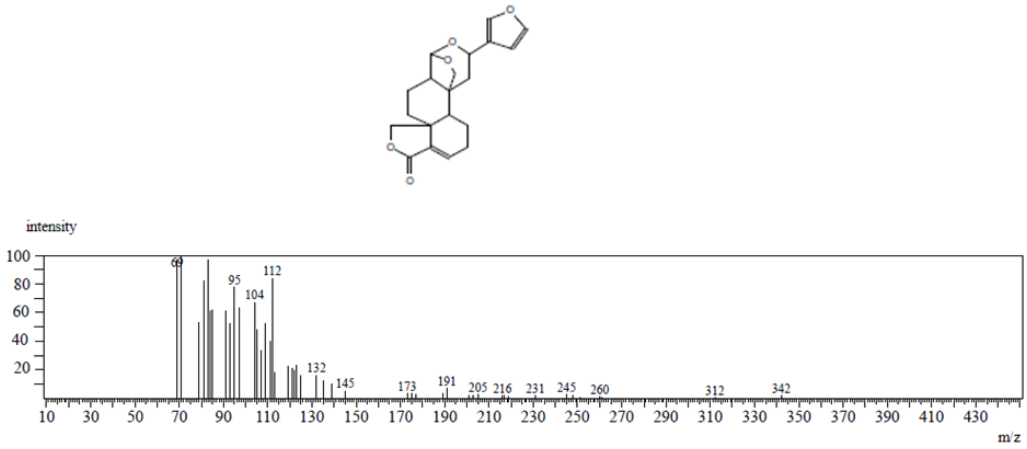

Figure 2: Bacchotricuneatin C.

Formula: $C_{28}H_{48}O$; MolWeight: 400; RetIndex: 2620

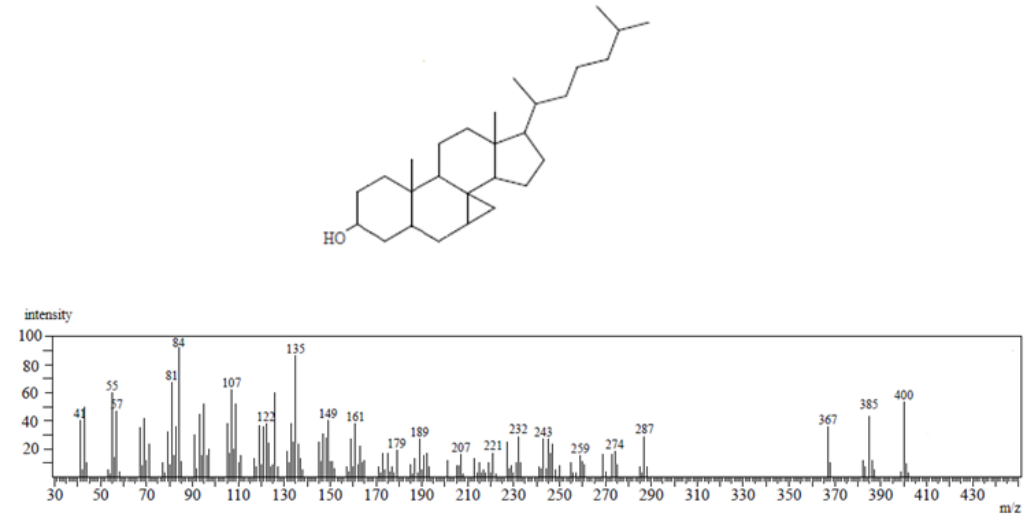

Figure 3: Cyclopropa[7,8]cholestan-3-ol, 3',7-dihydro-,(3.beta.,5.alpha.,7.beta.,8.alpha.).

Formula: $C_{19}H_{34}O_4$; MolWeight: 326; RetIndex: 2188

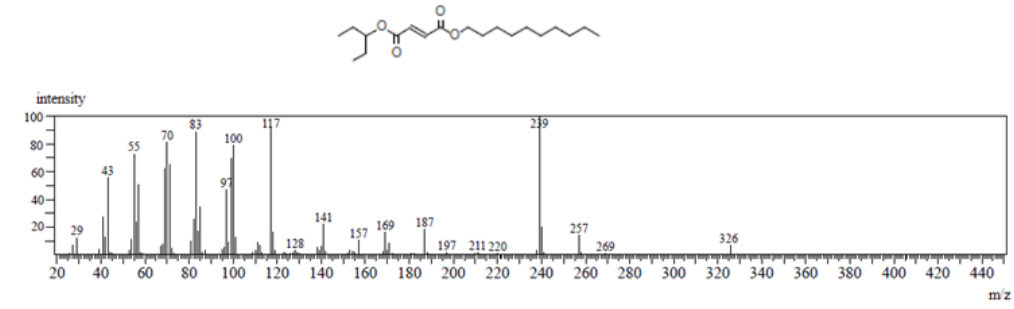

Figure 4: Fumaric acid, decyl 3-pentyl ester.

Formula: $C_{19}H_{20}O_6$; MolWeight: 344; RetIndex: 2665

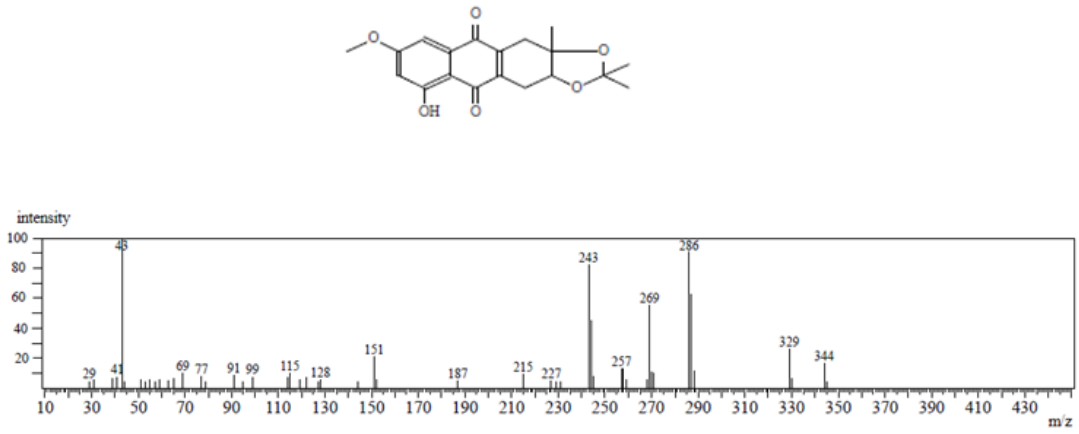

Figure 5: Anthra[2,3-d]-1,3-dioxole-5,10-dione, 3a,4,11,11a-tetrahydro-9-hydroxy-7-methoxy-2,2,3a-trimethyl-, cis.

Formula: $C_{31}H_{48}O_4$; MolWeight: 484; RetIndex: 3324

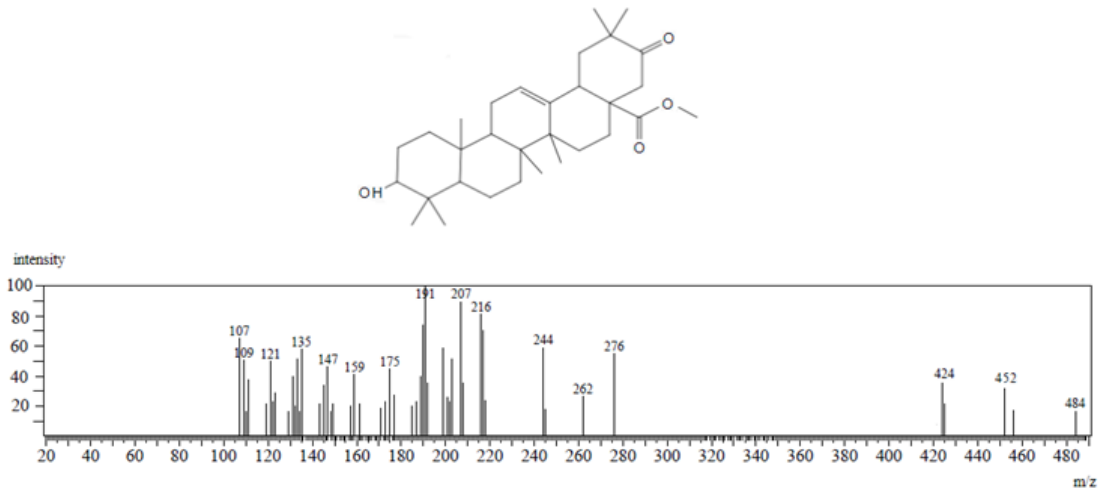

Figure 6: Olean-12-en-28-oic acid, 3.beta.-hydroxy-21-oxo-, methyl ester.

times. This study may stimulate further detailed investigation on the phytochemical and biological properties of the seeds.

CONCLUSION

This is the preliminary report on the phyto-composition of ethanolic extract of seeds of *Apium graveolens*. The result validates the remedial importance of the plant by revealing the presence of diverse bioactive compounds. It is suggested that further research is needed to isolate and elucidate the structure of bioactive moieties that can ensure lead clinical trials for the development of safe and effective drugs based on plant origin. Curing various ailments with minimal side effects is a need of the present scenario.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

A. graveolens: *Apium graveolens*; **MCF-7:** Michigan Cancer Foundation-7; **MTT:** (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide); **GC-MS:** Gas chromatography-Mass spectrometry; **MFP-PARC:** Minor Forest Produce Processing and Research Centre; **EI:** Electron ionization; **NIST:** National Institute of Standards and Technology; **DMSO:** Dimethyl sulfoxide; **IC_{50}:** Half maximal inhibitory concentration.

SUMMARY

The objective of this study was to identify main phytocompounds and to evaluate the *in vitro* anti-proliferative effect of the ethanolic extract of *Apium graveolens* seeds against MCF-7 breast cancer cell lines using the MTT colorimetric assay. Further, the main bioactive components were elucidated by Gas Chromatography–Mass Spectrometry (GC–MS). Commercial mass spectral library was used for the depiction of individual phytocomponents.

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