ANTIMICROBIAL ACTIVITIES OF LEAF OF VITEX DONIANA AND CAJANUS CAJAN ON SOME BACTERIA

Ejikeme Nwachukwu* Henrietta O. Uzoeto

Department of Microbiology, Michael Okpara University of Agriculture, Umudike, P. M. B. 7267, Umuahia, Nigeria.

*Corresponding author, Email drejik@yahoo.com

ABSTRACT: Antimicrobial activities of acetone, ethanol, methanol, hot and cold water extracts of leaves of Vitex doniana and Cajanus cajan on Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilis and Staphylococcus aureus were investigated. V. doniana and C. cajan contain alkaloid, tannins, saponins, carbohydrates and proteins at varying levels. Antimicrobial activity was particularly high in acetone extracts of V. doniana against S. typhi (19.71mm), followed by methanol extracts of V. doniana against E. coli (14.61mm) and ethanol extracts of V. doniana against S. typhi (13.66mm). Ethanol, methanol and acetone extracts of C. cajan inhibited growth of E. coli to 11.90 mm, 11.69 mm and 10.2 mm respectively. P. aeruginosa was generally resistant to all the extracts accept acetone extract of V. doniana. The minimum inhibitory concentration (MIC) of the extracts was determined. MIC for acetone extracts of C. cajan against E. coli was 0.78mg/ml. The kinetic of kill of individual cells by addition of extract to culture broth indicated decrease in the number of the viable counts during the period of monitoring. Analysis of variance showed that there was no significant difference (p=0.05) between the antimicrobial activity of standard antibiotic gentamicin and acetone extract of V. doniana or C. cajan on the isolates. This result suggests that the acetone extracts of V. doniana and C. cajan have antimicrobial properties which can be pharmaceutically exploited. [Researcher. 2010; 2(3):37-47]. (ISSN: 1553-9865).

Key words: Antibacterial activity, plant extracts, phytochemicals

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications (Baris *et al*., 2006). Medicinal plants are rich bio-resources of drugs (Hammer *et al*., 1999). A number of interesting outcomes have been found with the use of a mixture of natural products or plant extracts to treat diseases (Gibbons, 2003). The antimicrobial properties of plants have been investigated by a number of researchers worldwide though thorough biological evaluation of plants extracts is vital to ensure their efficacy and safety. These factors are of importance if plant extracts are to be accepted as valid medical agents for the treatment of infectious diseases (Tanaka *et al*., 2006) especially in light of the emergence of drug-resistant microorganisms.

Vitex doniana belongs to the family Verbenaceae and is commonly called black plum while is locally known as 'utakiri' in Eastern Nigeria. Chemical constituents of the plant include glycosides, flavonoids, alkaloids, essential fatty acid (Arokiyaraij *et al*., 2009). *Cajanus cajan* belongs to the family Fabaceae, commonly called pigeon pea and locally known as 'fiofio' in Eastern Nigeria. It is an important grain legume crop. *Cajanus cajan* is widely used as food. They contain high level of protein and the important amino acids methionine, lysine and tryptophan. The green leave of *Cajanus cajan* is usually used traditionally as medicine, in the treatment of stomach or intestinal disorder. This study was designed to evaluate the extracts of *Vitex doniana* ('utakiri') and *Cajanus cajan* ('Fiofio') as antimicrobial agents as well as the phytochemical characteristics.

MATERIALS AND METHODS

Sources of plant

Vitex doniana and Cajanus cajan were collected from a bush at Umulogho in Obowo Local Government Area of Imo State, Nigeria.

Extraction of plant

Fresh leaves of *V. doniana* and *C. cajan* were collected and air-dried. Fifty grams of the dried leaves of each of the plant species was separately soaked in 200 ml of ethanol, methanol, acetone, hot (100ºC) and cold water for 36 h at room temperature (28 ± 2ºC) with occasional shaking (Parekh and Chanda, 2007). Each portion was filtered using Whatman filter paper No.1. The filtrates were collected in different beakers. The filtrates were evaporated to dryness in a steady air-current for about 24 h in a previously weighed evaporation dishes (porcelain dishes). After evaporation, the dishes were re-weighed and the differences in their weights before and after evaporation were calculated and recorded. The plant extracts (residues) were stored in a clean sterile container for further use.

Phytochemical screening

The extracts of the plant were screened for tannins, alkaloids, saponins, cardiac glycosides and flavonoids using the method of Harborne, 1998.

Sources of Microorganisms

Pure cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Bacillus substilis* were obtained from bacteriology laboratory of Federal College of Veterinary and Medical Laboratory Technology (FCVMLT) Vom, Jos Plateau State. They were sub-cultured and re-identified to ensure the purity of the isolates. The inoculum size of each test strain was standardized according to the *National Committee for Clinical Laboratory Standards* (NCCLS, 1998).

Antimicrobial Assay

The inoculum size of each isolate was standardized (NCCLS, 1998). Each isolate was inoculated into Mueller Hinton broth (Oxoid) and incubated for 3 - 6 h to obtain a suspension of 0.5 Macfarland turbidity standards (1×10^6 cfu/ml). Antibacterial screening was by agar well diffusion method. A 1.0 ml volume of the standard suspension (1×10^6cfu/ml) was spread evenly on Mueller Hinton agar plates using sterile glass rod. Subsequently, 6 mm diameter wells were bored in the agar and a 100µl volume of each plant extract reconstituted in 50% Dimethyl sulfoxide (DMSO) to a concentration of 100 mg/ml was placed into triplicate wells. The plates were incubated at 37ºC for 24 h and the inhibition of bacterial growth was measured to the nearest mm. Gentamycin was used as positive control while DMSO (0.1ml) served as negative control.

Determination of Minimum Inhibitory Concentration (MIC) of the crude extracts

The minimum inhibitory concentration (MIC) was determined by adopting the techniques as specified by *National Committee for Clinical Laboratory Standards* (NCCLS, 1998). A twofold serial dilution of the reconstituted extract was prepared. Each dilution was seeded with bacterial suspension (1×10^6cfu/ml) and incubated for 24 h at 37°C. MIC was determined as the highest dilution (i.e. lowest concentration) of the extract that showed no visible growth.

Determination of Minimum Bactericidal Concentration (MBC) of the crude extracts

MBC were determined by first selecting tubes that show no growth during MIC determination. A loopful from each of the tube was subcultured on the sterile Muller Hinton agar and incubated for 24 hours at 37°C. The MBC was determined as the least concentration showing no visible growth on subculture (NCCLS, 1998).

Determination of kinetic of kill of the test organisms by the extract

The kinetic of kill of the test organisms by the extract was determined by microbroth dilution technique. Solutions of the acetone extract of *Vitex doniana* were prepared in DMSO to obtain concentrations of 0.39 mg/ml, 0.78 mg/ml, 1.56 mg/ml and 3.13 mg/ml for the experiment. Aliquot of 1.0 ml of each of the concentrations was added to 9 ml of broth containing appropriate suspension (1×10^6 or 5×10^5) of test organism. Similarly 6.25 mg/ml, 12.50 mg/ml, 25.00 mg/ml and 5.00 mg/ml were prepared for *Cajanus cajan.* Two sets of control tubes were set up. One set of the control experiment contained 50 µg of gentamicin and the other water. All cultures were incubated in a shaker water bath at 37ºC and samples were drawn every 30 min intervals for 120 min. Each sample was diluted 10 fold and was plated in duplicates in agar. After 24 h period of incubation at 37ºC, the viable bacterial colonies were counted and results obtained were compared with the controls. The results were expressed as positive or negative $\log_{10}$ value.

RESULTS

The total yield of extracts of *Vitex doniana* using acetone recorded the highest value of 7.76 mg (15.92%) (Table I). Hot water extract of *Vitex doniana* yielded 5.75 mg (11.50%) while methanol showed the least yield of 4.45 mg (8.90%). Phytochemical screening of *V. doniana* and *C. cajan* revealed that alkaloid, tannins, sponnins were present in almost all the extracts while glycosides and flavoniods were present or absent in some plant extracts (Table 2). The antibacterial activity of different extracts of *V. doniana* and *C. Cajan* is shown in Table 3. The extracts of *V. doniana* and *C. cajan* obtained by acetone, methanol and ethanol solvents generally inhibited the growth of *E. coli*, *S. aureus* and *S. typhi*. Hot or cold water extracts showed strong or weak effect in some bacteria. The MIC indicated acetone extract of *V. doniana* with highest sensitivity at 0.78 mg/ml. The MBC of the plant extract is shown in Table 6. Growth of *Salmonella typhi* 5 x 10^5cfu/ml at different extract concentrations is shown in Table 7. Gentamain used as positive control showed significant reduction of viable cells. *Staphylococcus aureus* showed the same reduction pattern of viable cells as observed in *Salmonella typhi* in the extracts of both *V. doniana* and *C. cajan*.

Table: 1 Yield of the crude extracts using different solvent

Plant	extraction solvent	yield (mg)	percent yield (%)
Vitex doniana	Methanol	7.31	14.62
	Ethanol	6.54	13.08
	Acetone	7.96	15.92
	Hot water	6.06	12.12
	Cold water	6.74	13.48
Cajanus cajan	Methanol	4.45	8.90
	Ethanol	4.82	9.64
	Acetone	5.16	10.32
	Hot water	5.75	11.50
	Cold water	4.65	9.30

Table 2 Phytochemical screening of crude plant extracts

Extract		*Vitex*	*doniana*				*Cajanus*	*Cajan*		
Test	Methanol	Ethanol	Acetone	Hot water	Cold water	Methanol	Ethanol	Acetone	Hot water	Cold water
Alkaloids	+++	++	+++	++	+	+++	++	+	+	-

Glycosides	++	+++	+++	+	-	-	-	-	+	+
Tannins	++	+	+	+++	+	+	+	+	++	++
Saponins	-	++	+	+++	-	++	++	++	+	+
Flavonoids	+	+	-	++	+	-	-	-	-	-
Carbohydrates	++	++	++	+++	++	+	+	+	+	+++
Protein	+	+	+	++	+	+	+	+	+	-

- = Not present
+ = Present in small amount (concentration)

++ = Moderately present

+++ = Present in large amount

Table 3 Zone of inhibition (in mm) of microorganisms by plant extracts

Plant	Solvent	Diameter of the zone of inhibition (mm)				
		E.coli	*S.aureus*	*P.aeruginosa*	*B.subtilis*	*S.typhi*
Vitex doniana	Methanol	14.61	10.36	-	3.60	10.54
	Ethanol	13.88	9.94	-	2.35	12.11
	Acetone	17.29	10.48	1.41	3.83	19.71
	Hot water	9.47	5.18	-	-	14.70
	Cold water	5.12	3.62	-	-	5.11
Cajanus cajan	Methanol	11.69	8.52	-	1.92	-
	Ethanol	11.90	7.46	-	1.08	9.85
	Acetone	10.25	8.55	-	1.23	8.46
	Hot water	2.04	-	-	-	2.09
	Cold water	-	-	-	-	3.50
	Gentamicin (100µg/ml)	23.04	26.18	5.84	24.30	27.15
	Ciprofloxacin (100µg/ml)	26.72	25.37	6.06	21.08	25.41

	DMSO (0.1ml)	-	-	-	-	-	

Table 4 Inhibitory effect of crude plant extracts on test organism at different concentrations.

Plant	Solvent	Test organism	Inhibition zone diameter (mm)					
			6.25	12.50	25.00	50.00	100.00	200.00
Vitex doniana	Methanol	*E.coli*	2.66	5.28	7.63	9.42	10.26	14.61
		S.aureus	--	1.04	4.55	10.00	10.21	10.31
		B.subtilis	--	--	--	0.08	1.91	3.61
		S.typhi	7.61	9.29	10.41	10.94	0.25	10.54
Cajanus cajan		*E.coli*	4.80	5.92	7.73	10.86	11.53	11.69
		S.aureus	--	--	0.66	3.65	6.14	8.52
		B.subtilis	--	--	--	--	0.07	1.92
		S.typhi	--	--	--	--	--	--
Vitex doniana	Ethanol	*E.coli*	5.04	5.12	5.45	6.84	10.50	13.88
		S.aureus	1.63	3.80	3.96	6.22	7.16	9.94
		B.subtilis	--	--	--	--	0.48	2.35
		S.typhi	2.16	2.60	2.82	10.60	11.00	12.11
Cajanus cajan		*E.coli*	6.31	6.44	6.92	9.15	10.41	11.90
		S.aureus	--	--	--	--	2.28	7.46
		B.subtilis	--	--	--	--	--	1.08
		S.typhi	--	4.00	4.55	6.83	3.53	9.85
Vitex doniana	Acetone	*E.coli*	10.77	11.12	11.95	13.46	16.85	17.29
		S.aureus	--	4.42	7.36	8.23	10.08	10.48
		B.subtilis	2.10	2.14	2.62	3.10	3.50	3.30
		S.typhi	11.20	11.76	13.41	15.50	18.30	19.71
Cajanus cajan		*E.coli*	--	1.80	5.65	8.94	9.16	10.25
		S.aureus	--	--	--	2.10	4.66	8.55
		B.subtilis	--	--	--	--	--	1.23
		S.typhi	6.19	6.30	7.42	7.88	8.23	8.46
Vitex doniana	Hot water	*E.coli*	--	--	--	5.55	6.63	9.47
		S.aureus	--	--	--	--	2.22	5.18
		B.subtilis	--	--	--	--	--	--
		S.typhi	9.92	10.40	10.86	12.14	12.69	14.70
Cajanus cajan		*E.coli*	--	--	--	--	--	1.04
		S.aureus	--	--	--	--	--	--
		B.subtilis	--	--	--	--	--	--
		S.typhi	--	--	--	--	1.04	2.09

Vitex doniana	Cold water	*E.coli*	--	--	--	--	2.00	3.12
		S.aureus	--	--	--	2.62	3.04	3.68
		B.subtilis	--	--	--	--	--	--
		S.typhi	--	--	--	--	--	--
Cajanus cajan		*E.coli*	--	--	--	--	--	--
		S.aureus	--	--	--	--	--	--
		B.subtilis	--	--	--	--	--	--
		S.typhi	--	--	--	--	--	3.69

Table 5 Minimum inhibitory concentration (mg/ml) of plant extract on test organism

Plant	Solvent	Test organism	MIC (mg/ml)
Vitex doniana	Methanol	*E.coli*	6.25
		S.aureus	12.50
		B.subtilis	100.00
		S.typhi	3.13
Cajanus cajan		*E.coli*	6.25
		S.aureus	50.00
		B.subtilis	200.00
		S.typhi	--
Vitex doniana	Ethanol	*E.coli*	6.25
		S.aureus	6.25
		B.subtilis	100.00
		S.typhi	6.25
Cajanus cajan		*E.coli*	3.13
		S.aureus	100.00
		B.subtilis	400.00
		S.typhi	12.50
Vitex doniana	Acetone	*E.coli*	0.78
		S.aureus	12.50
		B.subtilis	12.50
		S.typhi	1.56
Cajanus cajan		*E.coli*	12.50
		S.aureus	50.00
		B.subtilis	400.00
		S.typhi	3.13
Vitex doniana	Hot water	*E.coli*	50.00
		S.aureus	100.00

		B.subtilis	--
		S.typhi	1.56
Cajanus cajan		*E.coli*	800.00
		S.aureus	--
		B.subtilis	--
		S.typhi	200.00
Vitex doniana	Cold water	*E.coli*	100.00
		S.aureus	200.00
		B.subtilis	--
		S.typhi	50.00
Cajanus cajan		*E.coli*	--
		S.aureus	--
		B.subtilis	--
		S.typhi	200.00

Table 6 Minimum bacterial concentration of plant extracts

Plant	solvent	test organism	MBC (mg/ml)
Vitex doniana	Methanol	*E.coli*	50.00
		S.aureus	100.00
		B.subtilis	800.00
		S.typhi	12.50
Cajanus cajan		*E.coli*	25.00
		S.aureus	400.00
		B.subtilis	1600.00
		S.typhi	--
Vitex doniana	Ethanol	*E.coli*	100.00
		S.aureus	100.00
		B.subtilis	1600.00
		S.typhi	50.00
Cajanus cajan		*E.coli*	50.00
		S.aureus	200.00
		B.subtilis	1600.00
		S.typhi	200.00
Vitex doniana	Acetone	*E.coli*	3.125
		S.aureus	100.00
		B.subtilis	400.00
		S.typhi	6.25
Cajanus cajan		*E.coli*	200.00
		S.aureus	400.00

		B.subtilis	1600.00
		S.typhi	400.00
Vitex doniana	Hot water	*E.coli*	400.00
		S.aureus	1600.00
		B.subtilis	--
		S.typhi	25.00
Cajanus cajan		*E.coli*	1600.00
		S.aureus	--
		B.subtilis	--
		S.typhi	1600.00
Vitex doniana	Cold water	*E.coli*	400.00
		S.aureus	1600.00
		B.subtilis	--
		S.typhi	800.00
Cajanus cajan		*E.coli*	--
		S.aureus	--
		B.subtilis	--
		S.typhi	1600.00

Table 7 Kinetic of kill of 5×10^5 cfu/ml of *Salmonella typhi* at multiple concentration of acetone extract of *Vitex doniana*

	Viable cell Reduction at:			
Concentration of extract(µg/ml)	30 mins	60 mins	90 mins	120 mins
0.39	+0.25	+0.31	+0.30	+0.30
0.78	+0.26	+0.25	+0.18	-1.01
1.56	+0.24	+0.20	+0.20	-1.36
3.13	-0.07	-0.26	-0.55	-1.60
0.00	+0.28	+0.33	+0.46	+0.82
Gentamicin(50µg)	-0.38	-0.81	-2.44	-3.50

Table 8 Kinetic of kill of 5×10^5 cfu/ml of *Salmonella typhi* at multiple concentration of acetone extract of *Cajanus cajan.*

Concentration of extract(µg/ml)	Viable cell Reduction at: 30 mins	60 mins	90 mins	120 mins
6.25	+0.68	+0.69	+0.61	+0.59
12.50	+0.30	+0.08	-0.41	-0.96
25.00	+0.14	-0.77	-1.12	-1.23
50.00	+0.15	-1.06	-2.10	-3.16
0.00	+0.70	+0.71	+0.88	+0.89
Gentamicin(50µg)	-0.39	-0.80	-2.44	-3.50

Table 9 Kinetic of kill of 1×10^6 cfu/ml of *Staphylococcus aureus* at multiple concentration of acetone extract of *Vitex doniana*

Concentration of extract(µg/ml)	Viable cell Reduction at: 30 mins	60 mins	90 mins	120 mins
0.39	+0.44	+0.78	+0.85	+0.84
0.78	+0.43	+0.66	+0.53	+0.82
1.56	+0.40	+0.29	+0.06	-0.17
3.13	+0.32	-0.74	-1.11	-1.42
0.00	+0.44	+0.80	+0.86	+1.20
Gentamicin(50µg)	-0.35	-0.66	-2.61	-3.35

Table 10 Kinetic of kill of 1×10^6 cfu/ml of *Staphylococcus aureus* at multiple concentration of acetone extract of *Cajanus cajan*

	Viable cell	Reduction at:		
Concentration of extract (µg/ml)	30 mins	60 mins	90 mins	120 mins
6.25	+1.16	+1.17	+1.15	+1.15
12.50	+1.16	+1.16	+1.13	+1.13
25.00	+1.15	+1.12	+1.10	+0.70
50.00	+1.15	+0.05	-0.05	-0.24
0.00	+1.16	+1.16	+1.48	+2.49
Gentamicin(50µg)	-0.35	-0.66	-2.61	-3.35

DISCUSSIONS

Antimicrobial activity of extracts of *V. doniana* and *C. cajan* using different extraction solvents was investigated. Different yields of the extracts were obtained probably because of solvents and the methods adopted. Cold maceration has generally been reported to give lower yield compared to other extraction solvents (Ibrahim *et al.*, 2001). The hot water extract gave slightly higher yield than the cold water probably because of higher solubility of the plant component in hot water.

The phytochemical characteristics in the two plant used showed that alkaloid, tannins, saponins, carbohydrate and protein are present in almost all solvent extracts of the two studied plant but at varying intensity while glycosides and flavonoid are present in some but absent in some. The phytochemical characteristics possessed by *V. doniana* and *C. cajan* may be attributed to their antimicrobial properties. This finding agrees with similar study by Kilani (2006) and Arokiyaraj *et al.* (2009)

Antimicrobial activity of the extracts on the bacteria revealed inhibition of growth, though the susceptibility pattern to the extracts was not uniform (Table 3 and 4). *S. typhi* was highly sensitivity to acetone extract (19.71 mm) of *V. doniana* followed by methanol extract (14.61 mm) on *E. coli*. Ethanol extract of *C. cajan* produced the least (1.08 mm) pattern of sensitivity. Result obtained agrees with the report of Arokiyaraj *et al*., (2009) in a similar study. *V. doniana* and *C. cajan* acetone, methanol, ethanol extracts generally produced a clear inhibitory effect on the bacteria

The low MIC values confirm high antimicrobial activity of the extracts. Acetone extracts of *V. doniana* against *E. coli* (0.78mg/ml) and *S. typhi* (1.5mg/ml) showed higher antimicrobial activity. This result supports the use of *V. doniana* in the treatment of dysentery and gastroenteritis (Kilani, 2006).

Kinetic of kill in Tables 7-10, demonstrated reduction in the number of viable bacteria at 30 minutes interval using different concentrations of extracts. High level of reduction was recorded as the concentration of extract increased comparable with standard antibiotics used. The growth inhibitory effect was concentration dependent (Achi, 2006). This is important in considering dosage and rate at which the extract inhibits the growth of organism (Egwari, 1999). Statistical analysis showed that there is no significant difference between the gentamicin and acetone extract of *V. doniana and C. cajan*. This means that the extracts were as effective as standard antibiotic used. The present study showed that extracts of leaf of *V. doniana* and *C. cajan* generally have antimicrobial properties against the test organisms.

REFERENCES

Achi OK. Composition and antibacterial activities of *Tetrapleura tetraptera* Taub. pod extracts. *Research Journal of Microbiology* 2006:1 (5): 416 – 422.

Arokiyaraj k, Perinbam P, Agastian R, Mohan K.. Phytochemical analysis and antibacterial activity of *Vitex agnus-castus*. *International Journal of Green Pharmacy* 2009:34:162-164.

Baris O, Gulluce M, Sahin F, Ozer H, Kilic H, Ozkan H, Sokmen M, Ozbek T. Biological activities of the essential oil and methanol extract of *Achillea biebersteinii* Afan (Asteraceae). *Turkey Journal of Biology* 2006: 30:65-73.

Egwari LO. Antibacterial activity of crude extracts of *Nauclea latifolia* and *Eugenia aromatica*. *West African Journal Pharmacology drug Res* 1999:15 (1-2): 55 – 59.

Gibbons S. An overview of plant extracts as potential therapeutics. *Expert Opin. Ther. Pat.* 2003:13(4): 489 – 497.

Hammer K.A, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology* 1999:86(6): 985

Harborne JB. *Phytochemical methods: A guide to modern technique of plant analysis.* Chapman and Hall publishers, London. 1998: 51-59.

Ibrahim MB, Owonubi MO, Onadapu JA. Antimicrobial effects of extracts of leaf, stem, and root bark of *Anogiessus leicarpus* on *S. aureus* NCTC 8198, *E. coli* NCTC 10418 and *P. vulgaris* NCTC 4636. *Journal of pharmaceutical research and development* 2001:2: 20-26.

Kilani AM. Antibacterial assessment of whole stem bark of *Vitex doniana* against some enterobacteriaceae. *African Journal of Biotechnology* 2006: 6(10): 958-959.

Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal Microbiology* 2000:31:247-256.

National Committee for Clinical Laboratory Standard (NCCLS). Methods for dilution in antimicrobial susceptibility test. Villanova. 1998.

Parekh J, Chanda SU. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkey Journal of Biology* 2007: (31): 53 – 58.

Tanaka JCA, da Silva CC, de Oliveira AJB, Nakamura CV, Dias Filho BP. (2006). Antibacterial activity of indole alkaloids from *Aspidosperma ramiflorum*. Brazalian *Journal of Med. Biology Res*. 2006:39(3): 387-391.

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