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African Journal of Pharmacy and Pharmacology

8 August, 2018
ISSN 1996-0816
DOI: 10.5897/AJPP
www.academicjournals.org

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African Journal of Pharmacy and Pharmacology

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Vol. 12(23), pp. 310-318, 8 August, 2018
DOI: 10.5897/AJPP2018.4947
Article Number: EAA2D9857998
ISSN: 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Cytotoxic potential of ethanol extract of *Parquetina nigrescens* on MCF-7, C4-2WT, HT 29 and HTC 116 cell lines

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Received 29 June, 2018; Accepted 16 July, 2018

The cytotoxic activity of ethanol extract of *Parquetina nigrescen* was investigated using a (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, methylene blue and Trypan Blue exclusion assay on four human cancer cell lines, MCF-7, HT 29, HTC 116 and C4-2WT. A mitochondrial enzyme in living cells, succinate-dehydrogenase cleaves the tetrazolium ring and converts the MTT to an insoluble purple formazan whose intensity is directly proportional to the presence of viable cells in the microwell plate. Results showed a significant ($p<0.05$) cytotoxic effect of the extract in a dose dependent manner. Cytotoxicity increased with increase in the concentration of the extract used. GI50 results calculated after MTT test showed the concentration of ethanol extract of *P. nigrescens* required for 50% inhibition of the different cell lines as follows: MCF-7 = 2.61 µg/ml, C4-2WT = 8.33 µg/ml, HCT 29 = 3.47 µg/ml and HCT 116 =1.75 µg/ml. For the methylene blue assay, the number of viable cells present was significantly reduced ($p<0.05$) with an increase in the concentration of the extract and duration of exposure of the cells to the extract. The result of trypan blue assay showed a significant reduction ($p<0.05$) in the total count of viable cells and a significant increase ($p<0.05$) in the total count of non-viable cells over 72 h post-treatment with an extract of *P. nigrescens*. Comparatively, results obtained indicate that there is a correlation between the various methods adopted in establishing the antiproliferative and cytotoxic activity of ethanol extract of *P. nigrescens* obtained in this study.

Key word: *Parquetina nigrescens*, cytotoxicity, human cell lines, MTT, trypan blue, methylene blue.

INTRODUCTION

Cancer is a generic term for a large group of diseases that can affect any part of the body and is characterized by the rapid creation of abnormal cells that grow beyond their usual boundaries. Abnormal proliferating cells can invade adjoining parts of the body and spread to other organs. This latter process is referred to as metastasizing

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and metastases are a major cause of death from cancer. The World Health Assembly in 2017, passed the resolution Cancer Prevention and Control through an Integrated Approach (WHA70.12) urges governments and WHO to accelerate action to achieve the targets specified in the Global Action Plan *and* 2030 UN Agenda for Sustainable Development to reduce premature mortality from cancer (WHO, 2013). Despite the promotion of synthetic chemistry as a method of drug discoveries and drug productions, the contribution of new and novel products from potential bioactive plants or their extracts for disease treatment and prevention is still vast (Kviecinski et al., 2008). Numerous phytochemical compounds found in plants with anticancer properties include: alkaloids, phenylpropanoids, and terpenoids (Kintzios, 2006; Park et al., 2008). Some plant-derived drugs like vinblastine, vincristine, taxol, and camptothecin, with antitumor potentials, have been reported to be efficacious (Yousefzadi et al., 2010). Plants contain the unlimited capacity to generate compounds that fascinates researchers in the quest for new and novel chemotherapeutics (Reed and Pellecchia, 2005).

Phytochemicals in plants over the past century have been a pivotal pipeline for pharmaceutical discovery. The importance of the active ingredients of plants in agriculture and medicine has stimulated significant scientific interest in the biological activities of these substances (Moghadamtousi et al., 2013). Despite these studies, a limited range of plant species has experienced detailed scientific inspection. The attainment of an understanding of natural products necessitates comprehensive investigations on the biological activities of these plants (Moghadamtousi et al., 2014). Certain African plants have a long history of use in ethnomedicine and are a rich source of active phytoconstituents that provide medicinal or health benefits against various diseases. One such plant with extensive traditional uses is *Parquetina nigrescens*.

Also known as bullock, *P. nigrescens* is a shrub found in equatorial West Africa and its leaves, roots and latex have been in traditional medicine practice for centuries (Owoyele et al., 2011). It occurs in secondary forest, savanna, vegetation bordering roads and gallery forest, also commonly growing on ant-hills. It grows on various types of soil, including marshy areas (Alvarez Cruz, 2012). *P. nigrescens* is used in traditional medicine in small amounts, as the plant is toxic, especially the latex. Many fatal accidents have been recorded. The plant or leaf decoction is taken as an enema to treat serious kidney problems, severe constipation and to induce abortion.

Sometimes freshly crushed leaves are taken as an emetic to treat severe constipation (Imaga et al., 2009). A leaf decoction or infusion of *P. nigrescens*, sometimes with parts of other plant species, is drunk to treat measles, intestinal worms, diarrhoea, dysentery, diabetes, menstrual disorders and venereal diseases. It is given to children in very small quantities, to treat respiratory diseases (Agbor and Odetola, 2005). A leaf decoction with honey added is drunk to treat fatigue, jaundice, stomach ulcers and anaemia, as a tonic. It is also taken to treat hypotension and to ease child birth. The body is washed with a leaf decoction to treat general fatigue. The leaves are a common ingredient in medications to treat insanity (Kayode et al., 2009). In Nigeria *P. nigrescens*, *Sorghum bicolor* (L.) Moench and *Harungana madagascariensis* Lam. ex Poir, (Jubi formular), is marketed as a constituent of a commercial herbal preparation to treat anaemia (Alvarez Cruz, 2012). Extract of *P. nigrescens* has been shown to have a high content of flavonoids, saponins, glycosides, cardiac glycosides, tannins, anthraquinones, phlo- batannins and oils and its antioxidative properties have been reported (Ayoola et al., 2011).

Chemoprevention by natural products may be considered a promising approach to cancer control and management (Karikas et al., 2010). Many studies have demonstrated antiproliferative, cytostatic and cytotoxic activities of phytochemicals against cancer cells (Cordaliza et al., 2007). In this study, the ethanol extract of *P. nigrescens* was tested as potential anticancer agent. The antitumoral activity of this plant extract was tested on four human cancer cell lines: MCF-7 (breast carcinoma cells), C4-2WT (prostate carcinoma cells, HCT 29 and HCT 116 (Colorectal carcinoma cells). Cytotoxicity tests implored include MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), methylene blue proliferative assay and trypan blue assay for cell count.

MATERIAL AND METHODS

Plant material and extraction

The dried leaves of *P.* nigrescens were collected from Ghana. Plant materials were ground to a powder form using an electric mill. The powdered sample was kept in an airtight container until required. About 50 g of the powdered leaves of *P. nigrescens* was macerated in 250 mL of aqueous ethanol (70:30) for 72 h. The vacuum pump was used for filtering and the ethanol plant material was dried over a water bath at 40°C and the resulting extract was kept in the refrigerator at -4°C.

Reagents

Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) and all of other chemicals and reagents used were obtained from Sigma Aldrich and are of analytical grade.

Cell lines

All cell lines used during the present study were obtained from

Tissue Culture Unit of Gene Regulation and RNA Biology Laboratory of the School of Pharmacy, University of Nottingham, United Kingdom. These cell lines were: 1) MCF-7 (breast carcinoma cells), 2) C4-2WT (prostate carcinoma cells, 3) HCT 29 and 4) HCT 116 (Colorectal carcinoma cells). The cells were cultured at 37°C in a humidified 5% CO_2 incubator.

The cell lines were cultured at 37ºC in an atmosphere of 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM L-glutamine and 10% foetal calf serum (FCS), and routinely sub-cultured twice weekly to maintain continuous logarithmic growth.

Ethanol extracts of *P. nigrescens* were prepared as 50 mg stock solutions dissolved in dimethyl sulfoxide (DMSO) and stored at -4ºC, for a maximum period of 4 weeks. Extract dilutions were made in culture medium immediately prior to use.

Preparation of extract stock and working solution

Fifty milligrams of the extract was dissolved in 1 ml of DMSO to give a stock solution of 50 mg/ml. A working stock of 500 µg/ml was freshly prepared from the 50 mg/ml stock solution using DMEM and various working concentrations of equal volume made by dilution with DMEM to obtain the desired concentration of the extract. The working concentration was prepared freshly and filtered through 0.45-micron filter before each assay. The remaining working solutions were discarded. DMSO of corresponding concentrations was used as a control.

Cytotoxicity screening

Growth inhibitory assays

3-(4,5-dimethylthiazol-2-yl)-2,5 phenyltetrazolium bromide (MTT): Cells were seeded into 96-well microtitre plates at a density of 3.0 - 4 x 10^3 per well and allowed 24 h to adhere. Before drugs were introduced (final concentration 0.1 µg to 100 µg/ml, n=6), extract dilutions as well as DMSO control were prepared using DMEM as diluents immediately prior to each treatment. Ethanol fractions of *P. nigrescens* were dissolved in DMSO and diluted with complete DMEM medium to get a range of test concentration (0.1 µg to 100 µg/ml). DMSO concentration was kept less than 0.1% in all the samples. Prepared dilutions were added to different wells, and cells were incubated for 72 h. Control groups received the same amount of DMSO. Viable cells at the time of extract addition were time zero; (T0), and following 72 h, the effect of exposure to extract were determined by cell-mediated 3-(4,5-dimethylthiazol-2-yl)-2,5 phenyltetrazolium bromide (MTT) reduction. MTT was added to each well (final concentration 400 µg/ml) and plates were incubated at 37ºC for 4 h to allow reduction of MTT by viable cell dehydrogenases to an insoluble formazan product. Well supernatants were aspirated and cellular formazan solubilised by addition of DMSO: glycine buffer (pH 10.5; 4:1). Cell growth and agent activity were determined by measuring absorbance at 580 nm using the BioTek Synergy HTX Multi-Mode Microplate Reader. The GI_{50} values of ethanol extract of *P. nigrescens* were calculated for the four different cell lines - MCF7-, HT 29, HTC 116 and C4-2WT and compared statistically with the control. The American National Cancer Institute renamed the IC_{50} value, the concentration that causes 50% growth inhibition as GI_{50} value to emphasize the correction for the cell count at time zero; therefore, the GI_{50} measures the growth inhibitory power of the test agent and is calculated thus:

$$OD\ GI_{50} = (Cont - To)/2+To \quad (1)$$

Insert computed OD GI_{50} value into Equation 2

$$GI50 = (HOD - OD\ GI_{50})/(HOD - LOD) * (HC - LC) + LC \quad (2)$$

Where, OD GI_{50} = Optical Density of GI_{50}; Cont = optical density of non- treated; To = optical density at time zero; HOD = high optical density within which GI_{50} falls; LOD = low optical density within which GI_{50} falls; HC = High Conc. within which GI_{50} falls; LC = Low Conc. within which GI_{50} falls. Viable cells were determined by the absorbance at 580 nm after MTT. Measurements were performed and the concentration required for a 50% inhibition of viability (GI50) was determined graphically.

Methylene blue proliferation assay

A modified method of Oliver et al. (1989) was adopted. The cells were counted in a haemocytometer and the cell suspension was diluted with DMEM to give a density of 5.0 x 10^3 per well. Cell suspensions were introduced into 96- microtitre plates using a repeating pipette with sterile tip. Cells were seeded for 24 , 48 and 72 h for each cell line and allowed 24 h to adhere before extract was introduced (final concentration 10 µg -100 µg/ml, n=6). Cells for day 0 were counted 3-4hrs giving time for cells to adhere and then methylene blue assay was carried out. Assays were carried out 24, 48 and 72 h respectively.

Fixation of cells: The culture medium in each well was removed by gentle vacuum aspiration using a Pasteur pipette with a fine angled tip. The cell layer was then fixed by adding 100 µl of 100% methanol to each well and let stand for 30 min.

Cell staining: The fixative was removed by gentle vacuum aspiration using a Pasteur pipette and 100 µl of filtered 1 % (w/v). Methylene blue was added to each well. Methylene blue stains only the viable (live) cells. After 30 min, excess dye was removed by another gentle vacuum aspiration using a Pasteur pipette. The remaining dye was then washed off by serially dipping the plate into each of four tanks of distil water, shaking the water off between each immersion. This was done in a uniform manner to minimize between-plate variation. After the last rinse and shake, the cell layer, still stained with methylene blue, was examined microscopically. To elute the dye, 100 µl of 1:1 (v/v) ethanol and 0.I M-HCl was added to each well. The plates were then agitated on a plate shaker for 30 min to release the fixed stain and the optical density was measured at 650 nm for each well by BioTek Synergy HTX Multi-Mode Microplate Reader. The photometer was blanked on the last two rows of control wells containing elution solvent alone. Results were reported based on the 72 h assay.

Trypan blue exclusion assay

A modified method stated by Karthik Raman (2016) was adopted for this study. The cells were counted in a haemocytometer and the cell suspension was diluted with DMEM to give a density of 10.0 X 10^3 per mL per well. Cell suspensions were introduced in triplicates into 6- well plates using a sterile disposable pipette. Cells were seeded for 24, 48 and 72 h for each cell line and allowed 24 h to adhere before extract was introduced (final concentration; 10 µg - 40 µg/ml, n=6). Assays were carried out 24, 48 and 72 hrespectively for each cell line.

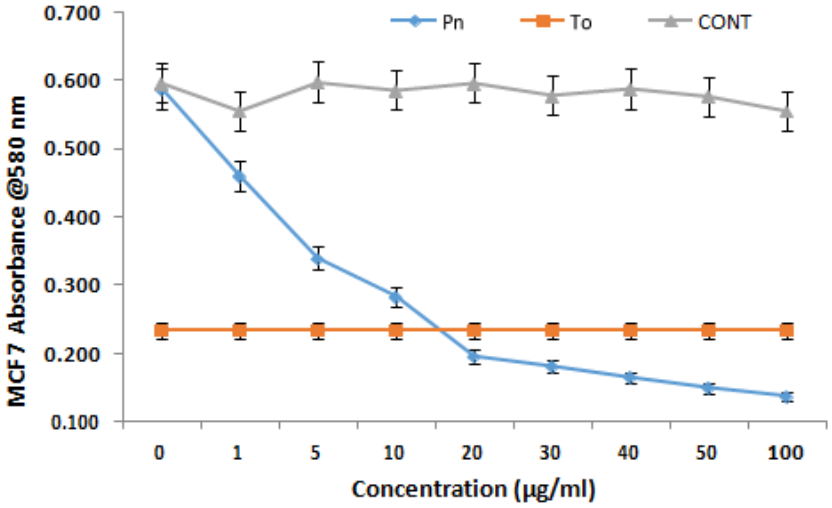

Figure 1. Cytotoxic effect ethanol extract of *P. nigrescens* on MCF 7 after 72 h treatment.

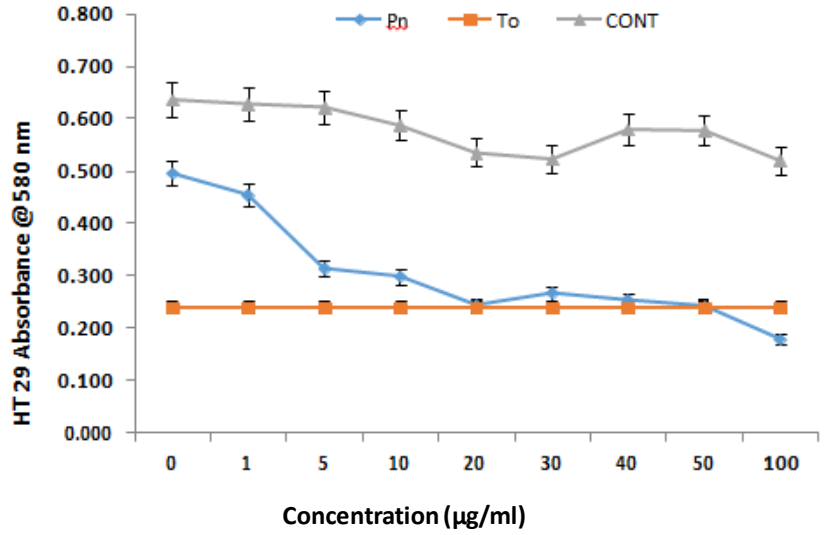

Figure 2. Cytotoxic effect ethanol extract of *P. nigrescens* on HT 29 after 72 h treatment.

The culture medium in each 6-well plate was removed by gentle vacuum aspiration using a Pasteur pipette with a fine angled tip. The wells were washed with warm sterile phosphate buffered saline (PBS) and aspirated off into the waste pot and 500 µl of 0.05% trypsin in 0.53 mM EDTA (enough to cover the cell surface) was added. This was incubated at 37°C for 5 min until the cells have dissociated. A tap to the side of the flask can encourage recalcitrant cells to let go. Cells were resuspended in 500 µl of fresh medium bringing the total volume to 1 mL. To check the concentration of dead cells, 95 µl cell suspension from each well was transferred into well labelled 0.5 ml Eppendorf tubes and 5 µl trypan blue added and count using the haemocytometer. Dead cells stained blue.

RESULTS

MTT

Concentrations of 0.1 to 100 g/mL of *P. nigrescens* extracts showed an increase ($p<0.05$) in cytotoxicity activity on MCF-7 C4-2WT, HCT 29 and HCT 116 as compared to the untreated control cells (Figures 1 to 4). The concentration required for a 50% inhibition of viability (GI_{50}) was determined by substituting the values in Equation 2 for calculation of GI_{50}. GI_{50} results calculated

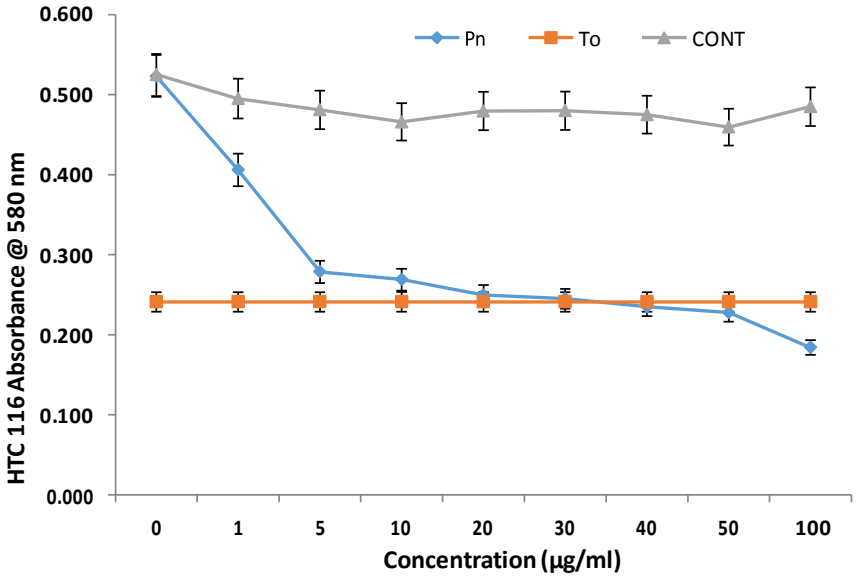

Figure 3. Cytotoxic effect ethanol extract of *P. nigrescens* on HTC 116 after 72 h treatment.

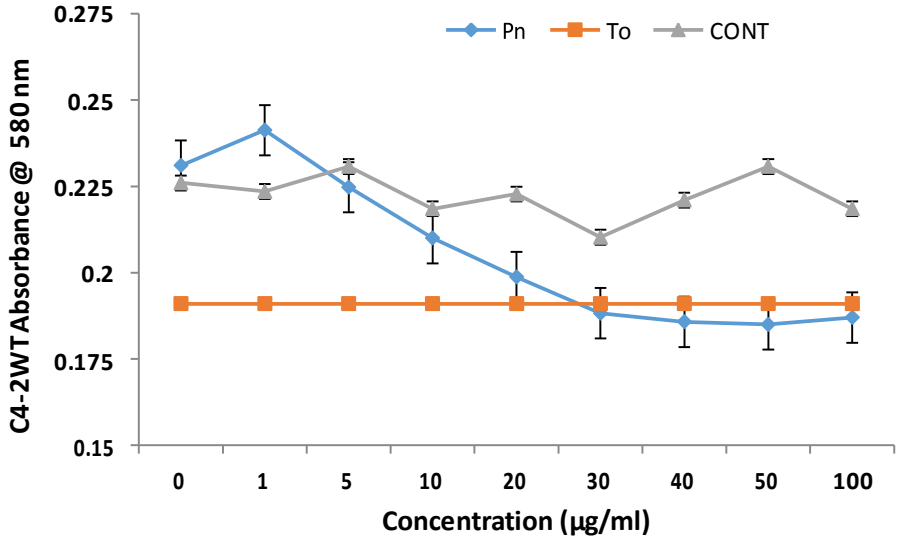

Figure 4. Cytotoxic effect ethanol extract of *P. nigrescens* on C4-2WT after 72 h treatment.

after MTT test showed the concentration of ethanol extract of *P. nigrescens* required for 50% inhibition of the different cell lines as follows: MCF-7 = 2.61 µg/ml, C4-2WT = 8.33 µg/ml, HCT 29 = 3.47 µg/ml and HCT 116 =1.75 µg/ml. However, according to the criteria of the American National Cancer Institute, the GI_{50} limit to consider a crude extract promising for further purification is lower than 30 µg/ml.

Methylene blue assay

The result of the methylene blue colorimetric micro titre plate assay for determining the response of monolayers of the different cell lines to ethanol extract of *P. nigrescens* showed linearity in the relationship between different concentrations of the extract used and their optical densities at 24, 48 and 72 h (Figures 5 to 7). This

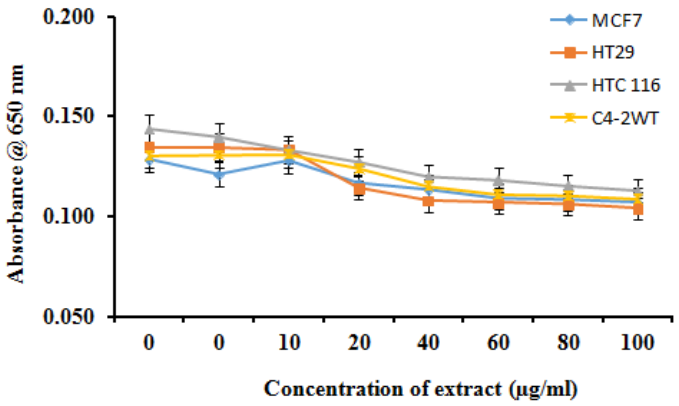

Figure 5. Optical density of viable cell lines 24 h after treatment with ethanol extract of *P. nigrescens.*

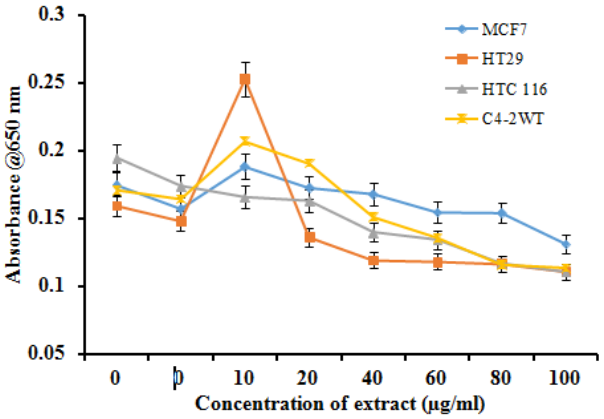

Figure 6. Optical density of viable cell lines 48 hrs after treatment with ethanol extract of *P. nigrescens.*

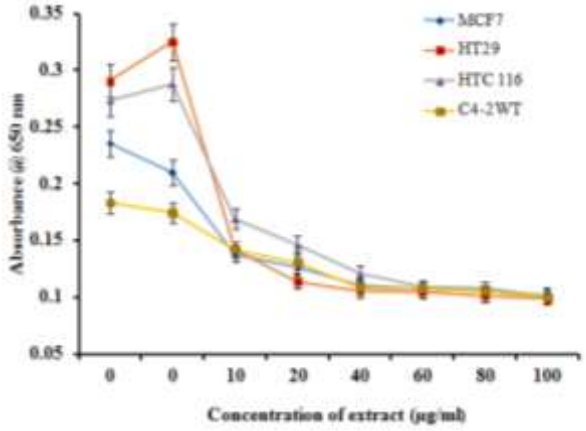

Figure 7. Optical density of viable cell lines 72 hrs after treatment with ethanol extract of ***P. nigrescens.***

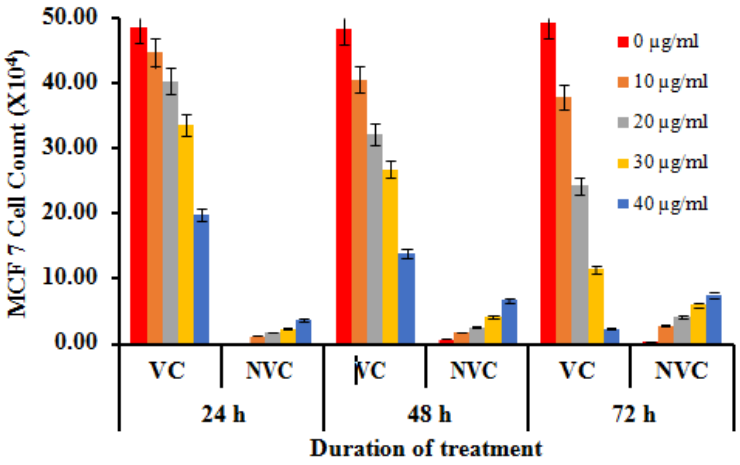

Figure 8. Cell count of viable (VC) and non-viable (NVC) MCF 7 cells after treatment with ethanol extract of *P. nigrescens.*

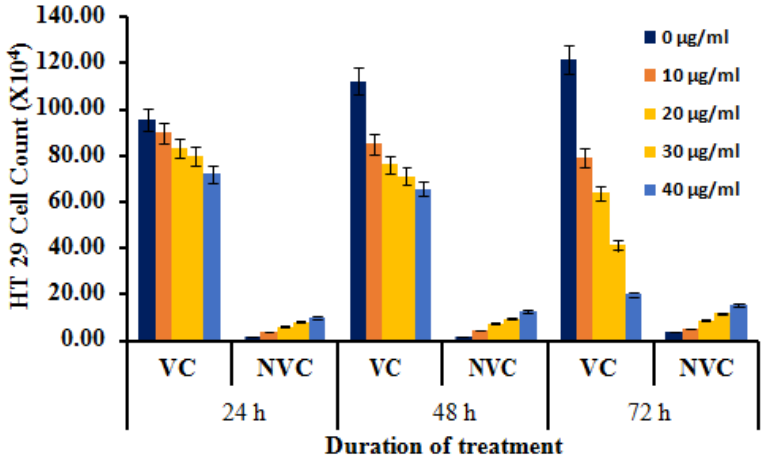

Figure 9. Cell count of viable (VC) and non-viable (NVC) HT 29cells after treatment with ethanol extract of *P. nigrescens.*

was confirmed for each cell line and when initial cell density was optimized to give exponential growth over the assay period, differences in response to different concentrations of the extract were obvious. The methylene blue colorimetric microtitre plates assay was found to be a simple, reliable, sensitive method with low variability, for determining the response of cultured cells lines to the inhibitory agent.

Trypan blue exclusion assay for cell count

Trypan blue exclusion assay counts the number of dead cells in a given cell population. Because trypan blue is a charged dye, it cannot permeate through living cells. So, simply incubating cells with trypan blue and looking under a microscope allows you to visually determine the number of viable cells (unstained), a number of non-viable cells (dark blue), and the number of damaged cells (slightly blue). Viable cells (VC) and non-viable cells (NVC) were counted for each concentration of extract and cell lines. Results obtained showed a significant decrease ($p<0.05$) in the total number of viable cells and a significant increase ($p<0.05$) in the total number of non-viable cells with an increase in extract concentration (Figures 8 to 11). Inhibition of cell growth and proliferation of cells by *P. nigrescens* occurred in a dose dependent manner.

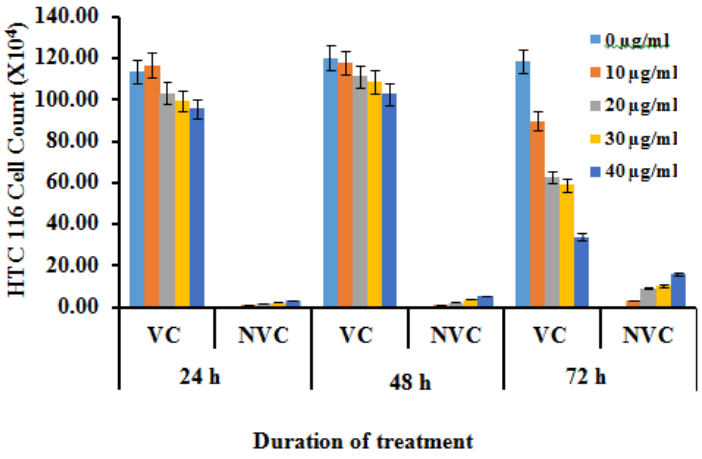

Figure 10. Cell count of viable (VC) and non-viable (NVC) HT 116cells after treatment with ethanol extract of *P. nigrescens.*

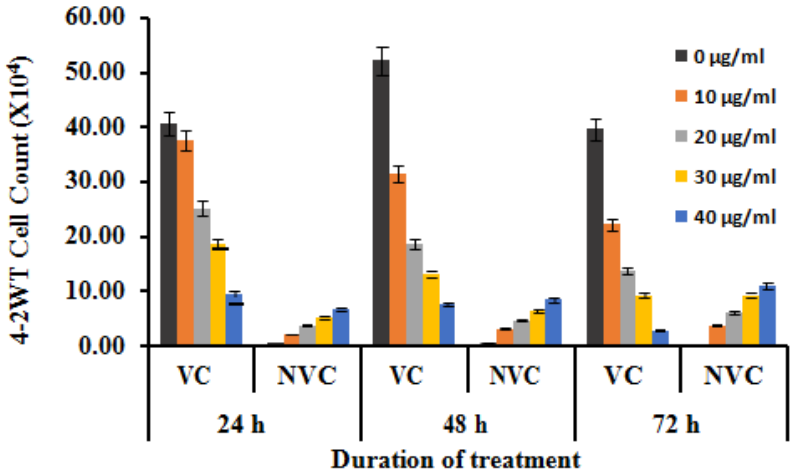

Figure 11. Cell count of viable (VC) and non-viable (NVC) C4-2WTcells after treatment with ethanol extract of *P. nigrescens.*

DISCUSSION

MTT reduction assay is carried out to evaluate the activity of mitochondrial and non-mitochondrial dehydrogenases of compounds as a potential indication of their cytotoxic effect (Döll-Boscardin et al., 2012). Also, a mitochondrial enzyme in living cells, succinate-dehydrogenase cleaves the tetrazolium ring and converts the MTT to an insoluble purple formazan. The amount of formazan produced is directly proportional to the number of viable cells. The *nigrescens* as a potential anticancer agent. Cytotoxicity may be due to loss of cellular function and viability either through necrosis or by apoptosis caused by *P. nigrescens* which is an exogenous or foreign agent to the affected cells. Mosmann (1983), showed that methanol cytotoxic activity of ethanol extract of *P. nigrescens* was investigated using an MTT assay on four human cancer cell lines, MCF-7, HT 29, HTC 116 and C4-2WT. Results showed a significant ($p<0.05$) cytotoxic effect of the extract in a dose dependent manner. Cytotoxicity increased with increase in the concentration of the extract used. Similarly, 50% inhibition of viability (GI_{50}) of the extract on the four cell lines was less than 30 µg/ml; this is below the criteria of the American National Cancer Institute and indicates the prospect of *P.* extract significantly inhibited cancer cell growth at a concentration of 100 µg/ml due to the presence of compounds in the extract. Similarly, Akiriti et al. (2014) in their *in- vitro* cytotoxicity study of methanolic fraction from *Ajuga bracteosa* wall ex. benth on MCF-7 breast

adenocarcinoma and hep-2 larynx carcinoma cell lines showed that significant cytotoxic activity was detected for the methanolic fraction of *Ajuga bracteosa* (aerial part) presenting IC_{50} values lower than 5 and 10 µg/ml against two cell lines (MCF-7 and Hep-2). Furthermore, Adu-Amoah et al. (2014), reported that significant reduction in the viability of the HaCaT keratinocytes was observed from treatment with 10, 50 and 100 81 µg/mL of leaf extract of *E. ivorense*, 0.1 to 100 µg/mL of bark extracts of *E. ivorense* ($p<0.0001$) and 100 µg/mL leaf and other aerial parts extract of *P. nigrescens* ($p<0.01$) as compared with the untreated cells.

The linearity of the methylene blue and Trypan Blue exclusion assay was carried out to demonstrate the presence of viable and non-viable cells in the media after 24, 48 and 72 h respectively. For the methylene blue assay, the number of viable cells present was significantly reduced ($p<0.05$) with an increase in the concentration of the extract and duration of exposure of the cells to the extract. The intensity of the medium is directly proportional to the concentration of the dye eluted from the viable cells and this is a function of the total number of viable cells present in the microplate wells. This observation suggests that cytotoxic effect of ethanol extract of *P. nigrescens* is dose and time dependent. On the other hand, the Trypan Blue exclusion cell count is a measure of the number of non-viable cells observed during cell count. The non-viable cells stained dark blue under the light microscope. A significant reduction ($p<0.05$) in the total count of viable cells and a significant increase ($p<0.05$) in the total count of non-viable cells over 72 h post treatment with an extract of *P. nigrescens* was an indication of non-proliferation of the cells due to the cytostatic or cytotoxic activity of the extract.

Conclusion

Results indicate that there is a correlation between the various methods adopted in establishing the anti-proliferative and cytotoxic activity of ethanol extract of *P. nigrescens* obtained in this study. However, this plant has shown pronounced cytotoxic activity against some human cell lines and will be evaluated further for the possible isolation of active anticancer compounds.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Vol. 12(23), pp. 319-328, 8 August, 2018
DOI: 10.5897/AJPP2018.4936
Article Number: 432B0F558003
ISSN: 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Cross-cultural adaptation and validation to Brazil of the "Medication Counseling Behavior Guidelines"

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Received 2 June, 2018; Accepted 18 July, 2018

This work aimed to translate cross-culturally adapts, and validate the "Medication Counseling Behavior Guidelines" instrument into Brazilian Portuguese. The process of cross-cultural adaptation was carried out using international recommendations. The generated versions were evaluated for the semantic, idiomatic, cultural, and conceptual equivalences and the pre-test was carried out with undergraduate pharmacy students. The reliability of the instrument was evaluated through inter-observer reliability, test-retest, and internal consistency tests. The final version was submitted for content validation. The process of cross-cultural translation and validation result in the Brazilian-Portuguese version of the tool was done. During the translation and back-translation stages, only grammatical changes were made to establish cross-cultural equivalence between the versions under analysis. Regarding the semantic evaluation, six items (15.4%) revealed less than 80% agreement between the judges and were adjusted. Agreement greater than 80% was verified for all items assessed as cultural and conceptual equivalences. In the pre-test, four items (10.2%) were modified. Inter-observers and test-retest reliability demonstrated good to excellent reproducibility for most items (ICC = 0.60-0.98) and internal consistency was considered high (Cronbach's alpha = 0.99). Psychometric evaluation demonstrated and confirmed the validity of the Brazilian-Portuguese version of the tool to assess patient counseling practices. The tool can be used by pharmacists and undergraduate pharmacy students to improve the quality of patient counseling.

Key words: Validation studies, health communication, counseling, simulation training.

INTRODUCTION

Pharmacists have been identified as important professionals in counseling patients regarding the rational use of medicines (Melo et al., 2017; Alaqeel and Abanmy, 2015). They are also considered strategic healthcare professionals to identify, solve, and prevent drug therapy problems (Castronovo et al., 2018; Huysmans et al., 2014). For this reason, pharmacists must establish an effective therapeutic relationship with patients. The quality

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of the pharmacist-patient relationship depends, above all, on the quality of the communication established between them over time. This relationship does not necessarily improve with professional experience but will be more effective as the professional receives more education and training on communication (Cantwell et al., 2011; Moore et al., 2013).

A key component in health education training has been the development and validation of communication skills assessment tools for use in encounters with simulated or real patients. In a systematic review, it was showed that communication assessment tools vary considerably in content, psychometric properties, and usability. Moreover, no revised instrument was well evaluated in all these categories (Schirmer et al. 2005). Although the fields of medicine and nursing have excelled in the development and use of validated instruments and standard assessment methods, there is growing concern about the authenticity and validity of clinical skills assessments of healthcare professionals and students (Barros et al., 2015; Cunha et al., 2017; Jesus et al., 2015).

In pharmacy field, some organizations have published specific guidelines about patient counseling since the 1960s (American Society of Health-System Pharmacists, 1997; De Young, 1996). However, the literature lacks studies on the validation of instruments and procedures used to evaluate the communication skills of pharmacists and undergraduate pharmacy students (Jesus et al., 2016; Wallman et al., 2013). An instrument applicable to this purpose is the “Medication Counseling Behavior Guidelines,” which is considered the first attempt to approach patient counseling skills within the context of pharmacist-patient communication (Federation International Pharmaceutical, 2005). It is a validated instrument for the English language, developed by the United States Pharmacopeia (USP) and is currently considered the most understandable model of approaching patient counseling for its completeness in evaluating pharmaceutical competencies in patient care (Federation International Pharmaceutical, 2005; Puumalainen et al., 2005). Thus, the aim of this study was to translate and cross-culturally adapt the instrument "Medication Counseling Behavior Guidelines" into Brazilian Portuguese.

MATERIALS AND METHODS

Study design

An adaptation and validation study was performed from March to December 2012 in the Northeast region of Brazil for the cross-cultural adaptation of the "Medication Counseling Behavior Guidelines" into Brazilian Portuguese. This instrument was designed by United States Pharmacopeia (USP) (Puumalainen et al., 2005) and contains 35 questions divided into four categories:

1) Counseling introduction: included items related to initial counseling, such as providing basic and pertinent information related to drugs, and understanding the clinical conditions of the patient;
2) Counseling content: covered items related to drug selection, instructions for use, storage, and general impression about the pharmacist's knowledge;
3) Counseling process: contained elements of non-verbal communication, counseling understanding, and overall impression of the counseling service;
4) Counseling conclusion: covered if the pharmacist addressed a consoling conclusion, verified patients' understanding, and prepared follow-up plan.

Participants

Pharmacy undergraduate students of three educational institutions of Sergipe state, Federal University of Sergipe (two campuses in different cities: São Cristovão and Lagarto) and Tiradentes University were selected to compose the sample. Students of both genders, volunteers, and that were enrolled in the second or third year of undergraduate Pharmacy course were randomly selected. Students who had not attended the “Pharmaceutical Services” subject were excluded because they had not learned yet the theoretical-practical references necessary for "Good Pharmaceutical Dispensing" (Marques and Lyra Junior, 2012). All students that agreed to participate were informed of the study’s purpose and invited to sign the Consent Form.

Cross-cultural adaptation

The protocol of cross-cultural adaptation was conducted using recommendations from international literature (Beaton et al., 2000; Gasparino and Guirardello, 2009; Guillemin et al., 1993; Guillemin, 1995; Pasquali, 2010):

Translation

The instrument was translated from English into Portuguese by two Pharmacy researchers, experts in communication with patients, fluent in English, and having Portuguese as their native language. These experts knew the objectives and conceptual framework of the study. The two translations were compared, and ambiguities or discrepancies in the translated words were addressed, generating a consensually translated version (Version 1) (Beaton et al., 2000).

Back translation

During the back-translation process, Version 1 of the instrument in Portuguese was translated again into the original language (English) by two different translators who did not participate in the previous stage, as well as by researchers and specialists in the field of Pharmacy. Translators had Portuguese as their native language, had lived in the USA and Australia for more than 25 years, and were fluent in both languages. These experts did not receive information about the objectives and concepts underlying the study (Guillemin et al., 1993). The two translations were compared, and the ambiguities or discrepancies were solved by a consensual translation (Version 2).

Expert panel

Two expert panels compared all versions (original, translated, and back-translated), evaluating the items according to semantic, idiomatic, cultural, and conceptual equivalences (Beaton et al., 2000). Two specific assessment forms were adapted and used for

analysis of equivalence: "Evaluation of semantic and idiomatic equivalents" and "Evaluation of cultural and conceptual equivalents" (Lino, 1998).

Expert panel 'A' was composed by five researchers specialized in the field of knowledge, selected by convenience. They evaluated the semantic and idiomatic equivalence comparing the original instrument with the translated and back-translated versions, and the evaluation form. This expert panel was asked to document the reason for each proposed change in the "Evaluation of semantic and idiomatic equivalents" form. At the end of this step, Version 3 of the instrument was generated.

Subsequently, expert panel 'B' evaluated cultural and conceptual equivalence by comparing the original scale with the translated version. It was considered as appropriate translation, the questions accepted by at least 80% of experts (Beaton et al., 2000; Fumimoto et al., 2001). The expert panel was formed by Pharmacy teachers and/or researchers in the field of knowledge and selected by convenience. In addition, each expert was native and resident of each of the five Brazilian regions. During this step, Version 4 of the instrument was generated.

Pre-test

This step consisted of administering the translated version of the instrument (Version 4) to a suitable sample of 40 Pharmacy undergraduate students attending a Brazilian public university, in Aracaju city, Sergipe state. The sample size of the pre-test was based on the literature, which suggests 30 to 50 individuals from the target population (Beaton et al., 2000; Gasparino and Guirardello, 2009).

Pharmacy students received guidance on using the scale and completing the instrument. Students completed the instrument by evaluating the pharmacist, based on an audiovisual recording from a simulated patient case. Each question of the instrument had a "not clear" answer option that could be checked by participants if the item was not easily understood. In this case, students could point out their critiques and suggestions regarding the content of unsuitable items. Students were also asked to evaluate the response scale, selecting the "not clear" option for the score that was considered ineffective to assess a certain item.

This step aimed to ensure the correction of possible inconsistencies in meanings, allowed the detection of errors, and confirmed whether the questions were comprehensible (Beaton et al., 2000; Gasparino and Guirardello, 2009). At the end of this step, Version 5 of the instrument was generated.

Reliability

Inter-observer reliability was evaluated comparing the results of two different independent researchers, as well as test-retest reliability, in which the same researcher applied the instrument twice to all subjects within a one-month interval (Melchiors et al., 2007).

In addition, the internal consistency of the instrument was evaluated, which refers to the degree of correlation between items and with the overall research result (Freitas and Rodrigues, 2005). One hundred and eighty-two undergraduate Pharmacy students of three higher education institutions in Sergipe state participated in this stage.

Content validation

After reliability tests, the instrument was submitted for content validation. This protocol was evaluated by expert panel 'C,' formed by five Pharmacy expert researchers selected by convenience. In the protocol, the items of the instrument were divided into two components: "Pharmacotherapeutic knowledge," that included the introduction and content of counseling, and "Communication skills," that contained the process and conclusion of counseling.

This expert panel was informed about the purpose of the instrument. They received instructions to evaluate the instrument regarding form, representativeness, and relevance of each item, considering the criteria established by Vituri and Matsuda (2009). During the evaluation, a dichotomous scale ("Yes" and "No") was used. In case of "No" response, the evaluator should point out their critiques and make suggestions about the changes that they considered most relevant. The items of the instrument were considered validated when the concordance between the expert panel was greater than or equal to 80% (Polit and Beck, 2003).

Statistical analysis

Data analysis was performed using BioEstat 5.0 software. Student's t-test was used to evaluate the differences between students' responses in the tests carried out by research 1 and 2 and in the test-retest. The inter-observer and test-retest reliability analyses used the intraclass correlation coefficient (ICC), adopting the criteria of Cicchetti and Sparrow (1981), which classifies the ICC into poor (<0.40), satisfactory (0.40-0.59), good (0.60-0.74), and excellent (0.75-1.00). The internal consistency was evaluated using Cronbach's alpha coefficient, whose values vary between 0 and 1; the closer the values are to 0, the less the items are related to each other (Rodriguez-Añez et al., 2008). Values between 0.75 and 0.90 indicate high internal consistency (Freitas and Rodrigues, 2005). A 95% confidence interval was adopted, and the differences were considered statistically significant when $p<0.05$.

Ethical aspects

This study was approved by the Research Ethics Committee of the University Hospital, Federal University of Sergipe (Brazil) under the registration code 'CAAE 08721412.8.0000.0058.

RESULTS

Participants

In this study, 235 students answered the instruments and their total completion took approximately 20 min. Of these students, 13 were eliminated because they did not answer the instrument in its entirety, presenting blank answers for one or more items. Thus, 222 students composed the final sample (40 students participated of the pre-test and 188 participated of the reliability tests). Most of the students were female (71.6%), with a mean age of 19.74 ± 1.93 years, and were in the second (79.7%) and third year (20.3%) of Pharmacy undergraduate course.

Cross-cultural adaptation

The translation and validation resulted in the Portuguese version of the instrument entitled "Guia Comportamental de Orientação sobre Medicamentos" (Appendix 1). This translated and validated instrument was composed of 35 questions that measure pharmaceutical competences in patient counseling.

Table 1. Comparison of the answers to the test applied by the researcher 1 and 2 and the test-retest applied by the researcher 1 regarding the questions concerning the Counseling introduction (mean ± standard deviation).

Variable	Test		p-value	R1		p-value
	R1	R2		Test	Retest	
Question 4	6.88 ± 2.04	7.09 ± 1.92	0.08	6.88 ± 2.04	7.12 ± 1.69	**0.02***
Question 5	5.01 ± 3.01	5.37 ± 2.82	0.04*	5.01 ± 3.01	5.06 ± 2.86	0.74
Question 6	5.87 ± 2.89	6.20 ± 2.53	0.03*	5.87 ± 2.89	5.87 ± 2.74	0.97

R1 = researcher 1; R2 = researcher 2. * = statistically significant difference for p <0.05.

The translation and back-translation steps were carried out without major difficulties, and all items of the instrument were translated. In this stage, only grammatical changes were made in some items to assure equivalence between words, languages and the cultural context, thus establishing cross-cultural equivalence between the versions under analysis. Regarding the evaluation of semantic and idiomatic equivalence, the most items obtained agreement ≥ 80%.

After the changes proposed by expert panel 'A,' the items of the instrument were submitted for cultural and conceptual equivalence evaluation to expert panel 'B,' who were natives and residents of the five Brazilian regions. There was an agreement among evaluators of 80% or more for all items. It is important to point out that all the modifications proposed by the expert panel were accepted.

The items of the original version of the instrument are measured by an 11-point Likert scale that are classified as follows: 0 and 1 = not done; 2 = poor; 3, 4 and 5 = unsatisfactory; 6 and 7 = satisfactory; and 8, 9, and 10 = excellent. Regarding the category "not done," it should be indicated when the item was applicable, but the patient counseling was not provided. However, this category (0 or 1) caused doubts regarding the duality of options to judge the counseling. As a result, the scale was reduced to 10 points with scores from 1 to 10, with score 1 classified as "not done". In addition to the Likert scale, a "not applicable" (N/A) item was part of instrument's structure and should be selected when counseling on a given issue was not done because it did not apply to the situation.

Pre-test

The instrument was considered adequate, with clear and easy-to-understand terms and expressions by the 40 undergraduate Pharmacy students who evaluated the content of the instrument. It was necessary to change items 3, 5, 29, and 32, because they were evaluated as "not clear". Items 3 and 29 had some of their wording replaced in order to guarantee the adequacy of the semantic equivalence. In items 5 and 32, explanatory expressions were included, to facilitate their clarity and comprehension. Regarding the assessment scale, no undergraduate Pharmacy student evaluated it as not suitable.

Reliability

Regarding the domain 'Counseling introduction,' there was a statistically significant difference in the tests applied by researchers 1 and 2 in two items (p <0.05) and in the test-retest there was a difference in only one item (p <0.05). Table 1 shows the items that presented a statistically significant difference in inter-observer reliability and the test-retest. In relation to inter-observer reliability, two items were considered excellent (ICC = 0.84-0.76, p <0.0001) and the others obtained a satisfactory to good ICC reproducibility (CI = 0.53 - 0.73, p <0.0001). Test-retest reliability showed good to excellent reproducibility for all items (ICC = 0.62-0.86, p <0.0001).

In relation to the domain 'Counseling content,' the answers provided to researcher 1 were different from those provided to researcher 2 in nine items (p <0.05) and also different from those provided in the retest for seven items (p <0.05). Table 2 shows the items that presented a statistically significant difference in inter-observer reliability and the test-retest. However, in this domain, inter-observer reliability presented responses with good to excellent reproducibility in 10 items (ICC = 0.61-0.83, p <0.0001). Only one item presented poor reproducibility (ICC = 0.35, p =0.0006) and tree were considered satisfactory (ICC = 0.42 - 0.56, p <.0.0001). On the other hand, the reliability of the test-retest obtained excellent reproducibility in eight items (ICC = 0.75-0.88, p <0.0001) and good reproducibility for the others (ICC = 0.49-0, 69, p <0.0001).

In the domains 'Counseling process' and 'Counseling conclusion,' there was a statistically significant difference in the items given to researchers 1 and 2 only for three items (p <0.05). When compared to the retest, only three items had a statistically significant difference (p <0.05). Table 3 shows the items that presented a statistically significant difference in inter-observer reliability and the

Table 2. Comparison of the answers to the test applied by the researcher 1 and 2 and the test-retest applied by the researcher 1 regarding the questions concerning the Counseling Content (mean ± standard deviation).

Variable	Test		p- value	R1		p-value
	R1	R2		Test	Retest	
Question 9	5.26 ± 2.86	5.84 ± 2.77	< 0.0001*	5.26 ± 2.86	5.63 ± 2.77	0.01*
Question 12	3.42 ± 3.08	4.37 ± 3.14	< 0.0001*	3.42 ± 3.08	3.87 ± 3.02	0.005*
Question 14	3.60 ± 3.16	3.80 ± 3.12	0.25	3.60 ± 3.16	3.89 ± 3.11	0.04*
Question 15	4.28 ± 3.01	4.70 ± 2.98	0.02*	4.28 ± 3.01	4.67 ± 2.86	0.02*
Question 16	2.50 ± 2.38	3.02 ± 2.72	0.001*	2.50 ± 2.38	2.80 ± 2.56	0.03*
Question 17	2.50 ± 2.41	3.02 ± 2.81	0.001*	2.50 ± 2.41	2.73 ± 2.54	0.09
Question 20	1.80 ± 1.99	2.28 ± 2.50	0.001*	1.80 ± 1.99	2.15 ± 2.44	0.004*
Question 21	2.10 ± 2.04	2.53 ± 2.48	0.005*	2.10 ± 2.04	2.47 ± 2.36	0.005*
Question 22	4.59 ± 3.06	4.90 ± 3.06	0.04*	4.59 ± 3.06	4.89 ± 2.93	0.05
Question 23	6.90 ± 2.21	7.14 ± 2.06	0.01*	6.90 ± 2.21	6.97 ± 2.17	0.53

R1 = researcher 1; R2 = researcher 2. * = statistically significant difference for p <0.05.

Table 3. Comparison of the answers to the test applied by the researcher 1 and 2 and the test-retest applied by the researcher 1 regarding the questions concerning the Counseling process and conclusion.

Variable	Test		p- value	R1		p-value
	R1	R2		Test	Retest	
Question 27	6.90 ± 2.14	6.43 ± 2.40	0.0006*	6.90 ± 2.14	6.82 ± 2.21	0.001*
Question 29	6.84 ± 2,46	6.48 ± 2.62	0.006*	6.84 ± 2.46	6.74 ± 2,55	0.03*
Question 30	7.65 ± 1.47	7.37 ± 1.80	0.01*	7.65 ± 1.47	7.54 ± 1.55	0.02*
Question 32	6.53 ± 2.91	6.33 ± 2.96	0.16	6.53 ± 2.91	6.79 ± 2.73	0.001*

R1 = researcher 1; R2 = researcher 2. * = statistically significant difference for p <0.05.

test-retest. In this domain, the inter-observer reliability presented excellent reproducibility (ICC = 0.76 - 0.98; p <0.00001) for all items, except for one item that represented good reproducibility (ICC = 0.73; p <0.0001). Test-retest reliability obtained excellent reproducibility for all items with ICC ranging from 0.85 to 0.98 (p <0.0001).

The evaluation of internal consistency resulted in high Cronbach's alpha coefficient values for all the items and domains evaluated (Introduction, Content, Process, and Conclusion of counseling) and also considering the general score, demonstrating the homogeneity of the test. Values of the internal consistency analysis by domain and considering the general score can be found in Table 4.

Table 4. Analysis of internal consistency by domain (Introduction, Content, Process and Conclusion of Counseling) and considering the general score of the instrument “Guia Comportamental de Orientação sobre Medicamentos”.

Domain	Cronbach alpha coefficient
Introduction	0.91
Content	0.92
Process	0.95
Conclusion	0.95
General	0.99

Content validation

All items in the instrument were relevant in representing the domain they intended to measure. However, expert panel 'C' suggested changes in six items (15.4%) that were accepted to improve the clarity and completeness of the instrument. Items 16, 23, and 25 were modified to assure semantic equivalence, and item 5 was modified to attain conceptual equivalence. In addition, explanatory expressions were added to items 14 and 18 to facilitate their clarity and understanding.

DISCUSSION

The need for tools to assess pharmacist's counseling and

communication skills during care justifies the interest in a Brazilian version of the tool. The instruments must meet two essential requirements: reliability and validity. Such reliable measures are replicable and consistent, that is, they generate the same results (Toffoli et al., 2016). Epstein et al., (2015) point out that the use of a foreign instrument without its adaptation may threaten the validity and accuracy of the evaluations carried out. In this sense, the process of cross-cultural adaptation was of primary importance in the validation process.

As proposed by Borsa et al. (2012), the cross-cultural adaptation of the instrument was carried out not only through a literal translation but also through the careful evaluation of its measures, considering the context and specific cultural aspects. For Brazil, in particular, this task is critical due to regional, social, and cultural differences which makes this task relevant (Pilz et al., 2014). In addition, grammar and vocabulary aspects were evaluated, and pronouns and verbal tenses were standardized to solve discrepancies in meaning and content between versions. Adaptations were also made, through the inclusion of terms and expressions appropriate to the reality of the five Brazilian regions. Thus, evaluations by the expert panels ensured semantic, idiomatic, cultural, and conceptual equivalence between the translated and original versions of the instrument.

Regarding the pretest, the purpose of this step was to ensure that the adapted version preserved equivalence with the original version, in addition to detecting errors and evaluating the suitability and comprehensibility of the items (Mesquista et al., 2012). In addition, Gasparino and Guirardello (2009) showed that the changes made at this stage contributed to improving the clarity and understanding of the instrument's items. Thus, the suggestions were important to guarantee the grammatical and semantic equivalence of the translated instrument.

By analyzing the mean of the undergraduate Pharmacy students' responses in the three moments that the instrument was evaluated (test research 1, test research 2, and retest), it was observed that some questions presented differences in students' answers. However, these differences were not sufficient to render the instrument unfeasible concerning its reliability. The inter-observer and test-retest reliability tests showed that the reproducibility of the instrument was considered good to excellent for most items evaluated according to Cicchetti and Sparrow criteria (1981).

In this study, high values of internal consistency for test-retest were obtained. The high values obtained in the reliability tests can be explained by the substantial number of items that composed the instrument, sufficient enough to reduce the sampling error, but not excessive to the point of causing impulsive and relapsing responses or increasing the incidence of unanswered items due to student fatigue or disinterest (Cronbach et al., 2004). In addition, the period between measurements was a factor to consider. Long periods favor the acquisition of new learning and in short periods, the results can be contaminated by the memory effect (Martins, 2006). Therefore, a one-month interval between test-retest applications was adopted, following recommendations described in the literature, to avoid interferences in the results (Melchiors et al., 2007).

In reference to content validation, the instrument has proved to be relevant to its purpose through evaluation by the expert panel. The content validation is determined by judging the proportion in which the items selected to measure a theoretical construct represent all the important facets of the concept measured. This measure also includes the apparent validity of the instrument, that is, the apparent consistency between what is to be measured and the chosen measuring instrument (Pilatti et al., 2010). Thus, it is possible to affirm that all items were considered validated as to their content, once they exceeded the standard of at least 80% of agreement as adopted.

The translation and validation of the instrument "Guia Comportamental de Orientação sobre Medicamentos" into Brazilian Portuguese met the requirements of adequacy, pertinence, and acceptability concerning each item of the instrument. Similarly, the requirements of reproducibility and linearity essential for the reliability of the instrument were satisfied. By analyzing the clarity and comprehensibility of the items it is possible to provide a validated instrument capable of evaluating the quality of pharmaceutical care to improve the activities of these professionals.

Conclusion

This study showed that the Brazilian Portuguese version of the instrument entitled "Guia Comportamental de Orientação sobre Medicamentos" had good validity and reliability performance. The stages of translation and back-translation were satisfactory in complying with conceptual requirements, considering the linguistic aspects and the meaning of the content in the Brazilian reality. Measurement reliability tests affirmed the instrument's ability to produce similar results in successive applications. Finally, the evaluation of content validation made it possible to state that the instrument's components were relevant in representing the domain that it intends to measure. Moreover, the tool can be used by pharmacists and undergraduate Pharmacy students to improve the quality of patient counseling.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors would like to thank the financial support, in the form of a scholarship, from the Coordination for the

Improvement of Higher Education Personnel (CAPES) and the Foundation for Research Support and Technological Innovation of the State of Sergipe (FAPTEC/SE).

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APPENDIX 1. INSTRUMENT *"Medication Counseling Behavior Guidelines"* (USP, 1997-1999). Cross-cultural adapted and validate to Brazilian Portuguese *GUIA COMPORTAMENTAL DE ORIENTAÇÃO SOBRE MEDICAMENTOS*

CATEGORIA 1: ÍTENS REFERENTES À INTRODUÇÃO DA ORIENTAÇÃO.

	N/A	Não feita	Péssimo	Insatisfatório			Satisfatório		Excelente		
1. No início, conduz a orientação, apresentando-se e identificando quem é o paciente ou o seu responsável	-	1	2	3	4	5	6	7	8	9	10
2. Explica a finalidade da orientação	-	1	2	3	4	5	6	7	8	9	10
3. Revisa a prescrição do paciente antes da orientação	-	1	2	3	4	5	6	7	8	9	10
4. Obtém informações prévias e pertinentes relacionadas ao medicamento (por exemplo, idade, alergias, outros medicamentos, gravidez, amamentação)	-	1	2	3	4	5	6	7	8	9	10
5. Adverte o paciente sobre o uso de outros medicamentos ou substâncias, incluindo medicamentos isentos de prescrição (MIPs), fitoterápicos e bebidas alcoolicas, os quais poderiam interagir com o medicamento prescrito (aumentando, diminuido ou anulando sua ação)	-	1	2	3	4	5	6	7	8	9	10
6. Avalia se o paciente tem outras condições clínicas as quais poderiam influenciar os efeitos desse medicamento ou a probabilidade de uma reação adversa	-	1	2	3	4	5	6	7	8	9	10
7.Avalia a compreensão do paciente (ou do responsável) sobre o(s) motivo(s) da farmacoterapia prescrita	-	1	2	3	4	5	6	7	8	9	10
8. Avalia quaisquer preocupações reais e/ou problemas potenciais do paciente	-	1	2	3	4	5	6	7	8	9	10

CATEGORIA 2. ÍTENS REFERENTES AO CONTEÚDO DA ORIENTAÇÃO.

	N/A	Não feita	Péssimo	Insatisfatório			Satisfatório		Excelente		
9. Discute o nome e a indicação do medicamento	-	1	2	3	4	5	6	7	8	9	10
10. Explica a posologia, incluindo o horário de utilização e a duração da terapia, quando apropriado	-	1	2	3	4	5	6	7	8	9	10
11. Auxilia o paciente (ou o responsável) no desenvolvimento de um plano de cuidados para incorporar a farmacoterapia à sua rotina	-	1	2	3	4	5	6	7	8	9	10
12. Explica quanto tempo levará para o medicamento fazer efeito	-	1	2	3	4	5	6	7	8	9	10
13.Discute as recomendações de armazenamento e instruções complementares (por exemplo, agitar bem, manter refrigerado)	-	1	2	3	4	5	6	7	8	9	10
14.Diz ao paciente (ou ao responsável) quando ele/ela deve voltar para adquirir novamente o medicamento	-	1	2	3	4	5	6	7	8	9	10
15.Enfatiza os benefícios da utilização do medicamento conforme prescrito	-	1	2	3	4	5	6	7	8	9	10
16.Alerta sobre os efeitos adversos potenciais (significativos) dos medicamentos	-	1	2	3	4	5	6	7	8	9	10

CATEGORIA 2.Cont'd.

17.Discute como prevenir ou controlar os efeitos adversos do medicamento, caso ocorram	-	1	2	3	4	5	6	7	8	9	10
18. Discute as precauções associadas ao uso do medicamento (por exemplo, evitar operar máquinas ou dirigir)	-	1	2	3	4	5	6	7	8	9	10
19.Discute as interações significativas entre medicamento-medicamento, medicamento-alimento e medicamento-doença	-	1	2	3	4	5	6	7	8	9	10
20. Explica detalhadamente o que fazer se o paciente esquecer de utilizar uma dose	-	1	2	3	4	5	6	7	8	9	10
21. Discute com o paciente (ou o responsável) os potenciais problemas em tomar o medicamento conforme prescrito (por exemplo, custo, acesso)	-	1	2	3	4	5	6	7	8	9	10
22. Ajuda o paciente (ou o responsável) a gerar soluções para os problemas potenciais	-	1	2	3	4	5	6	7	8	9	10
23. Fornece informações detalhadas sobre a farmacoterapia	-	1	2	3	4	5	6	7	8	9	10

CATEGORIA 3. ÍTENS REFERENTES AO PROCESSO DA ORIENTAÇÃO.

	N/A	**Não feita**	**Péssimo**	**Insatisfatório**			**Satisfatório**			**Excelente**	
24. Usa linguagem acessível ao paciente (ou ao responsável)	-	1	2	3	4	5	6	7	8	9	10
25. Usa conhecimentos embasados na literatura para dar suporte durante a orientação ao paciente (ou ao responsável)	-	1	2	3	4	5	6	7	8	9	10
26. Responde com compreensão e empatia	-	1	2	3	4	5	6	7	8	9	10
27. Apresenta fatos e conceitos em uma ordem lógica	-	1	2	3	4	5	6	7	8	9	10
28. Mantém o controle e o direcionamento da orientação	-	1	2	3	4	5	6	7	8	9	10
29. Investiga informações adicionais (por exemplo, hábitos de vida, crenças)	-	1	2	3	4	5	6	7	8	9	10
30. Utiliza perguntas abertas	-	1	2	3	4	5	6	7	8	9	10
31. De maneira geral, apresenta comportamentos não-verbais efetivos:	-	1	2	3	4	5	6	7	8	9	10
31 a. Contato visual apropriado	-	1	2	3	4	5	6	7	8	9	10
31 b. Voz é audível; tom e velocidade da fala são bons	-	1	2	3	4	5	6	7	8	9	10
31 c. Linguagem corporal, posturas e gestos confirmam a mensagem falada	-	1	2	3	4	5	6	7	8	9	10
31 d. Distância entre o profissional de saúde e o paciente (ou o responsável) é apropriada	-	1	2	3	4	5	6	7	8	9	10
32. Verifica a compreensão do paciente (ou do responsável), por meio de feedback (retorno da informação)	-	1	2	3	4	5	6	7	8	9	10
33. Resume, reconhecendo e/ou enfatizando os pontos-chave da informação	-	1	2	3	4	5	6	7	8	9	10
34. Fornece uma oportunidade para preocupações ou perguntas finais	-	1	2	3	4	5	6	7	8	9	10
35. Ajuda o paciente (ou o responsável) a planejar o acompanhamento e os próximos passos da farmacoterapia	-	1	2	3	4	5	6	7	8	9	10

CATEGORIA 4. ÍTENS REFERENTES À CONCLUSÃO DA ORIENTAÇÃO.

	N/A	Não feita	Péssimo	Insatisfatório			Satisfatório		Excelente		
32. Verifica a compreensão do paciente, através de feedback (retorno da informação)	-	1	2	3	4	5	6	7	8	9	10
33. Resume, reconhecendo e/ou enfatizando os pontos-chave da informação	-	1	2	3	4	5	6	7	8	9	10
34. Fornece uma oportunidade para preocupações ou perguntas finais	-	1	2	3	4	5	6	7	8	9	10
35. Ajuda o paciente a planejar o acompanhamento e os próximos passos	-	1	2	3	4	5	6	7	8	9	10

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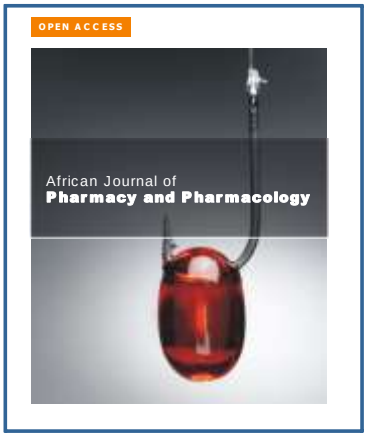

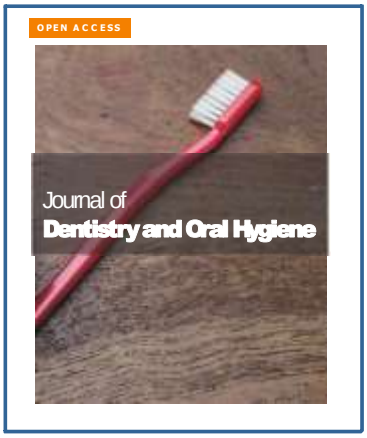

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