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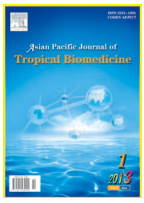

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Anti-bacterial activity and brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables from Bangladesh

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PEER REVIEW

Peer reviewer

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Comments

This is a good paper where authors evaluated the antibacterial activity against 12 different gram positive and gram negative bacteria and brine shrimp lethality bioassay of 14 edible vegetables from Bangladesh.
The results are interesting where it has been found that most of the vegetables are effective against gram negative bacteria rather that gram positive bacteria.
(Details on Page 5)

ABSTRACT

Objective: To investigate the antibacterial and cytotoxic activity of fourteen different edible vegetables methanolic extract from Bangladesh. **Methods:** The antibacterial activity was evaluated using disc diffusion assay method against 12 bacteria (both gram positive and gram negative). The plant extracts were also screened for cytotoxic activity using the brine shrimp lethality bioassay method and the lethal concentrations (LC_{50}) were determined at 95% confidence intervals by analyzing the data on a computer loaded with "Finney Programme". **Results:** All the vegetable extracts showed low to elevated levels of antibacterial activity against most of the tested strains (zone of inhibition=5-28 mm). The most active extract against all bacterial strains was from *Xanthium indicum* which showed remarkable antibacterial activity having the diameter of growth inhibition zone ranging from 12 to 28 mm followed by *Alternanthera sessilis* (zone of inhibition=6-21 mm). All extracts exhibited considerable general toxicity towards brine shrimps. The LC_{50} value of the tested extracts was within the range of 8.447 to 60.323 μg/mL with respect to the positive control (vincristine sulphate) which was 0.91 μg/mL. Among all studied extracts, *Xanthium indicum* displayed the highest cytotoxic effect with LC_{50} value of 8.447 μg/mL. **Conclusions:** The results of the present investigation suggest that most of the studied plants are potentially good source of antibacterial and anticancer agents.

KEYWORDS
Bangladeshi vegetables, Antibacterial, Disc diffusion, Brine shrimp lethality

1. Introduction

Bangladesh is a developing and over populated country where the most of the people are living below the poverty level especially in rural area. Most of the people in Bangladesh are habituated with the local and indigenous food. Vegetables are very common in their everyday food menu and play an important role in the food chain. More than sixty, indigenous and exotic, vegetables are grown in Bangladesh, most of which are very cheap, available and popular to people for daily food intake. The enriched food value as well as medicinal property are still unexplored and may play a pivotal role in the development of potential new drugs for chemotherapy which might help to overcome the growing problem of resistance and also the toxicity of the currently available commercial antibiotics. The traditional medicinal methods, especially the use of vegetable may play a vital role to cover the basic health needs in the

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developing countries like Bangladesh.

In the present study we screened fourteen vegetables for their antibacterial as well as cytotoxic properties. Most of them are reported for their individual as well as combined medicinal properties. *Alternanthera sessilis* was reported for antioxidant activity[1], hepatoprotective activity[2], antimicrobial and wound healing activites[3], antiviral activity[4]. *Amaranthus tricolor* was reported to have cyclooxygenase and human tumour cell growth inhibitory activities[5]. *Benincasa hispida* was found to have antiulcerogenic activity, bronchospasm inhibition activity, antioxidant and angiotensin converting enzyme inhibition capacity, hypoglycemic and antihyperglecemic activity[6-10], *Corchorus olitorius* was found to have antibacterial activity, antioxidant and antidiabetic effects, anti-angiogenic effects, anorectic activity, glucose and lipid lowering activity[10-14]. anticonvulsion and antisteroidogenic activity, cytotoxic and antimalarial activity[15-18] for the source of histamine release inhibitors and phenolic antioxidants[19,20]. *Diplazium esculentum* was found to have antioxidant and cytotoxic activity[21,22].

Enhydra fluctuans was reported for antioxidant, analgesic, antimicrobial activity, and antibacterial activity[23-26]. *Glinus oppositifolius* (L.) was found to contain immunomodulating peptic polymer, terpinoid saponine having antiprotozoal activity, and bioactives having antioxidant property[27-29]. *Ipomoea aquatica* Forsk was reported for hypoglycemic activity, cytotoxic activity[30-32]. *Lagenaria siceraria* was reported for its diuretic activity[33], antihypertensive and cardioprotective effect[34]. *Nymphaea nouchalli* was reported for hypoglecemic antitumor and antimicrobial activity[35-37]. *Portulaca oleracea* Linn was reported for analgesic and anti-inflammatory effects, wound healing activity, neuropharmacological activity[38-40]. *Spinacia oleracea* Linn has protective effect against radiation-induced oxidative stress, antimutagenic activity. *Xanthiom indicum* has antitrypanosomal activity, antitumor and anti-inflammatory activity[41-45].

The plant kingdom comprises many species of plants containing substances of medicinal value, which are yet to be explored. A large number of plants are constantly being screened for their possible medicinal value[46]. In recent years, multiple drug resistance in human pathogenic microorganism has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of various diseases[47]. Therefore, it is of great interest to carry out a screening of these plants in order to unfold the dimension of use as antimicrobial and cytotoxic activity and to reveal the active principle by isolation and characterization of their constituents.

2. Materials and methods

2.1. Drugs and chemicals

Methanol and DMSO (dimethyl sulfooxide) were purchased from Merck, Germany. Kanamycin was collected from Square Pharmaceuticals Ltd., Bangladesh. Vinchristin sulphate was purchased from Cipla Ltd., Goa, India.

2.2. Collection of plant material

The fresh plants were collected during the month of December-January (2007-2008) from different areas of Dhaka city and their identity were authenticated from the Bangladesh National Herbarium, Dhaka where a voucher specimen was deposited having different accession number which is given here in parenthesis: *Alternanthera sessilis* Linn (DACB-32915), *Amaranthus tricolor* (DACB-34186), *Benincasa hispida* (DACB-34185), *Corchorus olitorius* (DACB-34187), *Diplazium esculentum* (DACB-34181), *Enhydra Fluctuans* (DACB-32910), *Glinus oppositifolius (*DACB-34183), *Ipomoea aquatica/Ipomoea alba* (DACB-32911 *Lagenaria siceraria* (DACB-34188), *Nymphaea nouchalli (*DACB-32912), *Portulaca oleracea* Linn (DACB-34182), *Spinacia oleracea* (DACB-32914), *Xanthium indicum* (DACB-34184). Leaves of *Corchorus olitorius* and whole herb for other plants were used for the experiments.

2.3 Extraction of plant materials

The whole plants were firstly sun dried for 5 d and then finally dried in an oven at 40-45 °C for 72 h. The dried plant parts were ground into coarse powders. About 100 grams of the powdered materials of the each plant part were extracted separately with Soxhlet Extractor for 2 d. Then each of the extracts were filtered using cotton and filter paper. The clear filtrates were then evaporated to dryness under vacuum in a rotary evaporator.

2.4. Determination of the antibacterial activity

2.4.1. Antibacterial assay

Twelve pathogenic bacterial strains were used as the test organisms for antibacterial screening of the dried extract, of which four gram were +ve and eight gram -ve. The antibacterial activity of the plant extracts against the test organisms was determined by disc diffusion method using Kanamycin disc 30 μg/disc as standard for comparison[48,49]. Disc diffusion methods are extensively used to investigate the antibacterial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoirs containing solutions of substances to be examined. In the case of solutions with a low activity, however, a large concentration or volume is needed. The limited capacity of discs means that holes or cylinders are preferably used[50].

After placing the paper discs bearing the sample, the plates were incubated at (35±2) °C for 2 d and the zones of inhibition were determined. Clear inhibition zones around the discs indicated the presence of antibacterial activity.

2.4.2. Collection of microrganisms

The bacterial species used in the present study were *Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus, Sarcina lutea, Salmonella paratyphi, Salmonella typhi, Vibrio parahemolyticus, Vibrio mimicus, Escherichia coli, Shigella dysenteriae, Shigella boydii* and *Pseudomonas aeruginosa*. These were collected as pure cultures from the Institute of Nutrition and Food Sciences, Dhaka University and International Center for Diarrheal Disease and Research, Bangladesh Dhaka, Bangladesh.

2.5. Determination of cytotoxicity

2.5.1. Brine shrimps

Brine shrimp, *Artemia salina* Leach, also known as sea monkeys, are marine invertebrates about 1 mm in size. Freeze-dried cysts were readily available at aquarium stores. The cysts lasted for several years and could be hatched without special equipment[51].

2.5.2. Brine shrimp lethality bioassay

The cytotoxic activity of the plant was evaluated using Brine shrimp lethality bioassay method where 6 graded doses (*viz* 200 μg/mL, 100 μg/mL, 50 μg/mL, 20 μg/mL, 10 μg/mL, and 5 μg/mL) were used[52]. Brine shrimps (*Artemia salina* Leach) nauplii (Ocean 90, USA) were used as test organisms. For hatching, eggs were kept in brine with a constant oxygen supply for 48 h. The mature nauplii were then used in the experiment. DMSO was used as a solvent and also as a negative control. Vincristine sulfate was used as a reference standard in this case. The numbers of survivors were counted after 24 h. Larvae were considered dead if they did not exhibit any internal or external movement during several seconds of observation. The larvae did not receive food. To ensure that the mortality observed in the bioassay could be attributed to bioactive compounds and not to starvation; we compared the dead larvae in each treatment to the dead larvae in the control.

2.6. Statistical analysis

The median lethal concentration (LC_{50}) and 95% confidence intervals of the test samples were calculated using the probit analysis method described by Finney[53], as the measure of toxicity of the plant extract.

3. Results

In the present study, methanolic extracts of fourteen vegetables were screened for their antibacterial activity by disc diffusion method using dried extracts of 500 μg/disc against 12 pathogenic bacterial species of both gram positive and gram negative organisms and the results were compared with the standard Kanamycin (30 μg/disc). The gram negative species were *Vibrio parahemolyticus, Pseudomonas sureus, Salmonella typhi, Salmonella paratyphi, Shigella dysenteriae, Shigella boydii, Vibrio mimicus* and *Escherichia coli*, gram positive species were *Sarcina lutea, Bacillus megaterium, Staphylococcus aureus* and *Bacillus subtilis*. Among all the extracts, *Xanthium indicum* and *Enhydra fluctuans* were active against all of the tested bacteria. On the other hand, *Ipomoea aquatica, Chenopodium album, Alternanthera sessilis, Portulaca grandifolia* and *Lagenaria siceraria* were active against most of the tested bacteria. The rest of the plants showed no or little antibacterial activities against the tested bacteria. Among the extracts, the largest spectrum of sensitivity was found in *Xanthium indicum* extract where higher potential of sensitivity were found against *Pseudomonas aeruginosa* and it was recorded as 28 mm (Table 1).

Xanthium indicum also showed potent inhibitory activity against other tested microorganisms with the zone of inhibition of 13-20 mm. On the other hand the weakest inhibitory activity was found against *Vibrio mimicus* by *Corchorus olitoruis* (inhibition zone diameter 5 mm). Furthermore the growth of *Salmonella paratyphi* (21 mm), *Salmonella typi* (20 mm), *Escherichia coli* (16 mm) and *Sarcina lutea* (13 mm) were strongly inhibited by *Alternanthera sessilis* (Table 1).

The LC_{50} values of the brine shrimp lethality bioassay obtained for these extracts and that of the positive control, Vincristine sulphate, have been presented in Table 2 and Table 3 respectively. All extracts exhibited significant toxicity towards brine shrimps. The LC_{50} values of the plant extracts were within the range of 8.447 to 60.323 μg/mL, whereas that of the positive control (vincristine sulphate) was 0.91 μg/mL, LC_{90}=6.523, 95% IC=0.46-1.79. So these extracts can be considered as a promising candidate for a plant derived anticancer compound. Among the extracts *Xanthium indicum* showed more cytotoxic effects with LC_{50} value 8.447 μg/mL whereas *Spinacia oleracea* was the least toxic among the tested vegetables having LC_{50} value 60.323 μg/mL.

Table 1
Antibacterial activity of fourteen different edible vegetables from Bangladesh.

Plants	Zone of inhibition (mm)											
	Gram negative bacteria									Gram positive bacteria		
	E. coli	*P. aeruginosa*	*S. typhi*	*S. paratyphi*	*S. dysenteriae*	*S. boydii*	*V. mimicus*	*V. parahemolyticus*	*B. subtilis*	*B. megaterium*	*S. aureus*	*S. lutea*
Enhydra fluctuans	6	6	6	8	6	6	6	6	6	6	6	6
Ipomoea aquatica / Alba	8	12	8	8	9	12	7	10	7	10	–	7
Nymphaea nouchalli	–	–	–	–	–	–	6	6	–	–	–	–
Chenopodium album	6	–	11	10	6	10	6	6	6	–	10	–
Spinacia oleracea	–	6	–	–	–	6	6	6	9	6	–	–
Alternanthera sessilis	16	6	20	21	–	6	–	6	7	6	6	13
Diplazium esculcutum	7	–	–	10	6	6	–	10	–	6	–	–
Portulaca grandifolia	11	9	–	9	–	–	6	–	–	9	8	9
Gilinus oppositifolius	6	8	6	7	–	–	–	–	–	–	6	6
Xanthium indicum	20	28	20	15	15	13	15	14	12	18	17	20
Benincasa hispida	–	6	–	–	–	–	–	6	–	–	–	–
Amaranthus tricolor	6	–	–	–	–	6	–	6	–	–	–	–
Corchorus olitorius	–	–	–	–	–	6	5	–	–	–	–	–
Lagenaria siceraria	6	–	–	7	6	6	6	10	6	8	–	–
Kanamycin	30	33	30	30	28	35	32	37	30	32	30	33

–: No inhibition zone (resistant). *E. coli: Escherichia coli; P. aeruginosa: Pseudomonas aeruginosa; S. typhi: Salmonella typhi; S. paratyphi: Salmonella paratyphi; S. dysenteriae: Shigella dysenteriae; S. boydii: Shigella boydii; V. mimicus: Vibrio mimicus; V. parahemolyticus: Vibrio parahaemolyticus; B. subtilis: Bacillus subtilis; B. megaterium: Bacillus megaterium; S. aureus: Staphylococcus aureus; S. lutea: Sarcina Lutea.*

Table 2
Cytotoxic activity of methanolic extract of fourteen different edible vegetables from Bangladesh on brine shrimp nauplii.

Tested material	Concentration tested (μg/mL)	Probit	LC_{50} (μg/mL)	LC_{90} (μg/mL)	95% confidence interval
Alternanthera sessilis	5, 10, 20	4.48, 4.75, 5.00	19.825	601.458	5.418-76.339
Amaranthus tricolor	5, 10, 20, 50, 100	4.16, 4.48, 5.00, 5.25, 5.52	28.319	467.834	12.003-66.811
Benincasa hispida	5, 10, 20, 50	4.16, 4.48, 4.75, 5.00	45.187	1522.153	13.563-150.541
Chenopodium album	5, 10, 20	4.48, 5.00, 5.52	10.000	55.080	5.096-19.623
Corchorus olitorius	5, 10, 20, 50	4.16, 4.48, 5.00, 5.25	26.254	352.338	10.797-63.839
Diplazium esculentum	5, 10, 20, 50, 100	4.48, 5.00, 5.00, 5.25, 5.52	18.561	1240.301	5.129-67.160
Enhydra fluctuans	5, 10, 20, 50	4.48, 5.00, 5.52,5.84	10.522	89.646	5.055-21.900
Glinus oppositifolius	5, 10, 20, 50	4.16, 4.48, 4.48, 5.52	27.650	279.769	12.525-61.039
Ipomoea aquatica / Alba	5, 10, 20, 50	3.72, 4.16, 4.75, 5.25	32.668	215.645	15.498-68.857
Lagenaria siceraria	5, 10, 20, 50	4.16, 4.48, 5.00, 5.00	35.835	962.421	11.623-110.481
Nymphaea nouchalli	5, 10, 20, 50, 100	4.48, 5.00, 5.00, 5.00, 5.52	21.864	2550.281	5.095-93.818
Portulaca grandifolia	5, 10, 20, 50	4.48, 5.00, 5.25, 5.84	11.647	109.832	5.405-25.100
Spinacia oleracea	5, 10, 20, 50	4.16, 4.48, 4.48, 5.00	60.323	2693.980	16.441-221.325
Xanthium indicum	5, 10, 20, 50	4.75, 5.00, 5.52, 6.28	8.447	55.720	4.008-17.800

Table 3
Cytotoxicity of Vincristine sulphate on brine shrimp nauplii.

Concentration (μg/mL)	Probit
0.000	-
0.060	0
0.125	3.77
0.250	4.23
0.500	4.56
1.000	4.85
5.000	6.23
10.000	8.09

4. Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay[54]. It seems very likely, therefore, that the antibacterial compounds extracted from *Xanthium indicum* may inhibit bacteria by a different mechanism than that of currently used antibiotics and may have therapeutic value as an antibacterial agent against multi-drug resistant bacterial strains[55]. Because infections caused by *Pseudomonas aeruginosa*, especially those with multi-drug resistance, are among the most difficult to treat with conventional antibiotics. From the results obtained, it appears that the antibacterial action of the extracts is more pronounced on gram-negative than on gram-positive bacteria in most cases or is even equal. These findings do not correlate with the observations of previous screenings of medicinal plants for antimicrobial activity, where most of the active plant extracts showed activity against Gram-positive strains only[56-59]. Because Gram-negative organisms were reported to be less susceptible to the action of antibacterials, since they possess an outer membrane surrounding the cell wall, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering[60-67]. Besides the difference in sensitivity might be ascribed to the difference in morphological constitutions between Gram-positive and Gram-negative organisms. Many plant species present inhibition zones of differing diameters; however, size difference of the inhibition zone depends primarily upon many factors for *e.g.* diffusion capacity of substances (present in the extracts) in the medium, antimicrobial activity of diffused substances, growth and metabolic activity of microorganisms in the medium[68]. However demonstration of antimicrobial activity against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds[69,70]. But the mechanisms behind the antibacterial activity are complex to understand and could be attributed to either inhibiting the cell division or to damaging the cell walls of bacteria; which however requires to be investigated in detail[71].

It was found that different parts of various plant extracts in our investigation were studied earlier and exhibited antimicrobial activity to some different extent. For instance, it was found that while *Alternanthera sessilis* whole plant was moderately active in *Escherichia coli* in our studies, but in an earlier report [3] the methanol extracts of leaves were more potential to *Escherichia coli* with no other activity to other organisms. Again *Corchorus olitorius* showed no activity except *Shigella boydi* and *Vibrio mimicus* while the methanol extracts of seeds were found to have potent antibacterial activity against many gram positive and gram negative bacteria. Likewise *Nymphaea nouchalli* showed no activity except *Vibrio mimicus* and *Vibrio parahemolyticus* whereas flowers and leaves of *Nymphea nouchalli* were found considerable antibacterial activity against *Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus* and *Escherichia coli*[37,72]. *Enhydra fluctuans* showed little activity in our studies which is in accordance with a previous study[25], while significant antibacterial activities were found in another report[26]. Furthermore, *Portulaca oleracea* Linn showed little or no activity against most of the tested bacteria while it was found high antimicrobial activities against *Helicobater pylori, Staphylococcus epidermidis, Staphylococcus aureus*, *Eschericia coli* and *Streptococcus mutans* in another studies[73].

The brine shrimp lethality bioassay (BSLB) has been used extensively in the primary screening of the crude extracts as well as the isolated compounds to evaluate

the toxicity towards brine shrimps, which could also provide an indication of possible cytotoxic properties of the test materials[52]. Because BLSB is the simple method useful for screening large number of extracts in the drug discovery process. The method allows the use of smaller quantity of the extracts and permits larger number of samples and dilutions within shorter time than using the original test vials[74]. Furthermore it has been established that the cytotoxic compounds generally exhibit significant activity in the BSLB, and this assay can be recommended as a guide for the detection of antitumour and pesticidal compounds because of its simplicity and low cost[75]. This bioassay has also a good correlation with the human solid tumour cell lines. The inhibitory effect of the extract might be due to the toxic compounds present in the active fraction that possess ovicidal and larvicidal properties. The metabolites either affected the embryonic development or slay the eggs. Therefore the cytotoxic effects of the plant extracts enunciate that it can be selected for further cell line assay because there is a correlation between cytotoxicity and activity against the brine shrimp nauplii using extracts[76]. Hence the present study supports BLSB as a reliable method for the assessment of bioactivity of Bangladeshi vegetables and lends support for their use in pharmacology. It was found that no investigation has been carried out for cytotoxic activities till now among our studied fourteen vegetables except *Diaplazium esculentum* and *Ipomoea aquatica*. In the present study, *Ipomoea aquatica* showed strong cytotoxic activity which is in accordance with the result obtained earlier[32]. On the other hand *Diaplazium esculentum* also displayed potent cytotoxic potential in our observations but in another report it was found to have no cytotoxic activity[77].

The *in vitro* cytotoxicity displayed by the plant extracts tested is an initial indicator of *in vivo* antitumour activity. However since a wide range of phytocompounds are capable of exhibiting nonspecific cytotoxicity, plant extracts with significant cytotoxic activity should be further assayed using animal models to confirm antitumour activity, and/or a battery of various cell lines to detect specific cytotoxicity. This step is necessary to eliminate cytotoxic compounds with little value for further investigation as anticancer agents[4].

The results obtained in the present study demonstrated that a number of Bangladeshi vegetables have promising antibacterial activity against tested microorganisms which can be used in the healing of various infectious diseases caused by resistant microorganisms. Especially there is a likelihood to obtain better therapeutic agent against microbial diseases from some of the studied plants such as *Xanthium indicum*, *Enhydra fluctuans*, *Ipomoea aquatica*, *Chenopodium album*, *Alternanthera sessilis*, *Portulaca grandifolia* and *Lagenaria siceraria*. Besides all the plant extracts showed moderate to potent cytotoxic activity as well which could serve for further ethnobotanical and phytochemical research to find the possible relationship between brine shrimp lethality and plant bioactivity. It is therefore recommended to explore and be studied in detail the nature and extent of active principles in each plant extract.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

In recent years, multiple drug resistance in human pathogenic microorganism has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of various diseases. Therefore, it is of great interest to carry out a screening of these plants in order to unfold the dimension of use as antimicrobial and cytotoxic activity.

Research frontiers

This study is on the edible vegetables of Bangladesh to explore antimicrobial and cytotoxic effects. These types of studies on plants are available but studies on edible vegetables of a particular ethnicity are few.

Related reports

From the results obtained, it appears that the antibacterial action of the extracts is more pronounced on Gram-negative than on Gram-positive bacteria in most cases or is even equal. These findings do not correlate with the observations of previous screenings of medicinal plants for antimicrobial activity, where most of the active plant extracts showed activity against Gram-positive strains only [56-59].

Innovations and breakthroughs

Data regarding the antibacterial and cytotoxic activity of edible vegetables is few. This study revealed that edible vegetables are effective against bacteria particularly gram negative bacteria.

Applications

The results from this study can be used for isolation and characterization of new antibacterial and cytotoxic agents. As the vegetables are edible and consumed by an ethnic group for a long period of time without any noticeable unwanted effects, the isolated compounds may be safe as well.

Peer review

This is a good paper where authors evaluated the antibacterial activity against 12 different gram positive and gram negative bacteria and brine shrimp lethality bioassay of 14 edible vegetables from Bangladesh.
The results are interesting where it has been found that most of the vegetables are effective against gram negative bacteria rather that gram positive bacteria.

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