



Research Paper

Investigation of Bioactive Constituents in the Seeds of Three Herbaceous wild grasses (*Pennisetum purpureum*, *Eleusine Indica* and *Pogonatherum crinitum*) using GC-MS.

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ABSTRACT

Grasses have enjoyed enormous comparative advantage due to their economic and nutritional values. While some are edible and well-studied, most wild species have been scientifically under studied. Matured seeds of *Pennisetum purpureum*, *Eleusine indica* and *Pogonatherum crinitum* obtained from the University of Port Harcourt Botanical garden and identified at the Plant Science and Biotechnology Department of the University of Port Harcourt were dried dehulled and the resultant mesocarp were separately ground into fine smooth particles. Ten grams of each pulverized sample were placed in Whatman no.4 filter paper, sealed and extracted in different Soxhlet extractor using 50 ml of dichloromethane as the solvent. The resultant extracts were concentrated and used for GC-MS analysis. The highest peaks in the chromatogram of the bioactive constituents in *P. purpureum*, *E. indica* and *P. crinitum* extracts were observed at 15.182 mins, 13.136 mins and 16.741 mins respectively. *n*-Hexadecanoic acid ($C_{16}H_{32}O_2$) and Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$) were predominant bioactive constituents in *P. purpureum* and *E. indica* seed extracts with percentage concentrations of 43.552 and 36.803, while 9,12-Octadecadienoic acid (Z,Z)- ($C_{18}H_{32}O_2$) was predominant in *P. crinitum* seed extract with a percentage concentration of 43.397. These bioactive constituents may not only improve the health conditions of animals grazed on these grasses, they may be promising the management of some human pathological conditions when isolated and purified.

KEY WORDS: Bioactive constituents, extraction, seeds, pathological condition, therapeutic properties.

Received 01 Apr., 2023; Revised 09 Apr., 2023; Accepted 11 Apr., 2023 © The author(s) 2023.
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I. INTRODUCTION

The prominence of plants to both human nutrition and management of human health was due to their high concentration of various bioactive compounds such as polyphenols, vitamins, organic acids, terpenes, sesquiterpenes etc. These bioactive compounds work vigorously to enhance the nutritional need and fight against several disease conditions such as cancer, atherosclerosis, diabetes, etc.[1] Considering the economic and nutritional values of foods provided by plants to both humans and animals, no other plant family can be of comparative important as the grasses. Grasses are monocotyledonous plants of the family *Poaceae* (also referred as Gramineae) with about 10,000 species distributed in more than 600 genera.[2]

Grasses are mostly annual plants or herbaceous perennials plants whose leafy parts dies off at the end of every growing season and regenerates at the next season through shoots developed from their underground bud banks (root systems or rhizome) mainly as a result of environmental changes.[3] Grasses grows virtually in all habitat that supports the growth of vascular plants and in most cases, they dominate specific natural vegetations such as prairies and steppes.[3] Some species are grown as agricultural crops, for feeding of both humans and domestic livestock, while others are important sources of forage for herbivorous animals. Species such as maize, wheat, rice, sorghum, barley, and sugar cane are well studied and regarded as most prominent amongst the grasses. However, there are other lesser studied species of grasses, whose seeds may house

important therapeutic bioactive compounds. Notable amongst such grasses are *Pennisetum purpureum*, *Eleusine indica* and *Pogonatherum crinitum*.

P. purpureum (Elephant grass), is a semi-perennial grass widely used as forage because of its short growth period, non-interference with human food produce and low cost of production. It is highly efficient in atmospheric carbon dioxide fixation (via photosynthesis) and has a stronger potential to successfully thrive in adverse environmental conditions.[4] *E. indica* is a short tufted annual or short-lived perennial pantropical grass, whose seeds are mostly considered as noxious.[5] Though its leaves can be used as a forage for livestock[6][7], its seeds and early stage soft vegetative parts are eaten as food and vegetable in famine affected regions.[8] The *P. crinitum* is an obovate-oblong seed producing perennial herbaceous grass species, that grows optimally in full sunny or partial shaded environment with a constantly humid well drained, slightly acidic to slightly alkaline soil.[9] Both in fresh and dry condition, the entire plant has been of use in traditional medicine for the treatment and management of various pathological conditions which are mainly attributed to its anti-inflammatory properties.[9] It is also used as fodder in livestock production. This present study investigates the bioactive constituents in the seeds of *P. purpureum*, *E. indica* and *P. crinitum* and their therapeutic relevance.

II. METHODOLOGY

a. Sample Collection and Preparation and Extraction

Matured seeds of *P. purpureum*, *E. indica* and *P. crinitum* were obtained from the University of Port Harcourt Botanical garden and identified at the Plant Science and Biotechnology Department of the University of Port Harcourt. The seeds were dried separately at room temperature in a neat, well ventilated closet and dehulled. The resultant mesocarp were separately ground into fine smooth particles using a BLG 450 Binatone electric blender and placed in labeled dry sterile universal sample bottles. A quantity of 10g of each pulverized seed were placed in Whatman no.4 filter paper staple-sealed and placed into separate Soxhlet extractor, placed on a dried distillation flask and 50 ml of dichloromethane was introduced into the distillation flask and set up using a retort stand clamp. A continuous jet of cold water was allowed to flow into the condenser and the heated dichloromethane (at 50 °C) was refluxed. The oily extracts were separated from the solvent and concentrated by evaporation at room temperature.

b. Determination of Bioactive Component of *P. purpureum*, *E. indica* and *P. crinitum*

The bioactive components were determined using the procedure described by Ohiri and Bassey [10]. A combined gas chromatograph model HP 6890 and mass spectrometer model 5973 (Agilent Technology) fitted with a capillary column HP-5 MS (5% phenylmethylsiloxane) 30.0 m x 250 µm x 0.25 µm with helium as the carrier gas was used. The initial column temperature was kept at 120°C for 5 minutes and increased at 5°C per minutes to 320°C and held for 5 minutes. A volume of 0.5 ml of each extract was separately diluted with 98% dichloromethane and 2 µl of each diluted sample was automatically injected into Agilent Tech model 5973 mass spectrometer. The compounds present were identified with Chem-Office software attached to the MS library, while the molecular formula, weights and name of the bioactive component were established using the database of National Institute of Standard and Technology.

III. RESULTS

Chromatogram of bioactive constituents of dichloromethane extract of *P. purpureum*, *E. indica* and *P. crinitum* seeds are shown in figure Ia-c. The highest peak in the chromatogram of bioactive constituents in *P. purpureum* extract were observed at 15.182 mins, 16.768 mins, 16.975 mins and 14.622 mins (see fig. Ia), while highest peaks in the chromatogram of bioactive constituents in *E. indica* were observed at 13.136 mins, 14.537 mins, 14.491 mins and 14.720 mins (see fig. Ib). Highest chromatogram peaks at 16.741 mins, 15.076 mins, 16.935 mins and 14.623 mins were observed in the dichloromethane extract of *P. crinitum* (see fig. Ic).

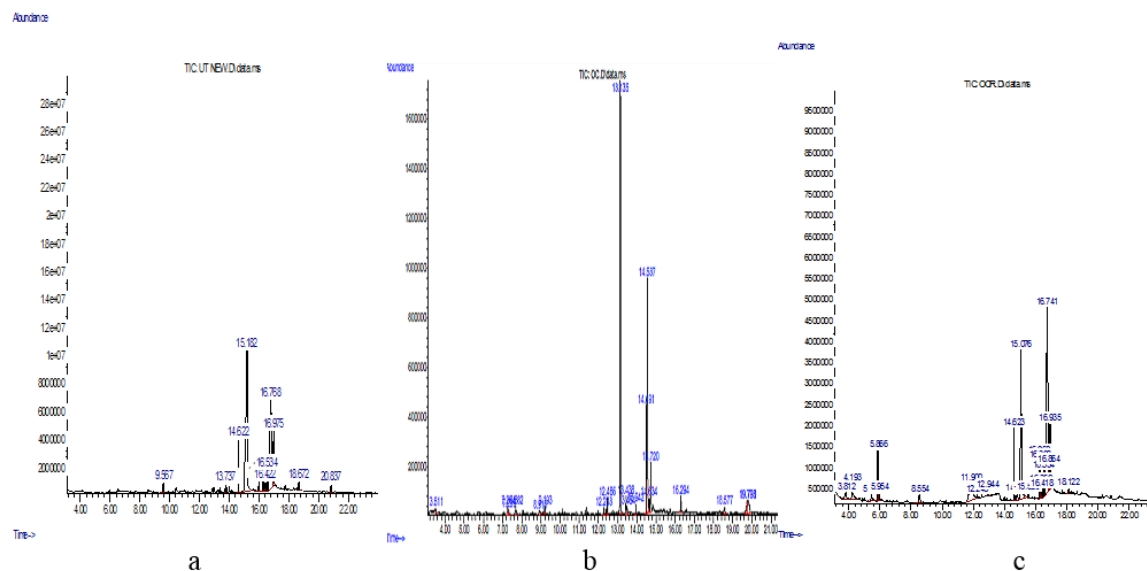


Fig I. Chromatogram of bioactive constituents in the seedsof: (a). *P. purpureum*(b). *E. Indica* and (c). *P. crinitum*

Amongst the 13 bioactive constituents observed in the dichloromethane extract of *P. purpureum* seeds (table I), n-Hexadecanoic acid ($C_{16}H_{32}O_2$) was predominant with a percentage concentration of 43.552, while 9,12-Octadecadienoic acid (Z,Z)- ($C_{18}H_{32}O_2$), Octadecanoic acid ($C_{18}H_{36}O_2$) and Pentadecanoic acid, 14-methyl-, methyl ester ($C_{17}H_{34}O_2$) were also predominant, with percentage concentrations of 33.663, 7.873 and 3.680 respectively (table 1).

Table I: Bioactive constituents in the seeds of *P. purpureum*

S/N	Compound	Retention Time (min)	Percentage concentration	Molecular formula	Molecular weight
1	Phthalimide	9.567	1.422	$C_8H_5NO_2$	147.1308
2	Bicyclo[3.1.1]heptane,2,6,6-trimethyl-, [1R-(1.alpha.,2.alpha.,5.alpha.)]-	13.737	0.526	$C_{10}H_{18}$	138.2499
3	Pentadecanoic acid, 14-methyl-, methyl ester	14.622	3.680	$C_{17}H_{34}O_2$	270.4507
4	n-Hexadecanoic acid	15.182	43.552	$C_{16}H_{32}O_2$	256.4241
5	Heptadecanoic acid	15.981	1.387	$C_{17}H_{34}O_2$	270.4507
6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.248	0.894	$C_{19}H_{34}O_2$	294.4721
7	10-Octadecenoic acid, methyl ester	16.307	2.333	$C_{19}H_{36}O_2$	296.4879
8	7-Oxabicyclo[4.1.0]heptane, 1,5-dimethyl-	16.422	0.793	$C_6H_{10}O$	98.1430
9	Heptadecanoic acid, 15-methyl-, methyl ester	16.534	1.431	$C_{19}H_{38}O_2$	298.5038
10	9,12-Octadecadienoic acid (Z,Z)-	16.768	33.663	$C_{18}H_{32}O_2$	280.4455
11	Octadecanoic acid	16.975	7.873	$C_{18}H_{36}O_2$	284.4772
12	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	18.672	1.106	$C_{16}H_{28}O_3$	268.3917
13	Phthalic acid, 2-ethylhexyl undecyl ester	20.837	1.339	$C_{23}H_{36}O_5$	392.5000

Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$) was the predominant bioactive constituent observed in theseeds of *E. Indica* with a percentage concentration of 36.803. 9-Octadecenoic acid (Z)-, methyl ester ($C_{19}H_{36}O_2$) and delta-Tocopherol ($C_{27}H_{46}O_2$) were also predominant in the caryopses of *E. Indica* with percentage concentrations of 24.073 and a total percentage concentration of 7.217, while Methyl stearate ($C_{19}H_{38}O_2$) had a percentage concentration of 4.437 (table II).

Table II. Bioactive constituents in the seeds of *E. Indica*

S/N	Compound	Retention time (min)	Percentage concentration	Molecular formula	Molecular weight
1	Benzene, 1,2-dichloro-	3.511	1.050	C ₆ H ₄ Cl ₂	147.0020
2	2-Methoxy-4-vinylphenol	7.264	1.020	C ₉ H ₁₀ O ₂	150.1745
3	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadeca methyl-	7.299	0.868	C ₁₆ H ₄₈ O ₇ Si ₈	577.2000
4	Phenol, 2,6-dimethoxy-	7.682	1.403	C ₈ H ₁₀ O ₃	154.1632
5	o-Cyanobenzoic acid	8.918	0.838	C ₈ H ₅ NO ₂	147.1308
6	3,5-Diisopropoxy-1,1,1,7,7,7-hexamethyl-3,5bis(trimethyl siloxy)tetrasiloxane	9.193	1.060	C ₁₈ H ₅₀ O ₇ Si ₆	547.1000
7	Decanoic acid, methyl ester	12.283	0.853	C ₁₁ H ₂₂ O ₂	186.2912
8	2-Pentadecanone, 6,10,14-trimethyl	12.466	1.568	C ₁₈ H ₃₆ O	268.4778
9	Hexadecanoic acid, methyl ester	13.135	36.803	C ₁₇ H ₃₄ O ₂	270.4507
10	n-Hexadecanoic acid	13.438	2.088	C ₁₆ H ₃₂ O ₂	256.4241
11	Phthalic acid, isobutyl 2-methoxyethyl ester	13.484	0.773	C ₁₄ H ₁₆ Cl ₂ O ₄	319.180
12	Hexadecanoic acid, 14-methyl-, methyl ester	13.942	0.999	C ₁₈ H ₃₆ O ₂	284.4772
13	Methyl 10-trans,12-cis-octadecadienoate	14.491	10.195	C ₁₉ H ₃₄ O ₂	294.472
14	9-Octadecenoic acid (Z)-, methyl ester	14.537	24.073	C ₁₉ H ₃₆ O ₂	296.4879
15	1,2-15,16-Diepoxyhexadecane	14.634	1.232	C ₁₆ H ₃₀ O ₂	254.4100
16	Methyl stearate	14.720	4.437	C ₁₉ H ₃₈ O ₂	298.5038
17	Hexadecanoic acid, 15-methyl-, methyl ester	16.294	2.136	C ₁₈ H ₃₆ O ₂	284.4772
18	Docosanoic acid, methyl ester	18.577	1.387	C ₂₃ H ₄₆ O ₂	354.6101
19	.delta.-Tocopherol	19.773	3.368	C ₂₇ H ₄₆ O ₂	402.6500
20	.delta.-Tocopherol	19.790	3.849	C ₂₇ H ₄₆ O ₂	402.6500

P. crinitum had 9,12-Octadecadienoic acid (Z,Z)- (C₁₈H₃₂O₂) as the most predominant amongst the 23 bioactive constituents in its seeds, with a total percentage concentration of 43.397. n-Hexadecanoic acid (C₁₆H₃₂O₂), 2,3-dihydroxycyclohexanone (C₆H₁₀O₃) and Octadecanoic acid (C₁₈H₃₆O₂) were also predominant with percentage concentrations of 18.292, 7.936 and 6.170 respectively (Table III).

Table III. Bioactive constituents in the seeds of *P. crinitum*

S/N	Compound	Retention time (min)	Percentage concentration	Molecular formula	Molecular weight
1	4-Pentyn-1-ol	3.812	1.452	C ₅ H ₈ O	84.1200
2	1-Pentene, 5-methoxy-	4.193	2.544	C ₆ H ₁₄ O	102.1748
3	Oxalic acid, isohexyl pentyl ester	5.474	1.064	C ₁₃ H ₂₄ O ₄	244.3300
4	Naphthalene	5.866	4.167	C ₁₀ H ₈	128.17
5	3(2H)-Oxazolecarboxylic acid, 2-(1, -dimethylethyl)-, methyl ester, (R)-	5.954	0.916	C ₁₆ H ₂₁ NO ₃	275.34
6	Benzene-D6	8.554	1.570	C ₆ D ₆	84.1488
7	2,3-dihydroxycyclohexanone	11.900	7.936	C ₆ H ₁₀ O ₃	130.1400
8	L-Phenylalanine, N-(trifluoroacetyl)-, methyl ester	12.248	0.174	C ₁₂ H ₁₂ F ₃ NO ₃	275.2238
9	Cyclohexane, 1R-acetamido-2,3-cis-epoxy-4-cis-formyloxy-	12.944	0.439	C ₁₁ H ₂₁ NO ₅ Si	243.37

10	Hexadecanoic acid, methyl ester	14.623	3.274	C ₁₇ H ₃₄ O ₂	270.4507
11	1,13-Tetradecadiene	14.820	0.890	C ₁₄ H ₂₆	194.3562
12	n-Hexadecanoic acid	15.076	18.292	C ₁₆ H ₃₂ O ₂	256.4241
13	Z-10-Pentadecen-1-ol	15.290	0.566	C ₁₅ H ₃₀ O	226.3981
14	2-Propenoic acid, 3-(1H-indol-3-yl)-, (E)-	15.526	0.527	C ₁₁ H ₉ NO ₂	187.1900
15	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.250	1.995	C ₁₉ H ₃₄ O ₂	294.4721
16	trans-13-Octadecenoic acid, methyl ester	16.303	1.941	C ₁₉ H ₃₆ O ₂	296.4879
17	9,17-Octadecadienal, (Z)-	16.358	0.931	C ₁₈ H ₃₂ O	264.4461
18	Oxirane, decyl-	16.418	0.550	C ₁₂ H ₂₄ O	184.3184
19	Heptadecanoic acid, 16-methyl-, methyl ester	16.534	1.205	C ₁₉ H ₃₈ O ₂	298.5038
20	9,12-Octadecadienoic acid (Z,Z)-	16.741	41.211	C ₁₈ H ₃₂ O ₂	280.4455
21	9,12-Octadecadienoic acid (Z,Z)-	16.864	1.853	C ₁₈ H ₃₂ O ₂	280.4455
22	Octadecanoic acid	16.935	6.170	C ₁₈ H ₃₆ O ₂	284.4772
23	9,12-Octadecadienoic acid (Z,Z)-	18.122	0.333	C ₁₈ H ₃₂ O ₂	280.4455

IV. DISCUSSION

The bioactive constituents observed in the seeds of these grasses indicates their possible therapeutic potentials. For instance, the high concentration of n-Hexadecanoic acid observed in *P. purpureum* (table I) mainly serves as a protective component to the seeds because of pesticidal, nematicide and insecticidal properties.[11]The nemato-phagocytic potential of n-Hexadecanoic in the seeds of *P. purpureum* may be of paramount importance to the abdominal health of ruminants that ingest these plant's part in the cause forage. n-Hexadecanoic acid has also shown some notable medicinal properties such as cancer preventive, antioxidant, hypochloesterolemic, antiandrogenic, haemolytic, and 5-Alpha reductase inhibitory properties.[12]The competitive inhibitory activity of n-hexadecanoic acid on phospholipase A₂ enzyme [13], is responsible for the traditional use of oils rich in n-hexadecanoic acid for the treatment/management of inflammations attributed to rheumatoid arthritis. [14]

The high concentration of 9,12-Octadecadienoic acid (Z,Z)- in the seeds of *P. purpureum* may be of protective effect to the plant. The release of 9,12-Octadecadienoic acid (Z,Z)- by dead ants, bees and cockroaches has been reported to serve as a repellent signal for others to exit the environment or to discourage them from entering the environment.[15]9,12-Octadecadienoic acid (Z,Z)- is an essential omega-6 fatty acid with strong potential for treatment of hyperlipidemia and atherosclerosis in humans.[16][17]There are prominent scientific evidence that the consumption of 9,12-Octadecadienoic acid (Z,Z)- (linoleic acid) by humans has the potential to reduce the risk of cardiovascular disease and diabetes,[18][19] thereby decreasing their associated mortality rate.[20]Cholesterol and low-density lipoprotein lowering potentials of 9,12-Octadecadienoic acid (Z,Z)- has been reported[21], while the American Heart Association advice on the reduction of cardio-vascular diseases by replacement of saturated fat with 9,12-Octadecadienoic acid.[22]

The high concentration of Hexadecanoic acid, methyl ester in the seeds of *E. Indica*(table II) also revealed potential therapeutic properties imbedded in it. The methyl ester of Hexadecanoic acid not only inhibits cellular phagocytic activity and nitric oxide (NO) production[11], it also causes a reduction in tumor necrosis factor alpha (TNF α), Interleukin 10 (IL-10) and Prostaglandin E₂ (PGE₂) without a reducing effect on ATP levels.[11]A high concentration of 9-Octadecenoic acid (Z)-, methyl ester (methyl oleate) was also observed in the seeds of *E. Indica*. 9-Octadecenoic acid (Z)-, methyl ester shares the same metabolic fate with cis-9-octadecenoic acid (oleic acid). Both compounds are first converted to oleoyl-CoA before being oxidized to acetyl-CoA. The produced acetyl-CoA is acted upon by acetyl-CoA dehydrogenase complex and the resultant citrate is funneled to the citric acid cycle, which tantamount to energy generation through the electron transport chain.[23] Acetyl-CoA can also be used in fatty acid synthesis, while oleoyl-CoA can serve as a precursor for glycerol and diacylglycerol synthesis.[23]Aside from serving as a metabolic precursor for the synthesis of other compounds, Octadecenoic acid (Z)-, methyl ester enhances fatty acid oxidation in the liver, thereby increasing energy production. It also increases ketone production, which also serves as an alternative energy source.[24] Therapeutically, Octadecenoic acid (Z)-, methyl ester has been reported to reduce cholesterol level and also show both anti-inflammatory and antioxidant effects.[25]

Another prominent bioactive constituent in theseed of *E. Indica* isdelta-Tocopherol (C₂₇H₄₆O₂). delta-Tocopherol is a variant of tocopherol, in which the chroman-6-ol core at position 8 is replaced by a methyl group.It is a plant metabolite and a food antioxidant mainly found in soybean oil and maize oil, with the potential of scavenging free radicals and protecting cells from oxidative damage.[26] Jiang *et al.*, [27] reported a synergetic ability of delta-tocopherol and gamma-tocopherol to elicit apoptotic reaction on androgen-sensitive prostate cancer cells within 72 hours of administration. This synergetic effect is induced by interrupting sphingolipid synthesis in the membranes of human prostate cancer cells.[28]

The predominance of 9,12-Octadecadienoic acid (Z,Z)- (C₁₈H₃₂O₂), in the seeds*P. crinitum*(table III) also reveals some therapeutic potentials embedded in this plant. 9,12-Octadecadienoic acid (Z,Z) has been reported to have both anti-arthritic and anti-inflammatory potentials.[29] Its cancer preventive, hepatoprotective and anti-coronary properties has been reported.[25] The high concentration of n-Hexadecanoic acidalso observed in the seeds *P. crinitum*may signify a possible specie relationship with *P. purpureum*. As reported earlier, n-Hexadecanoic acid has a wide spectrum of biochemical and therapeutic propertieswhich ranges from its antibacterial and antifungal properties, [30] to its ability to act directlyon T cells, thereby elicits immuneresponse modulation.[31]Lawrence *et al.*,[32] reported the ability of dietary conjugated n-Hexadecanoic acid to suppressed synthesisof inflammatory mediators such as prostaglandin E₂, IL-6, IL-1β, Nitric oxide and TNFα as a resultant effectof Phospholipase A₂ (PLA₂) inhibition.[13]This indicates that some pathological conditions which are attributed to excessive and uncontrolled production ofinflammatory mediators such as rheumatoid arthritis, bronchial asthma,ulcerative colitis, psoriasis and Crohn’s diseasescan be controlled through consumption of n-Hexadecanoic acid rich diets.

V. CONCLUSION

The presence of notable bioactive constituents of therapeutic relevance observed in theseeds of these grasses were majorly synthesized as either protective compounds or energy store for potential germinating seedlings. However, these bioactive constituents may have a contributive effect on the health of ruminants that regularly graze on both their leaves and seeds. Integration of these seeds into human diets may also help in the control ofpathological conditions associated to unregulated secretion of inflammatory mediators. However, their isolation and purification may be promising in the development of novel pharmaceutical products for treatment of adverse pathological conditions.

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