Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v37i1.22525

Evaluation of *in vitro* biological properties of *Senna occidentalis* (L.) Link

Márcia Lombardo[1*], Sumika Kiyota[2], Edna Tomiko Miyake Kato[1], Monica Beatriz Mathor[3], Terezinha de Jesus Andreoli Pinto[1] and Telma Mary Kaneko[1]

*[1]Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Professor Lineu Prestes, 580, 05508-900, São Paulo, São Paulo, Brazil. [2]Centro de Pesquisa e Desenvolvimento de Sanidade Animal, Instituto Biológico, São Paulo, São Paulo, Brazil. [3]Centro de Tecnologia das Radiações, Instituto de Pesquisas Energéticas e Nucleares, São Paulo, São Paulo, Brazil. *Author for correspondence. E-mail: marcialombardo@yahoo.com.br*

ABSTRACT. *Senna* species have been widely used by American, African and Indian ethic groups mainly in the treatment of feebleness, constipation, liver disorders and skin infections. *Senna occidentalis* (L.) Link is a perennial shrub native to South America and indigenous to tropical regions throughout the world. Current study evaluated the antimicrobial activity of aqueous and hydroalcoholic extracts from *S. occidentalis* prepared from different parts of the plant. Antimicrobial activity was assessed against standard pharmaceutical microorganisms by spectrophotometry and microdilution technique. *Escherichia coli* was sensitive only to compounds extracted from seeds which may be proteinaceous. A broader antimicrobial spectrum was demonstrated by the hydroalcoholic extract of seeds, mostly against *Pseudomonas aeruginosa*. The *in vitro* toxicity using mouse fibroblasts indicated that the extract might be a biocompatible ingredient for topical formulations, while the hydroalcoholic extract of aerial parts demonstrated to be potentially cytotoxic.

Keywords: *Cassia occidentalis*, Leguminosae, traditional medicine, antibacterial, antifungal, fibroblasts.

Avaliação *in vitro* de propriedades biológicas de *Senna occidentalis* (L.) Link

RESUMO. Espécies de *Senna* são amplamente utilizadas por tribos americanas, africanas e indianas, principalmente para tratar a fraqueza, a constipação, as desordens do fígado e também em preparações tópicas para infecções de pele. A *Senna occidentalis* (L.) Link é um arbusto perene nativo da América do Sul encontrado em regiões tropicais. Este trabalho avaliou a atividade antimicrobiana de extratos aquosos e hidroalcoólicos de diferentes partes da planta. A atividade antimicrobiana foi estabelecida frente aos microrganismos padrões farmacêuticos por espectrofotometria e técnica de microdiluição. A *Escherichia coli* apresentou sensibilidade apenas a componentes extraídos das sementes, os quais podem ser de natureza proteica. O espectro mais amplo de atividade antimicrobiana foi obtido com o extrato hidroalcoólico das sementes, principalmente contra *Pseudomonas aeruginosa*. A toxicidade *in vitro* utilizando fibroblastos de camundongo indicou que este extrato pode ser um ingrediente biocompatível para formulações de uso tópico. Já o extrato hidroalcoólico de partes aéreas demonstrou ser potencialmente citotóxico.

Palavras-chave: *Cassia occidentalis*, Leguminosae, medicina popular, antibacteriana, antifúngica, fibroblastos.

Introduction

Cassia is a large genus of the family Fabaceae Lindl. (Leguminosae), highly relevant in folk medicine. Several species have been used for centuries by American, African, and Indian tribes, principally as a laxative, hepatoprotective, antimalarial or antimicrobial medicine (LOMBARDO et al., 2009). Scientific data have revealed that *Cassia* spp. comprise a rich source of phenolic derivatives with important biological and pharmacological properties (VIEGAS JÚNIOR et al., 2006).

The species *Senna occidentalis* (L.) Link (syn.: *Cassia occidentalis* L.) is a perennial shrub native to South America and indigenous to tropical regions throughout the world frequently considered a weed growing in wastelands. Although the legume releases a volatile substance with a characteristically fetid odor, its seeds are a substitute for coffee beans in some regions in Northeastern Brazil, Central America, Africa and India (CORRÊA, 1926).

Numerous reports have demonstrated that *S. occidentalis*, which often grows in pastures and among cereal crops, is poisonous to animals that accidentally ingest large amounts of its seeds or food rations contaminated with them (TAKEUTI et al., 2011). According Hueza et al. (2007), even small quantities of seeds in the diet of birds were able to

cause immunotoxic effects, including depletion of lymphoid cells on the spleen and bursa of Fabricius.

In traditional medicine, *Senna* spp. have several therapeutic indications. It is commonly employed to heal skin disorders by topical applications, demonstrating a possible role in the treatment of mycoses, parasitic diseases and eczemas (CACERES et al., 1993; OGUNKUNLE; LADEJOBI, 2006). In Brazilian folk medicine, leaves and seeds of *S. occidentalis* are employed as a topical antifungal agent, especially in the treatment of wounds and mycoses such as ringworms (*tinea corporis*) and the skin eruption *ptiriase versicolor* (FENNER et al., 2006).

Since *S. occidentalis* extract may be used in medical topical preparations, particularly as a natural preservative agent, current study examines the antibacterial and antifungal activities of aqueous and hydroalcoholic extracts prepared from different segments of the plant and checks the toxic effect of their extracts on fibroblasts.

Material and methods

Plant collection

Senna occidentalis was collected from a culture collection maintained at the Instituto Biológico, São Paulo, Brazil. The species was authenticated by Dr. Inês Cordeiro and deposited under the number SP-363817 in the Maria Eneida Fidalgo Herbarium of the Instituto de Botânica, São Paulo, São Paulo State, Brazil. Aerial parts (including flowers, young pods, stems, petioles and predominantly leaves) were dried at 35°C and seeds were dried at room temperature. Aerial parts and seeds were pulverized separately with a grinder and stored at room temperature in closed containers until use.

Preparation of extracts

The hydroalcoholic extract of the aerial parts (HAAP) was obtained with ethanol 75% (v v^{-1}) by slow percolation at room temperature, following the Farmacopeia Brasileira (2010). The ethanol extract was evaporated at 40°C in a rotary evaporator and lyophilized. The seeds' hydroalcoholic extract (HAS) was obtained under the same conditions described above. Seeds were then macerated with 10% (w v^{-1}) phosphate buffer (0.2 M; pH 7.3) for 12 h at 4°C by stirring to obtain the aqueous extract of seeds (AS). Starting from the AS, protein fractionation based on polarity characteristics was performed. The macromolecules showed interesting biological properties in previous studies (LOMBARDO et al., 2004). AS was centrifuged at 10,000 rpm for 30 min. at 4°C. The supernatant was subjected to protein fractionating by precipitation with ammonium sulfate at 25, 50 and 75% saturation (DEUTSCHER, 1990). The protein fractions (F25, F50 and F75) were dialyzed against deionized water and lyophilized.

Phytochemical analysis

Anthraquinones and flavonoids in ethanol extracts (HAAP and HAS) were evaluated by thin layer chromatography (TLC) using silica plates 20 x 15 cm (Merck™ silica gel 60), ethyl acetate: methanol: water (10:1.35:1) as mobile phase and 1,8-hydroxyanthraquinone and quercetin as references. Plates were analyzed under UV light (365 nm) and sprayed with KOH 0.1N or Natural Products reagent (NP) number 28 (WAGNER; BLADT, 1996). The protein content of the aqueous extract of seeds (AS) and its fractions (F25, F50 and F75) was determined by the Lowry method, using bovine serum albumin (BSA) as reference. The Lowry method consists of a colorimetric reaction in which occurs the binding of copper (II) ions with the peptide nitrogens, followed by the reduction of the Folin Ciocalteu reagent by tyrosine, tryptophan and polar amino acids, under alkaline conditions. The chromogenic groups formed have an intense blue color with absorption at 660 nm (WATERBORG, 2009).

Antimicrobial activity

Sample preparation

The ethanol extracts were solubilized in DMSO/MeOH (1:1) at 60 mg mL^{-1} (w v^{-1}). The aqueous extract and its fractions were solubilized in phosphate buffer at 20 mg mL^{-1} (w v^{-1}).

Microbial strains

Antimicrobial activity was performed against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231), and *Aspergillus brasiliensis* (ATCC 16404), formerly *Aspergillus niger* (*A. brasiliensis* Varga et al. deposited as *A. niger* van Tieghem, anamorph). These organisms were obtained from the Laboratório de Materiais de Referência, Instituto Nacional de Controle de Qualidade em Saúde (INCQS), Rio de Janeiro, Brazil. Bacteria were cultured on Tryptone Soy Agar (TSA, Difco™) for 24h at 37°C. *C. albicans* and *A. brasiliensis* were cultured on Sabouraud Dextrose Agar (SDA, Difco™) for 48 h and 5 days respectively, at 25°C. The microbial growth was recovered in a physiological saline solution by adding polysorbate 80 at 0.05% (w v^{-1}) and glass beads to re-suspend *A. brasiliensis*. The number of

Colony-Forming Units (CFU mL^{-1}) of each microbial suspension was determined by pour plate count, following according to United States Pharmacopoeia (2012). Standardized suspensions at a concentration of 5 x 10^4 CFU mL^{-1} were prepared with Müller Hinton broth (Himedia™) for bacteria and Sabouraud Dextrose Broth (SDB, Difco™) for fungi. Cell viability of each inoculum was verified by pour plate count.

In vitro antimicrobial assay

Antibacterial and antifungal activities were determined by spectrophotometry, microdilution protocol, sterile 96-well microplates and ELISA reader (OSTROSKY et al., 2011). Each well was filled to a final volume of 200 μL. Quadruplicates of the extracts (10-20 μL) were incubated with 180-190 μL of bacterial or fungal inoculum. Samples were tested at concentrations (mg mL^{-1}): 0.05, 0.10, 0.20, 0.30 and 0.40. Solutions of chloramphenicol, amycacin, or nystatin at 1 mg mL^{-1} were used as positive antibiotic-containing controls (0.01 mg $well^{-1}$). Sample diluents without micro-organisms were included as negative controls. Wells in each microtiter dish were included to monitor microbial growth (no extracts) and broth sterility (no microorganism). Microplates were incubated for 24h at 37°C (bacteria) or for 48h at 25°C (fungi). Growth of bacteria and *C. albicans* was determined by measuring turbidity values at 630 nm. Growth of *A. brasiliensis* was qualitatively evaluated by visually inspecting and subculturing plating.

Cytotoxic activity

Sample preparation

The ethanol extracts were solubilized in DMSO/MeOH (1:1) at 300 mg mL^{-1} (w v^{-1}), sterilized by filtration (0.45 μm) and then diluted in D-10 medium (DMEM - Dulbecco's modified Eagle's medium Gibco™, supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and glutamineat 4 mM). Samples were tested at concentrations (mg mL^{-1}): 0.005, 0.090, 0.190, 0.375, 0.750, 1.500, 3.000 and 6.000.

Cells

Cytotoxicity was evaluated against Balb/C NIH-3T3 fibroblasts, using sterile 96-well microplates and ELISA reader (FUNARI et al., 2007). After cell cultivation, each well was filled with 120 μL of D-10 medium containing approximately 8 x 10^4 cells mL^{-1} and plates were incubated for 24h at 37°C in a humidified atmosphere and 5% CO_2. The medium was removed and cells were treated with 100 μL of samples.

In vitro toxicity assay

Cell viability was assessed with MTS assay which consists of a colorimetric reduction of tetrazolium salt (MTS) to formazan by metabolically active cells. Cells remained in contact with the extracts for 24 and 48h at 37°C in a humidified atmosphere and 5% CO_2. The wells were then washed, filled with 100 μL of D-10, 20 μL of the Promega™ solution MTS/PMS (phenazine methosulfate) and incubated for 2 h. The absorbance was measured at 490 nm and the toxicity of the extracts on fibroblasts was evaluated by comparing it with that of untreated cells.

Results and discussion

The hydroalcoholic extract of the aerial parts (HAAP) yield was approximately two-fold higher than the hydroalcoholic extract of seeds (HAS). Anthraquinones were not detected by TLC in the aerial parts. According to Rai and Shok (1983), anthraquinones contents in *S. occidentalis* seeds are much greater than in leaves and roots. Aerial parts and seeds both contained flavonoids (Table 1). The abundance of flavonoids in *S. occidentalis* leaves was demonstrated by Purwar et al. (2003).

Table 1. Extraction yield and phytochemical characterization of *Senna occidentalis* hydroalcoholic extracts.

Plant part	Yield[3]	Anthraquinone	Flavonoid
HAAP[1]	0.23	(-)	(+)
HAS[2]	0.12	(+)	(+)

HAAP (hydro-alcoholic extract of aerial parts); HAS (hydro-alcoholic extract of seeds); [1]Aerial parts included 0.65 g of flowers, 2.65 g of young pods, 8.56 g of stems, and 133.38 g of leaves and petioles; [2]156 g of seeds; [3]Values are expressed in g extract g^{-1} plant parts; (+) Presence; (-) Absence.

Source: Author's data.

Plants of the legume family are a rich source of dietary protein. In addition to the nutritional aspects, lectins and proteins function in the plant´s defense and may have antimicrobial properties, (LOMBARDO et al., 2004). Since proteins and peptides were extracted from *S. occidentalis* seeds after the hydroalcoholic extraction, sugars and pigments were eliminated. Table 2 shows protein concentration and protein yield of the aqueous extracts of seeds (AS) and fractions (F25, F50 and F75). The results revealed a predominance of proteins and peptides from *S. occidentalis* seeds with hydrophilic characteristics, i.e., very polar molecules which require a high degree of salt saturation for the removal of water during precipitation. Therefore, F75 had the highest protein concentration.

Table 2. Protein yield of *Senna occidentalis* seeds.

Sample	Protein concentration[1]	Protein yield[2]
AS	0.58	8.70
F25	0.39	2.24
F50	0.21	0.83
F75	1.29	2.13

[1]Estimated values in mg mL^{-1} by the Lowry method and a standard solution of BSA at 1 mg m L^{-1}; [2]Values expressed as mg protein g^{-1} seeds; AS (Aqueous extract of seeds); F25 (Fraction 25); F50 (Fraction 50); F75 (Fraction 75).

Source: Author's data.

In current assay, antimicrobial activities were evaluated by the microdilution technique, a sensitive and reproducible method that enables the analysis of a large number and small amounts of samples. Results demonstrated that *S. occidentalis* extracts have antimicrobial activity against bacterial and fungal pathogens (Table 3).

Although none of the extracts showed any activity against *C. albicans*, the hydroalcoholic extracts moderately inhibited *A. brasiliensis*. The HAS (0.3 mg mL^{-1}) inhibited the growth of *S. aureus* (47%), *E. coli* (35%) and *P. aeruginosa* (78%). HAAP (0.3 mg mL^{-1}) showed activity against *S. aureus* (52%) and *P. aeruginosa* (59%), but *E. coli* was not inhibited by this extract. AS (0.1 mg mL^{-1}) inhibited the growth of *S. aureus* (42%) and *E. coli* (32%) but had no effect on *P. aeruginosa*. An inhibition less than 20% was considered an irrelevant activity.

Table 3. Antimicrobial activity of *Senna occidentalis*.

Sample	Concentration (mg mL^{-1})[2]	Inhibition (%)[1]				
		S. aureus[3]	*E. coli*[3]	*P. aeruginosa*[3]	*C. albicans*[3]	*A. brasiliensis*
AS	0.1	42	32	<20	<20	(-)
F25	0.1	<20	<20	<20	<20	(-)
F50	0.1	<20	<20	<20	<20	(-)
F75	0.1	<20	46	<20	<20	(-)
HAS	0.3	47	35	78	<20	(+/-)
HAAP	0.3	52	<20	59	<20	(+/-)

[1]Inoculum concentration of 10^4 CFU/well; [2]The highest concentrations with the best activity (mg mL^{-1}); [3]Optical density reduction (%); (-) no visual inhibition; (+/-) moderate visual inhibition; AS (Aqueous extract of seeds); F25 (Fraction 25); F50 (Fraction 50); F75 (Fraction 75); HAS (Hydroalcoholic extract of seeds); HAAP (Hydroalcoholic extract of aerial parts).

Source: Author's data.

The above findings show that multiple antimicrobial compounds with diverse activity or mechanism of action are present in the seeds and foliar parts of *S. occidentalis*. Interestingly, the protein fraction F75 (0.1 mg mL^{-1}) showed a moderate but specific activity against *E. coli*, suggesting that this fraction is responsible for the anti-*E. coli* activity of the aqueous extract of seeds (AS). This result indicated that *E. coli* was sensitive to bioactive compounds from *S. occidentalis* seeds (AS and HAS) and some of them may be proteinaceous.

The hydroalcoholic extracts were selected to examine *in vitro* toxicity due to their best antimicrobial performance, discussed previously. While HAS promoted a satisfactory dose-dependent decrease in cellular viability at 24 and 48h, which was < 50% until the concentration of 0.38 mg mL^{-1}, all concentrations of HAAP were toxic to fibroblasts cells within 24h (Figure 1). On the other hand, the high cytotoxic potential of HAAP revealed an interesting activity to be explored, similar to the anticancer substances derived from natural products (SHARMA et al., 2000; CALDERÓN et al., 2006).

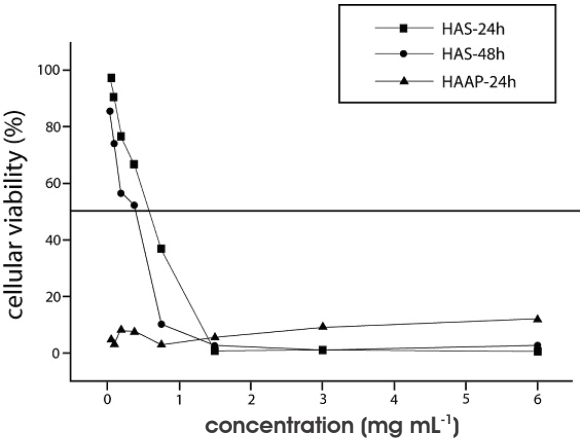

Figure 1. Toxicity of *Senna occidentalis* hydroalcoholic extracts against fibroblast cells. Extracts concentration (mg mL^{-1}): 0.005; 0.090; 0.190; 0.375; 0.750; 1.500; 3.000 and 6.000.

Source: Author's data.

Conclusion

The antimicrobial screening of *Senna occidentalis* plant parts showed that the hydroalcoholic extract of the seeds displayed a broad spectrum of activity, with pronounced inhibition to *Pseudomonas aeruginosa*. At the same time, the *in vitro* toxicity profile of this extract indicated that it might be safe for topical use up to the effective concentration. Results suggest that further analysis of *S. occidentalis* bioactive constituents and its effectiveness in pharmaceutical formulations should be undertaken, particularly when the importance of this species as an antimicrobial for ethnic groups is taken into account.

Acknowledgements

The authors would like to thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for postgraduate scholarship granted to Lombardo M. The authors are deeply grateful to Dr. Lima M.E.L., Ostrosky E.A. and Lopes P.S., for their assistance.

References

CACERES, A.; LOPEZ, B.; JUAREZ, X.; DEL AGUILA, J.; GARCIA, S. Plants used in Guatemala for the treatment of dermatophytic infections. 2. Evaluation of

antifungal activity of seven american plants. **Journal of Ethnopharmacology**, v. 40, n. 3, p. 207-213, 1993.

CALDERÓN, A. I.; VÁZQUEZ, Y.; SOLÍS, P. N.; CABALLERO-GEORGE, C.; ZACCHINO, S.; GIMENEZ, A.; PINZÓN, R., CÁCERES, A., TAMAYO, G., CORRÊA, M.; GUPTA, M. P. Screening of Latin American plants for cytotoxic activity. **Pharmaceutical Biology**, v. 44, n. 2, p. 130-140, 2006.

CORRÊA, M. P. **Dicionário das plantas úteis do Brasil e das exóticas cultivadas**. Rio de Janeiro: Imprensa Nacional, 1926.

DEUTSCHER, M. P. **Guide to protein purification**: **methods in enzymology**. San Diego: Academic Press, 1990.

FARMACOPEIA BRASILEIRA. Rio de Janeiro: Fiocruz, 2010.

FENNER, R.; BETTI, A. H.; MENTZ, L. A.; RATES, S. M. K. Plantas utilizadas na medicina popular brasileira com potencial atividade antifúngica. **Revista Brasileira de Ciências Farmacêuticas**, v. 42, n. 3, p. 369-394, 2006.

FUNARI, C. S.; FERRO, V. O.; MATHOR M. B. Analysis of propolis from *Baccharis dracunculifolia* D.C. (Compositae) and its effects on mouse fibroblasts. **Journal of Ethnopharmacology**, v. 111, n. 2, p. 206-212, 2007.

HUEZA, I. M.; LATORRE, A. O.; RASPANTINI, P. C. F.; RASPANTINI, L. E. R.; MARIANO-SOUZA, D. P.; GUERRA, J. L.; GÓRNIAK, S. L. Effect of *Senna occidentalis* in broiler chickens. **Journal of Veterinary Medicine-Series A**, v. 54, n. 4, p. 179-185, 2007.

LOMBARDO, M.; KIYOTA, S.; KANEKO, T. M. Ethnic, biological and chemical aspects of *Senna occidentalis* (Fabaceae). **Revista de Ciências Farmacêuticas Básica e Aplicada**, v. 30, n. 1, p. 9-17, 2009.

LOMBARDO, M.; IKUNO, A. A.; BALDASSI, L.; FERREIRA, V. C. A.; KIYOTA, S. Evaluation of protein fractions from *Senna occidentalis* seeds extracts for cytotoxic, antiviral and antibacterial activities. **Virus Reviews and Research**, v. 9, n. 2, p. 61-68, 2004.

OGUNKUNLE, A. T. J.; LADEJOBI, T. A. Ethnobotanical and phytochemical studies on some species of *Senna* in Nigeria. **African Journal of Biotechnology**, v. 5, n. 21, p. 2020-2023, 2006.

OSTROSKY, E. A.; MARCONDES, E. M. C.; NISHIKAWA, S. O.; LOPES, P. S.; VARCA, G. H. C.; PINTO, T. J. A.; CONSIGLIERI, V. O.; BABY, A.R.; VELASCO, M. V. R.; KANEKO, T. M. *Rubus rosaefolius* extract as a natural preservative candidate in tropical formulations. **AAPS PharmSciTech**, v. 12, n. 2, p. 732-737, 2011.

PURWAR, C.; RAI, R.; SRIVASTANA, N.; SINGH, J. New flavonoid glycosides from *Cassia occidentalis*. **Indian Journal of Chemistry Section B**, v. 42, n. 2, p. 434-436, 2003.

RAI, P. P.; SHOK, M. Anthraquinone glycosides from plant parts of *Cassia occidentalis*. **Indian Journal of Pharmaceutical Sciences**, v. 45, n. 2, p. 87-88, 1983.

SHARMA, N.; TRIKHA P.; ATHAR M.; RAISUDDIN, S. *In vitro* inhibition of carcinogen-induced mutagenicity by *Cassia occidentalis* and *Emblica officinalis*. **Drug Chemistry and Toxicology**, v. 23, n. 3, p. 477-484, 2000.

TAKEUTI, K. L.; RAYMUNDO, D. L.; BANDARRA, P. M.; OLIVEIRA, L. G. S.; BOABAID, F. M.; BARRETO, L.; DRIEMEIER, D. Outbreak of poisoning by *Senna occidentalis* in grazing cattle. **Acta Scientiae Veterinariae**, v. 39, n. 1, p. 954-957, 2011.

UNITED STATES PHARMACOPOEIA. Rockville: The US Pharmacopoeial Convention, 2012.

VIEGAS JÚNIOR, C.; REZENDE A.; SILVA, D. H. S.; CASTRO-GAMBÔA, I.; BOLZANI, V. S. Aspectos químicos, biológicos e etnofarmacológicos do gênero *Cassia*. **Química Nova**, v. 29, n. 6, p. 1279-1286, 2006.

WAGNER, H.; BLADT, S. **Plant drug analysis**. New York: Springer, 1996.

WATERBORG, J. H. The Lowry method for protein quantification. In: WALKER, J. M. (Ed.). **The proteins protocols handbook**. New York: Humana Press, 2009. p. 7-10.

Received on December 2, 2013.
Accepted on December 4, 2014.