Review Article

PHYTOPHARMACOLOGICAL PROPERTIES OF AEGLE MARMELOS AS A POTENTIAL MEDICINAL TREE: AN OVERVIEW

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ABSTRACT

Aegle marmelos family rutaceae is highly reputed ayurvedic medicinal tree commonly known as the bael. It is medium sized tree growing throughout the forest of India of altitude 1200 meter. It is found all over India, from sub-Himalayan forest, Bengal, central and south India. The different parts of this plant contain number of coumarins, alkaloids, sterols and essential oils. Various parts of this plant such as leaves, roots, seed, bark and fruit, possess anti-microfilarial, antifungal, analgesic, anti-inflammatory, antipyretic, hypoglycaemic, antidyslipidemic, immunomodulatory, antiproliferative, wound healing, anti-fertility, and insecticidal activity. Various phytopharmacological evaluations have been reported in this literature for the important potential of the Aegle marmelos.

Keywords: Aegle marmelos, Phytochemicals, Pharmacological properties.

INTRODUCTION

History: Bael or Bengal quince is a deciduous sacred tree, associated with Gods having useful medicinal properties, especially as a cooling agent. This tree is popular in 'Shiva' and 'Vishnu' temples and it can be grown in every house. Its leaves are trifoliate symbolizing the 'Thrimurthies'-Brahma, Vishnu, Shiva, with spear shaped leaflets resembling "Thrisoolam" the weapon of Lord Shiva. Many legends, stories and myths are associated with this tree. The leaflets are given to devotees as 'prasadam' in Shiva temples and as 'Tulasi' in Vishnu temples.

Distribution: Bael tree is native to India and is found growing wild in Sub-Himalayan tracts from Jhelum eastwards to West Bengal, in central and south India.

Documented Species Distribution: Native range: India Exotic range: Bangladesh, Egypt, Malaysia, Myanmar, Pakistan, Sri Lanka, Thailand

Local Names: English (bael fruit, Indian bael, holy fruit, golden apple, elephant apple, Bengal quince, Indian quince, stone apple); Burmese (opesheet, ohshit); French (oranger du Malabar, cognassier du Bengale, bel indien); German (Belbaum, Schleimapfelbaum, Baelbaum); Gujarati (bili); Hindi (baelputri, bela, sirphal, siri-phal, kooralam); Indonesian (maja batuh,maja); Javanese (modjo); Khmer (bnau); Lao (Sino-Tibetan) (toum); Malay (bilak,bel, bila,maja pahit); Portuguese (marmelos); Thai (matum, mapin, tum); Vietnamese (trai mam, mbau nau)

Family: Rutaceae

Botanical Description: Aegle marmelos is a slow-growing, medium sized tree, up to 12-15 m tall with short trunk, thick, soft, flaking bark, and spreading, sometimes spiny branches, the lower ones drooping. Young suckers bear many stiff, straight spines. A clear, gummy sap, resembling gum arabic, exudes from wounded branches and hangs down in long strands, becoming gradually solid. It is sweet at first taste and then irritating to the throat. The deciduous, alternate leaves(see fig.1), borne singly or in 2's or 3's, are composed of 3 to 5 oval, pointed, shallowly toothed leaflets, 4-10 cm long, 2-5 cm wide, the terminal one with a long petiole. New foliage is glossy and pinkish-maroon. Mature leaves emit a disagreeable odor when bruised. Fragrant flowers (see fig.2), in clusters of 4 to 7 along the young branch lets, have 4 recurved, fleshy petals, green outside, yellowish inside, and 50 or more Greenish-yellow stamens. The fruits (see fig.3) are round, pyriform, oval, or oblong, 5-20 cm in diameter, may have a thin, hard, woody shell or a more or less soft rind, gray-green until the fruit is fully ripe, when it turns yellowish. It is dotted with aromatic, minute oil glands. Inside, there is a hard central core and 8 to 20 faintly defined triangular segments, with thin, dark-orange walls, filled with aromatic, pale orange, pasty, sweet, resinous, more or less astringent, pulp. Embedded in the pulp are 10 to 15 seeds, flattenedoblong, about 1 cm long, bearing woolly hairs and each enclosed in a sac of adhesive, transparent mucilage that solidifies on drying.¹

Figure 1: Aegle marmelos leaf





Figure 2: Aegle marmelos flowers



Figure 3: Aegle marmelos fruits



Phytochemicals

Bael is reported to contain a number of coumarins, alkaloids, sterols and essential oils. Roots and fruits contain coumarins such as scoparone, scopoletin, umbelliferone, marmesin and skimmin. Fruits, in addition, contain xanthotoxol, imperatorin and alloimperatorin and alkaloids like aegeline and marmeline identified as N-2hydroxy-2-[4 - (3',3'-dimethyl allyloxy) phenyl] ethyl cinnamide. b- sitosterol and its glycoside are also present in the fruits. Roots and stem barks contain a coumarin aegelinol. Roots also contain psoralen, xanthotoxin, 6,7dimethoxy coumarin, tembamide, mermin and skimmianine. Leaves contain the alkaloids - O-(3,3dimethyl allyl)-halfordinol, N-2-ethoxy-2 (4-methoxy phenyl) ethyl cinnamide, N-2-methoxy-2-(4-3',3'-dimethyl allyloxy) phenyl] ethyl cinnamide, N- 2- [4-(3',3'-dimethyl allyloxy) phenyl] ethyl cinnamide, N-2-hydroxy-2-[4-(3',3'dimethyl allyloxy) phenyl] ethyl cinnamide, N-4-methoxy steryl cinnamide and N-2-hydroxy-2-(4- hydroxy phenyl) ethyl cinnamide. Mermesinin, rutin and b-sitosterol - b-Dglucoside are also present in the leaves.² A series of phenylethyl cinnamides, which included new compounds named anhydromarmeline (1), aegelinosides A and B were isolated from Aegle marmelos leaves as alfaglucosidase inhibitors. The structures of new compounds were characterized by spectroscopic data and chemical degradation of compounds isolated, anhydroaegeline (2) revealed the most potent inhibitory effect against alfaglucosidase with IC50 value of 35.8 IM. The present result also supports ethnopharmacological use of A. marmelos as a remedy for diabetes mellitus.³ A rare alkaloid, shahidine (1), having an unstable oxazoline core has been isolated as a major constituent from the fresh leaves of Aegle marmelos. It is moisture-sensitive, and found to be the parent compound of aegeline and other amides; however, it is stable in dimethyl sulfoxide. Its structure was established by spectroscopic analysis. Biogenetically, oxazolines may be considered as the precursor of hydroxy amides and oxazoles found in plants. Shahidine (1) showed activity against a few Gram-positive bacteria.⁴ From dry leaves of Aegle marmelos, four new alkaloids, N-2-[4-(3', 3'-dimethylallyloxy)phenyl] ethyl cinnamide, N-2hydroxy-2-[4-(3',3'-dimethylallyloxy)phenyl] ethyl cinnamide, N-4-methoxystyryl cinnamide and N-2hydroxy-2-(4-hydroxyphenyl) ethyl cinnamide were isolated and characterized. Also isolated were aegeline and a purple compound whose structure has not yet been established.⁵ From the unripe fruits of Aegle marmelos, a new alkaloid named marmeline was isolated and N-2-hydroxy-2-[4-(3',3'identified as dimethylallyloxy)phenyl] ethyl cinnamide. Aegline, imperatorin, alloimperatorin and xanthotoxol were also present.⁶ The purified polysaccharide isolated from the cambium layer of a young bael (Aegle marmelos) tree contains galactose, arabinose, rhamnose, xylose, and glucose in the molar ratios of 10.0:9.8:1.4:1.9:1. Methylation analysis and Smith degradation studies established the linkages of the different monosaccharide residues. The anomeric configurations of the various sugar units were determined by oxidation of the acetylated polysaccharide with chromium (VI) trioxide. The oligosaccharides isolated from the polysaccharide by graded hydrolysis were characterized. The structural significance of these results is discussed.⁷ The crude carbohydrate material isolated from bael (Aegle marmelos) seeds was resolved into four fractions. The homogeneous fraction contained 38.5% of carbohydrate and 60.6% of protein, and its carbohydrate moiety consisted of glucose, galactose, rhamnose, and arabinose in the molar ratios of 40:3:1:2. The linkages among various monosaccharide residues were established through methylation analysis and Smith-degradation studies. The anomeric configurations of the glycosyl groups and the structure at the glycosyl-amino acid iunction were also determined. From the results of these experiments, a partial structure of the glycoprotein has been proposed.⁸ Purified hemicellulose isolated from a young bael (Aegle marmelos) tree with 2.5m sodium hydroxide contained d-xylose and 4-O-methyl-dglucoronic acid in the molar ratio of 7.43:1; traces of glucose, galactose, rhamnose, and arabinose were also present. The linkages between the monosaccharide units were determined by methylation analysis of a hemicellulose fraction (II A) and carboxyl-reduced, hemicellulose II A, and the results were corroborated by those from periodate oxidation and Smith degradation. The anomeric configurations of the d-xylopyranosyl residues were determined by chromium (VI) trioxide oxidation of the acetylated, carboxyl-reduced



hemicellulose, and the aldobiouronic acid obtained from graded hydrolysis was characterized. These experiments clearly revealed the structure of this hemicellulose.⁹ The homogeneous, neutral polysaccharide isolated from the crude polysaccharide of the fruit pulp from bael (Aegle marmelos) contains arabinose, galactose, and glucose in the molar ratios of 2:3:14. The linkages among the different monosaccharide residues were established through methylation analysis and Smith-degradation studies of the polysaccharide. The anomeric configurations of the different glycosyl groups were determined by study of the chromium trioxide oxidation of the acetylated polysaccharide. Results of these experiments have been discussed in order to assess the structure of the neutral polysaccharide.¹⁰ A new 7-[7-(2,6-dihydroxy-7-methoxy-7geranyloxycoumarin methyl-3-octaenyloxy) coumarin] named marmenol (1) has been isolated from the leaves of methanolic extract of Aegle marmelos belonging to the family Rutaceae. In addition to marmenol, several known compounds have also been obtained for the first time from the same source. They include: praealtin D, trans-cinnamic acid, valencic acid, 4-methoxy benzoic acid, betulinic acid, N-pcisand trans-coumaroyltyramine, montanine, and rutaretin. The structures of marmenol and known constituents were established with the help of NMR spectroscopy. However, structure of 1 was further confirmed via 2-D NMR experiments.¹¹ Antifungal 2-isopropenyl-4-methyl-1-oxaconstituents, cyclopenta[b]anthracene-5,10-dione and (+)-4- (20hydroxy-30-methylbut-30-enyloxy)-8H-[1,3]dioxolo[4,5h]chromen-8-one in addition to known compounds imperatorin, b-sitosterol, plumbagin, 1-methyl-2-(30methyl-but-20-enyloxy)-anthraquinone, b-sitosterol glucoside, stigmasterol, vanillin and salicin were isolated during phytochemical investigation on seeds of Aegle marmelos Correa.¹²

Pharmacological properties

Acute and sub-acute toxicity studies: This study was designed to elucidate the toxicity of the widely used plant Aegle marmelos in rats. The total alcoholic, total aqueous, whole aqueous and methanolic extracts isolated from the leaves of A. marmelos and studied their toxic effects. Acute, subacute and LD50 values were determined in experimental rats. The dead animals were obtained from primary screening studies, LD50 value determination experiments and acute studies subjected to postmortem studies. The external appearance of the dead animals, the appearance of the viscera, heart, lungs, stomach, intestine, liver, kidney, spleen and brain were carefully noted and any apparent and significant features or differences from the norm were recorded. Following the chronic administration of A. marmelos for 14 days, the vital organs such as heart, liver, kidney, testis, spleen and brain were carefully evaluated by histopathological studies and any apparent and significant changes or differences from the norm were studied. From the acute administration of A. marmelos, the LD50 values were

determined using graphical method. The hearts stopped in systolic stand-still in the acute experiments. There were no remarkable changes noticed in the histopathological studies after 50 mg/kg body wt of the extracts of A. marmelos when administered intraperitoneally for 14 successively. Pathologically, neither days gross abnormalities nor histopathological changes were observed. After calculation of LD50 values using graphical methods, we found a broad therapeutic window and a high therapeutic index value for A. marmelos extracts. Intraperitoneal administration of the extracts of the leaves of A. marmelos at doses of 50, 70, 90 and 100 mg/kg body wt for 14 consecutive days to male and female Wistar rats did not induce any short-term toxicity. Collectively, these data demonstrate that the extracts of the leaves of A. marmelos have a high margin of drug safety.13

Contractile activity: The effect of the alcoholic extract of the leaves of Aegle marmelos Corr. on guinea pig isolated ileum and tracheal chain was investigated, as this plant is used traditionally to treat asthma and related afflictions. These effects were investigated using the isolated organ bath method. 1 mg/ml and 2 mg/ml doses of the alcoholic extract of this plant produced a positive relaxant effect in isolated guinea pig ileum and tracheal chain, respectively. In addition, they antagonized the contractions, which are produced by histamine. Because the alcoholic extracts elicited the antagonistic effect against histamine and also relaxed the histamine-induced contractions, it can be concluded that relaxations induced by A. marmelos in both guinea pig ileum and tracheal chain were due to the depression of H1- receptors. Since we observed a complete relaxation of the guinea pig ileum and tracheal chain produced by the extract, we investigated its antagonistic effect against histamine. These results were due to the presence of one or more antihistaminic constituents present in the alcoholic extract of this plant, therefore supporting to the traditional use of A. marmelos in asthmatic complaints.¹⁴

Anti-microfilarial activity: Methanolic extract of roots of *vitex negundo* L. and extracts of leaves of *vitex negundo* L. *Ricinus communis* L. and Aegle marmelos corr. were explored for possible antifilarial effect against Brugia malayi microfilariae. It was observed that among the herbal extract, root extract of *vitex negundo* L and leaves extract of Aegle marmelos Corr. At 100 ng/ml concentration showed complete loss of motility of microfilariae after 48 hrs of incubation. Thin layer chromatography of the extracts revealed the presence of alkaloids, saponins and flavonoids in the roots of *vitex negundo* L and coumarin in the leaves of *Aegle marmelos Corr.*¹⁵

Antifungal activity: A new anthraquinone, 1-methyl-2-(3'-methyl-but-2'-enyloxy)-anthraquinone (1) has been isolated from seeds of Aegle marmelos Correa and was characterized on the basis of spectral analysis (UV, IR, 1H NMR, 13C NMR, 2D NMR and mass spectroscopy). The compound exhibited significant antifungal activity against



pathogenic strains of Aspergillus species and Candida albicans in disc diffusion assay (MIC value of 6.25 µg/disc), microbroth dilution and percent spore germination inhibition assays (MIC value of 31.25–62.5 µg/ml).¹⁶ The antifungal activity of essential oil isolated from the leaves of bael (Aegle marmelos (L.) Correa ex Roxb; Rutaceae) has been evaluate using spore germination assay. The oil exhibited variable efficacy against different fungal isolates and 100% inhibition of spore germination of all the fungi tested was observed at 500 ppm. However, the most resistant fungus, Fusarium udum was inhibited 80% at 400ppm. Kinetic studies showed concentration as well as time dependant complex inhibition of spore germination by essential oil.¹⁷

Analgesic: The methanol extract of leaves of *Aegle marmelos* at a dose level of 200 and 300 mg/kg showed significant analgesic activity on acetic acid-induced writhing and tail flick test in mice.¹⁸

Anti-inflammatory, antipyretic and analgesic: The serial extracts of the leaves of Aegle marmelos Corr. were investigated for anti-inflammatory property. The analgesic and antipyretic properties were also evaluated. The most of the extracts derived from the plant Aegle marmelos caused a significant inhibition of the carrageenan-induced paw oedema and cotton-pellet granuloma in rats. The extracts also produced marked analgesic activity by reduction the early and late phases of paw licking in mice. A significant reduction in hyperpyrexia in rats was also produced by the most of the extracts. This study was established anti-inflammatory, antinociceptive and antipyretic activities of the leaves of Aegle marmelos.¹⁹

Hypoglycaemic effects: The hypoglycaemic effect of the water extract of the fruits of Aegle marmelos was examined in streptozotocin-induced diabetic Wistar rats. Oral administration of the water extract (125 and 250 mg kg-1) twice a day for 4 weeks resulted in significant reductions in blood glucose, plasma thiobarbituric acid reactive substances, hydroperoxides, ceruloplasmin and -tocopherol and a significant elevation in plasma reduced glutathione and Vitamin C in diabetic rats. The effect of the extract at a dose of 250 mg kg-1 was more effective than glibenclamide in restoring the values of these parameters. The results of this study clearly showed the hypoglycaemic activity of the fruit extract.²⁰ The aqueous extract of Aegle marmelos seeds was administered orally at different doses (100, 250 and 500 mg/kg) to normal as well as sub (fasting blood glucose (FBG) normal; glucose tolerance abnormal) and mild (FBG 120-250 mg/dl) diabetic rats. The dose of 250 mg/kg was found to be most effective dose and it decreases blood glucose level (BGL) by 35.1% in normal healthy rats after 6 h of administration. The same dose also showed a marked reduction in BGL of 41.2% in sub and 33.2% in mild diabetic rats in glucose tolerance test (GTT) after 2 h. Treatment of severely (FBG >250 mg/dl) diabetic rats for 14 days with a dose of 250 mg/kg reduces the fasting blood glucose by 60.84% and urine sugar by 75% than

their pretreatment levels. It brought about fall in level of total cholesterol (TC) by 25.49% with increase of 33.43% in high density lipoprotein (HDL) and decrease of 53.97 and 45.77% in low density lipoprotein (LDL) and triglyceride (TG), respectively. These results clearly indicate that aqueous seed extract of *Aegle marmelos* possess antidiabetic and hypolipidemic effects in diabetic rats.²¹

Antidyslipidemic activity: From the leaves of A. marmelos an alkaloidal-amide, Aegeline 2, was isolated and found to have antihyperglycemic activity as evidenced by lowering the blood glucose levels by 12.9% and 16.9% at 5 and 24 h, respectively, in sucrose challenged streptozotocin induced diabetic rats (STZ-S) model at the dose of 100 mg/kg body weight. Aegeline 2 has also significantly decreased the plasma triglyceride (Tg) levels by 55% (P < 0.001), total cholesterol (TC) by 24% (P < 0.05), and free fatty acids (FFA) by 24%, accompanied with increase in HDL-C by 28% and HDL-C/TC ratio by 66% in dyslipidemic hamster model at the dose of 50 mg/kg body weight. The reasonable mapping of compound 2 to validated pharmacophoric hypothesis and 3D QSAR model with an estimated activity (283 nM) suggest that the compound 2 might be a b3-AR agonist.²²

Immunomodulatory activity: The aim of the present study was to investigate the immunomodulatory action of methanolic extract of Aegle marmelos fruit (FEAM) in experimental model of immunity. Methods: Cellular immunity was carried out by neutrophil adhesion test and carbon clearance assay, whereas, humoral immunity was analyzed by mice lethality test and indirect haemagglutination assay. FEAM dose was selected by Stair case method (up and down) and administered at 100 and 500 mg/kg orally. The Ocimum sanctum (OSE, 100 mg/kg, p.o) was used as standard. FEAM at 100 and 500 mg/kg produced significant increases in adhesion of neutrophils and an increase in phagocytic index in carbon clearance assay. Both high and low doses of FEAM significantly prevented the mortality induced by bovine Pasteurella multocida in mice. Treatment of animals with FEAM and OSE significantly increased the circulating antibody titre in indirect haemagglunation test. Among the different doses, low one was more effective in cellular immunity models than the high. However, all the doses exhibited similar protection in humoral immunity procedures. From the above findings, it is concluded that FEAM possesses potential for augmenting immune activity by cellular and humoral mediated mechanisms more at low dose (100 mg/kg) than high dose (500 mg/kg).23

Antiproliferative activity: In the present paper we show that extracts from *Aegle marmelos* Correa are able to inhibit the *in vitro* proliferation of human tumor cell lines, including the leukemic K562, T-lymphoid Jurkat, Blymphoid Raji, erythroleukemic HEL, melanoma Colo38, and breast cancer MCF7 and MDAMB- 231 cell lines. Molecules present within the studied *Aegle marmelos* C. extracts were identified by gas-chromatography/mass-

spectrometry analysis; three derivatives (butyl p-tolyl sulfide, 6- methyl-4-chromanone and butylated hydroxyanisole) were found to exhibit strong activity in inhibiting *in vitro* cell growth of human K562 cells. The antiproliferative activity of these compounds was found to be comparable to that of known antitumor agents, including cisplatin, chromomycin, cytosine arabinoside and 5-fluorouracil. In addition, the antiproliferative activity of butyl-p-tolyl sulfide, 6-methyl-4-chromanone and 5-methoxypsolaren was associated to activation of the differentiation pattern of K562 cells.²⁴

Wound healing activity: Effect of topical and intraperitoneal administration of methanolic extract of Aegle marmelos ointment and injection was studied respectively on two types of wound models in rats, the excision and the incision wound model. Both the injection and the ointment of the methanolic extract of Aegle marmelos produced a significant response in both of the wound type tested. In the excision model the extract treated wounds were found to epithelialize faster and the rate of wound contration was higher, as compared to control wounds. The extract facilitated the healing process as evidenced by increase in the tensile strength in the incision model. The results were also comparable to those of a standard drug nitrofurazone.²⁵

Anti-fertility: Fifty percent ethanolic extract from the leaves of A. marmelos was prepared. The effect of A. marmelos leaf extract on the reproductive system of male albino rats was investigated at three different doses, namely, 100, 200 and 300 mg-1 kg -1 day-1 for each rat for 60 days. Recovery was also investigated after a withdrawal of 120 days. All the major accessory sex organs shed weight post administration of the extract. There was a marked reduction in motility and density of the sperm derived from cauda epididymis of the treated animals. A. marmelos reduced fertility of male rats by 100% at the 300-mg dose level. Serum testosterone levels also decreased significantly in all the experimental groups. The protein, glycogen and lipid peroxidation content of the testes was significantly reduced at the highest dose level; a highly significant increase in testicular cholesterol was observed along with a highly significant reduction in the sialic acid contents of testes, epididymis and seminal vesicles. Blood tests did not point to distress in any of the vital organs. Withdrawal of the extract restored all the altered parameters including organ weights, fertility, testosterone levels and tissue biochemistry to control levels after 120 days. The leaf extract of A. marmelos suppresses fertility in male rats. Complete recovery of fertility was observed following the withdrawal of drug. Absence of any deleterious effect on the vital organs points to the safe use of the extract.²⁶

Insecticidal activity: Experiments were carried out to determine the potential of using essential oil from leaves of *Aegle marmelos* to control insect infestation of stored gram from *Callosobruchus chinensis* (L.) (Bruchidae) and wheat from *Rhyzopertha dominica* (F.) (Bostrychidae), *Sitophilus oryzae* (L.) (Curculionidae) and *Tribolium*

castaneum (Herbst) (Tenebrionidae). After introducing the test insects, stored gram and wheat samples were fumigated with essential oil of Aegle marmelos at 500 µg/mL (ppm). The oil significantly enhanced feeding deterrence in insects and reduced the grain damage as well as weight loss in fumigated gram and wheat samples infested with all insects except T. castaneum. The essential oil at different doses significantly reduced oviposition and adult emergence of C. chinensis in treated cowpea seeds. The oil protected stored gram from C. chinensis and wheat from R. dominica and S. oryzae for two years. Limonene (88 %) was found to be the major component in the oil through GC-MS analysis. Regression analysis of data on individuals in treated cowpea confirmed that significant reduction of oviposition and adult emergence of C. chinensis decreased with increase in doses. The findings emphasize the efficacy of A. marmelos oil as fumigant against insect infestations of stored grains and strengthen the possibility of using it as an alternative to synthetic chemicals for preserving stored grains.²⁷

Miscellaneous: The effect of the aqueous, alcoholic and petroleum ether extracts of *A.marmelos* have been studied for t h e hypoglycaemic and other pharmacological actions. The aqueous and alcoholic extracts at 500 mg/kg dose produced hypoglycaemia in normal fasted rabbits, but t h e petroleum ether extract did not. The aqueous extract revealed cardiac stimulant, smooth-muscle relaxant and uterine stimulant properties. The alcoholic extract showed cardiac depressant, smooth muscle relaxant and uterine relaxant properties. Daily administration for six weeks showed necrosis and congestion of liver and kidney, with both the extracts. But more pronounced with the aqueous extract.²⁸

CONCLUSION

Numerous phytochemical and pharmacological studies have been conducted on different parts of *Aegle marmelos*. The present literature supports the potential of *Aegle marmelos* as a medicinal tree. In view of the nature of the plant, more research can be done to investigate the unexplored and unexploited potential of this plant.

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