PHYTOCHEMISTRY AND IN VITRO ANTIPLASMODIAL PROPERTIES OF AQUEOUS AND ETHANOL STEM BARK EXTRACTS OF Jatropha curcas (Physic Nut)

Abdulahi A Imam¹, Amina Shehu², Ibrahim A Khalil³ and Hadiza Abdullahi²

¹Department of Biochemistry, Faculty of Bio-medical Sciences, Bayero University, Kano
P.M.B3011 Kano-Nigeria

² Department of Biochemistry, Faculty of Basic Medical Sciences, Northwest University, Kano

³ Department of Biochemistry, Faculty of Science, Federal University, Dutse, Jigawa State-Nigeria

*Corresponding author: aaimam.bch@buk.edu.ng

ABSTRACT

The increasing prevalence and distribution of malaria is due to a number of factors, one of which is the emergence and spread of drug resistant parasites. Efforts are now being directed towards the discovery and development of new chemically diverse antimalarial agents. The present study reports on the phytochemistry and in vitro antiplasmodial properties of aqueous and ethanol stem bark extract of Jatropha curcas. Maceration method of extraction was used. The two extracts were assayed at various concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml and 1.25 mg/ml for antiplasmodial activity against Plasmodium falciparum and were also screened for the presence of phytochemicals following standard procedure. The phytochemicals detected from both extracts were alkaloids, cardiac glycosides, antheranol glycosides, saponins, phytosterols, phenols, flavonoids, protein and amino acids and diterpenes. The result of antiplasmodial activity revealed that both the aqueous and ethanol stem bark extracts of Jatropha curcas were effective against the malaria parasite. However, the aqueous extract showed statistically (P<0.05) higher activities than the ethanol extract. At extract concentration of 10mg/ml, both the ethanol and aqueous extracts produced the highest parasite clearance rate after 72 hours incubation period, with percentage elimination of 77%. From these observations, Jatropha curcas is likely to contain promising chemical compounds which can be utilized as an effective plant-based chemical therapy for the treatment of malaria.

Keywords: Antiplasmodial, Jatropha curcas, Phytochemistry, Plasmodium falciparum,

INTRODUCTION

Plants extracts used as traditional medicine continues to provide for over 80% of the world’s population health coverage, especially in the developing world (Igbinosa et al., 2009). Phytochemicals (plant derived compounds) are natural alternatives to synthetic compounds which have been attracting much interest. The treatment of various diseases are carried out by using extracts of plants and this forms the basis for all traditional systems of medicine (Mbakwem-Aniebo et al., 2012).

The word Jatropha is derived from a Greek word Jatrus meaning Doctors and Trope which means Nutritional food (Aina et al., 2016). Jatropha curcas or physic nut is a non-edible multipurpose shrub belonging to the family Euphorbiaceae. It is an uncultivated non-food wild-species which grows in tropical and sub-tropical regions of the world (Lasalita-Zapico et al., 2012). Different parts of the plant have been reported to contain phenolics, flavonoids, saponins, glycosides, tannins and alkaloids. And these phytochemicals are said to have potentials as antimicrobial, anti-inflammatory, anticancer and antioxidant principles (Oloyede et al., 2012).

The extracts from different parts such as root, stem bark and leaves of J. curcas plant have been used in ethnomedicine (Aina et al., 2016). Stems of young leaves have been used to successfully treat urinary infections. The roots are used to make an antidote for snake bites, decoction as mouth wash for bleeding gums and tooth ache as well as for eczema, scabies and ringworm. Tea from the bark of J. curcas is given to people with leprosy and rheumatism. The seeds contain between 27% to 40% oil which can be processed to produce a high-quality biodiesel fuel usable in a standard diesel engine (Agbogidi et al., 2013).

Malaria is one of the most serious pathogenic diseases in endemic areas of the world, particularly in Africa, Asia, and Latin America. In Africa alone, it is estimated that more than 300 million people are infected annually by the parasite, Plasmodium falciparum, and over one million deaths have been recorded in children less than five years (Omoregie and Sisodia, 2012). Malaria control efforts are having a positive impact, thus contributing to the declining global prevalence of malaria in recent years (Chinsembu, 2015).

Control of malaria is still one of the world’s chief current public health challenges, particularly in sub-Saharan Africa where it is still responsible for 10% of the total disease burden (Bornman et al., 2012). Malaria is estimated to cause between 300 and 500 million clinical cases with about 700,000 to 1.6 million deaths annually and most of these deaths occur in sub-Saharan Africa (Ahorlu and Koram, 2012).
In Nigeria, malaria accounts for 60% of outpatient visits, 30% hospitalization, and is estimated to be responsible for about 11% of overall maternal mortality, 25% of infant mortality, and 30% of under-five mortality. The disease is particularly virulent among the under-five years of age and pregnant women, due to their low levels of immunity (Ebong et al., 2012).

Although the burden of malaria is decreasing, parasite resistance to current antimalarial drugs threaten the prospects of malaria elimination in endemic areas (Chinsembu, 2015). In Africa, the use of indigenous plants plays an important role in the traditional methods of malaria treatment by providing good sources for the detection of novel antiparasitoid compounds (Omoregie and Sisodia, 2012). Recent research on the leaves of J. curcas has revealed that the aqueous and methanolic extracts following phytochemical detection and by antiparasitoid sensitivity test were highly effective against the malaria parasite. Thus J. curcas could serve as an effective chemotherapy for the treatment of malaria (Imam et al., 2016). Previous studies have shown efficacy of J. curcas against most vector-borne diseases (Ohimain et al., 2014). It has been reported that different parts of this biodiesel producing plant including the leaves, the oil, sap, stem bark and root have numerous health benefits including skin infections, antidote for snake bites, leprosy and rheumatism, treatment of tooth ache and muscular pains, pile, fever, jaundice, gonorrhea, constipation, heart burn and as purgative as well as contraceptive principle (Agbogidi et al., 2013). It is also maintained that J. curcas has antimalarial, insecticidal, anticancerous and anti-tumour properties (Agbogidi et al., 2013). Development of resistance to almost all known antimalarial drugs including the once highly effective ACT-based therapies has necessitated the need for new compounds for malaria management and since plants has always provided promising and effective therapeutic compounds in the past, it becomes necessary to screen and study many plant species for novel and more effective therapy for malaria (Imam et al., 2016), hence this study.

**MATERIALS AND METHODS**

**Collection of Plant Materials**

*Jatropha curcas* sample was collected from Ringim Local Government Area, Jigawa state in July, 2016 during the raining season. The plant was identified by botanists at the herbarium section of the Department of Biological Sciences, Bayero University Kano, where an Accession Number BUKHAN 0060 was assigned to it.

**Preparation and Processing of Plant Extracts**

The stem barks of *J. curcas* were collected and air dried under shade at room temperature in the Department of Biochemistry, Bayero University Kano, Nigeria for 16 days. The extraction method used was maceration. The samples were ground into coarse powder using motor and pestle and stored in an air tight bottle prior to analysis. After weighing 170g of the ground sample of the stem bark, it was dissolved in 1100 mL of aqueous solution in an air tight bottle. 150g of the sample was also weighed and dissolved in 1000 mL ethanol solution in an air tight bottle. Both were shaken and kept for 48 h. The extracts were filtered using a cheese cloth and Whatman filter paper No. 1 (24 cm), to obtain filtrates of the respective solvents of aqueous and ethanol. The aqueous extract was concentrated to dryness using a freeze drier while the ethanol extract was concentrated in a rotary evaporator and allowed to dry at ambient temperature (Veeramuthu et al., 2006).

**Phytochemical Screening and Quantification**

Qualitative phytochemical analysis was carried out to identify plant secondary metabolites such as alkaloids, carbohydrates, cardiac glycosides, anthranol glycosides, saponins, phytosterols, phenols, tannins, flavonoids, proteins and amino acids and diterpenes using standard procedures using standard procedures described by Tiwari et al., (2011); and Parekh and Chanda (2007). Some of the detected phytochemicals were quantified using standard procedures described by Obadanomi and Ochuko (2001); Sudduraju and Becker (2003); Jia et al., (1999) Oluwaniyi et al., (2007) and Makkar et al., (2007).

**Preparation of Plasmodium falciparum Culture Medium**

The media was prepared by dissolving 10.4g of the powdered material into one litre of distilled water and then autoclaved at 121°C for 15mins as instructed by the manufacturers. Venous blood (2ml) from the main vein of white healthy rabbit’s pinnae was withdrawn using a disposable 5ml syringe (BD 205 WG). This was defibrinated by allowing it to settle for at least one hour (Dacie and Lewis, 1968). The defibrinated blood was centrifuged at 1500rpm using spectre merlin centrifuge for 10mins and the supernatant layer was collected in a sterilized tube. The sediment was further centrifuged at 1500rpm for 5mins and the supernatant layer was added to the first test tube. The sediments were discarded and the serum collected was supplemented with the salt of RPMI 1600 medium (Trager, 1982).

**Preparation of the Test Concentrations**

An electronic digital balance was used to measure 20mg of each of the extracts and then dissolved in 1ml of dimethylsulphoxide (DMSO) in separate vials (stock solution). Using serial doubling dilution, four different concentrations (10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml) of each extract were prepared.

**In vitro Assay of the Activity of the Extracts on Plasmodium falciparum Culture**

0.1ml of test solution and 0.2ml of the culture medium were added into a tube containing 0.1ml of 5% parasitemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to the tested fractions was determined microscopically after incubation for 24, 48 and 72 hours at37°C. The incubation was undertaken in a bell jar glass containing a lighted candle to ensure the supply of required quantity of carbon dioxide (about 5% Oxygen gas, 2% and about 93% nitrogen gas as demonstrated by Muktar et. al (2006).
Determination of Antimalaria Activity
At the end of each incubation period, a drop of a thoroughly mixed aliquot of the culture medium was smeared on microscopic slides and stained by Geimsa’s staining techniques. The mean number of erythrocytes appearing as blue discoid cells containing life rings of the parasite (that appeared red pink) was estimated and the average percentage elimination by the samples was determined. The activity of the tested samples was calculated as the percentage elimination of the parasites after each incubation period, using the formula below:

\[
\% = \frac{\text{N} - \text{Nx}}{\text{N}} \times 100
\]

Where, \(\%\) = Percentage activity of the extracts  
\(\text{N}\) = Total number of cleared RBC  
\(\text{Nx}\) = Total number of parasitized RBC  

Note: RBC = Red Blood Cells (Muktar et al., 2006).

Statistical Analysis
Values are expressed as mean ± standard error of mean (SEM). Data was analyzed by ANOVA and significant differences between multiple variables were analysed using multivariate analysis. Values of \(P<0.05\) were considered statistically significant. All analysis were carried out using the Graph Pad InStat statistical package.

RESULTS
Physical Properties of the Plant Extracts
Two extracts were obtained from \(J.\ curcas\) stem bark. The aqueous stem bark extract (ASBE) of the plant was reddish-brown in colour, oily and gummy in texture. The ethanol stem bark extract (ESBE) of the plant was a greenish-black in colour and powdery in texture. Percentage yields of 6.00% (ASBE) and 2.55% (ESBE) were obtained as shown in Table 1.

<table>
<thead>
<tr>
<th>Property</th>
<th>ASBE</th>
<th>ESBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of plant sample (g)</td>
<td>150</td>
<td>170</td>
</tr>
<tr>
<td>Weight of plant extract (g)</td>
<td>9.00</td>
<td>4.34</td>
</tr>
<tr>
<td>Percentage yield (%)</td>
<td>6.00</td>
<td>2.55</td>
</tr>
<tr>
<td>Colour of extract</td>
<td>Reddish-brown</td>
<td>Greenish-black</td>
</tr>
<tr>
<td>Texture of extract</td>
<td>Gummy</td>
<td>Powdery</td>
</tr>
</tbody>
</table>

(ASBE)=Aqueous stem bark extract and (ESBE)=Ethanol stem bark extract

Qualitative Phytochemical Analysis
Result of phytochemical screening of aqueous extract showed the presence of alkaloids, cardiac glycosides, anthranol glycosides, saponins, phytosterols, phenols, protein and amino acids and diterpenes. However, carbohydrates, flavonoid and tannins were found to be absent in the aqueous stem bark extract of \(J.\ curcas\). Result of phytochemicals of ethanol extract showed the presence of alkaloids, cardiac glycosides, saponins, phenols, flavonoids and diterpenes. Carbohydrates, anthranol glycosides, phytosterols, protein and amino acids and tannins were absent in the ethanol stem bark extract of a \(J.\ Curcas\) (Table 2).

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>ASBE</th>
<th>ESBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthranol glycosides</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Protein and amino acids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Diterpenes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: + =Present; - =Absent; ASBE=Aqueous stem bark extract and ESBE=Ethanol stem bark extract

Quantitative Phytochemical Analysis
The quantitative estimation of these secondary metabolites showed that ethanol extract has a higher amount of flavonoid, phenols and saponins than the aqueous extract. Saponin was found to have the highest quantity (164.9024 mg DE/g ± 0.5616 extract in aqueous extract and 173.3504 mg DE/g ± 0.2639 extract in ethanol extract), followed by cardiac glycosides (4.4700 g% ± 0.0447), flavonoids (2.2436 mg RE/g ± 0.0227 extract in ethanol extract), alkaloids (2.2000 g% ± 0.5657) and the least quantity was found in phenols (0.0397 mg GAE/g ± 0.0001 extract in aqueous extract and 0.0917 mg GAE/g ± 0.0008 extract in ethanol extract).

In Vitro Antimalarial Activity of Extracts
The results of antimalarial activity of the extracts are shown in Table 3. Both the aqueous and ethanol stem bark extracts have reflected a remarkable activity at most concentrations with few exceptions. The highest antiplasmodial activity was obtained with both the ethanolic and aqueous extract, in which the microscopic examination of stained slides at 10mg/ml showed a percentage elimination of 77% after 72 hours.
The least antiplasmodial activity was observed with the ethanol extract at a concentration of 1.25mg/ml yielding a percentage elimination of 38%. This is shown in the table below. The development stage of the malaria parasite at which both the aqueous and ethanol extracts at 10mg/ml concentration and 24 hours incubation kill the malaria parasite is at the schizont stage by disrupting the Plasmodial cell membrane.

### Table 3: In Vitro Antimalarial Activity of *J. curcas* Aqueous and Ethanol Extract with Percentage Elimination after 24, 48 and 72 hours Incubation

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Conc. (mg/ml)</th>
<th>Mean no. of parasites before incubation</th>
<th>Average no. of parasites during incubation</th>
<th>Mean no. of parasites after incubation</th>
<th>Percentage elimination at the end of incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control-</td>
<td>10</td>
<td>1.0±0.2</td>
<td>1.0±0.2</td>
<td>0.0±0.0</td>
<td>93%</td>
</tr>
<tr>
<td>(Artemisin Combination Therapy)</td>
<td>-</td>
<td>12.0±1.9</td>
<td>15.0±3.2</td>
<td>19.3±3.6</td>
<td>0%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>10</td>
<td>1.6±0.5^c,n</td>
<td>2.6±0.2^p,n</td>
<td>2.6±0.7^c*,n</td>
<td>77%</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.4±0.5^c,n</td>
<td>3.4±0.9^t*,p,n</td>
<td>7.2±2.0^t*,c*,e,n</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>10.4±2.6^c*,p,n</td>
<td>4.0±1.1^t*,p,n</td>
<td>1.0±0.0^t*,c*,e,n</td>
<td>49%</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>11.6±3.1^c*,p,n</td>
<td>4.2±0.9^t*,p,n</td>
<td>3.0±1.0^t*,c*,e,n</td>
<td>38%</td>
</tr>
<tr>
<td>Aqueous</td>
<td>10</td>
<td>2.2±0.7^a</td>
<td>3.0±0.5^p,n</td>
<td>1.8±0.4^c*,n</td>
<td>77%</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.6±0.5^c,n</td>
<td>5.0±1.0^t*,p,n</td>
<td>1.2±0.4^t*,c*,e,n</td>
<td>74%</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>3.8±0.7^p,n</td>
<td>2.2±0.4^p,n</td>
<td>2.6±0.5^c,n</td>
<td>71%</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>5.2±2.8</td>
<td>6.6±1.8^a</td>
<td>0.4±0.2^c*,n</td>
<td>59%</td>
</tr>
</tbody>
</table>

n = 5 (number of samples); Values are expressed in mean ± S.E.M.; Data analyzed using one way Anova;
- t- Compares significance over time interval at the same concentration and same extract at values of P<0.05.
- c- Compares significance at different concentrations on the same time perceived and same extract at values of P<0.05.
- e- Compares significance of different extracts under the same concentration and same time interval at values of P<0.05.
- p- Significantly different from the positive control at values of P<0.05.
- n- Significantly different from the negative control at values of P<0.05.
**DISCUSSION**

Preliminary qualitative phytochemical screening of the aqueous and ethanol stem bark extracts of *J. curcas* revealed the presence of alkaloids, cardiac glycosides, anthranol glycosides, saponins, phytosterols, phenols, flavonoids, protein and amino acids and diterpenes. Phytochemicals, also called non-nutritive chemicals compounds are secondary metabolites occurring naturally in medicinal plants with various disease defensive properties (Djeridane et al., 2006). The presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids were previously reported in the stem bark of *J. curcas* grown in Southern part of Nigeria (Igbinosa et al., 2009). However, the geographical location of the plant used in the present study was different and tannin was also found to be absent. The phytochemical screening of the aqueous, ethanolic and methanolic extracts of the *J. curcas* has also been reported, but the authors failed to
categorize the bioactive compounds in the three solvents (Igbinosa et al., 2009). Sharma et al., (2012) also reported the presence of bioactive compounds such as alkaloids, saponins, tannins, terpenoids, steroids, glycosides, phenols and flavonoids in the extracts of root, stem and leaf of the plant in methanol extracts. From table 2, the aqueous extract revealed the presence of more secondary metabolites when compared with the ethanol extract. Both extracts revealed the presence of alkaloids, cardiac glycosides, phenols, saponins and diterpenes whereas carbohydrates and tannins were absent in both extracts. Anthranol glycosides, phytosterols and proteins and amino acids were only found in aqueous extract whereas flavonoid was only found in ethanol extract. These variations could have arisen due to differences in polarity index of the solvents. The variations in these extracts affects the quantity and secondary metabolite composition and thus depends upon type of extraction, time of extraction, temperature, nature of solvent, solvent concentration and most importantly polarity (Ncube et al., 2008). Solvents are either polar or non-polar. Polarity is roughly measured using the dielectric constant of the solvent. Polarity of a solvent is attributed to its attraction power to different compounds in a solution where polar solvents extract polar compounds and non-polar solvents extract non-polar compounds. Quantitative investigation revealed J. curcas to have saponins in the highest amount. High saponin value indicated that oils are normal triglycerides and very useful in production of liquid soap and shampoo (Goodrum, 2002). Low saponin levels are considered safe and non-toxic as high saponin levels have been associated with gastroenteritis, manifested by diarrhoea and dysentery (Awe and Sodipo, 2001). Saponins are amphipathic glycosides which are grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions (Hostettmann and Marston, 1995). Most saponins function as antioxidants, because they possess a special moiety (2,3-dihydri-2,5-dihydroxy-6-methyl-4-pyran-4-one) which act by forming hydroperoxide intermediates which scavenges free radicals (Hu et al., 2002). Saponins also possess haemolytic action on human erythrocytes (Baumann et al., 2000). Saponins with acyl residues or oxide-ring moiety tend to show haemolytic activity (Oda et al., 2000). A moderate level of cardiac glycosides was found, which is a class of natural product used to increase the cardiac contractile force in patients with congestive heart failure and cardiac arrhythmias (Hauptman et al., 1999). Flavonoids that have been used for centuries in oriental medicine (Di Carlo 1999). Flavonoids which have been implicated in antiparasomal activity in many plants (Okonk et al., 2006). Alkaloids also exhibit toxicity against foreign cells in culture and are widely studied in cancer research. On the other hand, saponins disrupt the cell wall providing toxic materials free access to the cell. The damage to the cell wall leads to leakages of valuable cell constituents that are usually stored inside (Oyi et al., 2007). Phenols and flavonoids act as cytoplasmic poisons with their inhibitory effect on cellular enzymes (Iwu, 1985). Flavonoids has been revealed to possess significant anti-parasitic activities against various strains of malaria parasite, trypanosome and leishmania (Al-Adhoery et al., 2010). Flavonoids also are known for their antimicrobial, cytostatic and anti-inflammatory properties (Hodek et al., 2002). The antimicrobial action of most of the Jatropha plant can also be attributed to the presence of phenolics (Kowalski &Kedzia, 2007; Igbinosa et al., 2007). These compounds act as acids with high antimicrobial action (Oyi et al., 2007). Aside from their very potent antibacterial action, phenolics also exhibit anticancer, antitumor and cytotoxic properties (Aiyelaagbe et al., 2007; Igbinosa, 2009). From Table 3, it can be seen that the average number of parasites after incubation increased as the concentration of the extract decreased. This indicates that higher concentrations of the extracts were found to be more effective on the parasites. The elimination rate for both the extracts was seen to be almost rapid within the first 24hrs, which then decreased steadily within the next incubation periods. Besides, previous studies on J. curcas leaves showed that it contained some phytochemicals which includes alkaloids, saponins, tannins, flavonoids, phenols, glycosides, steroids and terpenoids. The aqueous and methanolic extracts showed a remarkable antiparasomal activity with high percentage eliminations of 95.4% (Imam et al., 2016). However in this study at the highest dose of 10mg/ml, both the ethanol and aqueous extracts showed percentage elimination of 77%. The least activity was observed with ethanol extract at 1.25mg/ml which yielded a percentage elimination of 38%. This shows that the leaf of J. curcas could be more effective than the stem bark in the treatment of malaria. Also the aqueous extract was observed to have higher percentage elimination at the various concentrations when compared with the ethanol extract. This could be due to the aqueous extract dissolving more phytochemicals than the ethanol extract. These observations suggest that the activity of the extracts may be cytotoxic for the malaria parasite, there by inhibiting their growth and development.
CAUTION
This study revealed the presence of various phytochemicals which was ascertained by both qualitative screening and quantitatively screening. The aqueous and ethanol bark extracts of J. curcas was found to have antiplasmodial properties at higher concentrations of both extracts. This suggests that J. curcas contains secondary metabolites with promising antimalarial properties which when fully harnessed could offer a novel therapy for the management of clinical malaria.

Recommendation
The promising antiplasmodial properties of J. curcas established in this study could form a basis for recommending herbal preparation of the plant for management of malaria. However, care should be taken in its usage until extensive toxicological studies is conducted to establish its safety or otherwise.

REFERENCES


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