In Vitro Cytotoxicity of *Aspilia Pluriseta* Schweinf. Extract Fractions

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Research note

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Abstract

Objectives: We and others have shown that *Aspilia pluriseta* is associated with various biological activities. However, there is a lack of information on *A. pluriseta* cytotoxicity. This has created an information gap about the safety of *A. pluriseta* extracts. As an extension to our recent publication on the antimicrobial activity and the phytochemicals characterization of *A. pluriseta* root extracts, here we report the missing data on cytotoxicity of tested extracts. We evaluated the potential cytotoxicity of the root extracts on Vero cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: We show that all solvent extracts (except methanolic solvent fractions) had cytotoxic concentration values that killed 50% of the Vero cells (CC_{50}) greater than 20 µg/mL and selectivity index (SI) of greater than 1.0. Taken together, we demonstrate that, *A. pluriseta* extract fractions' earlier reported bioactivity are within the acceptable cytotoxicity and selective index limits. This scientifically validates the potential use of *A. pluriseta* in the discovery of safe therapeutics agents.

Introduction

Plant-derived products and compounds have been used to treat and manage wide range of diseases and infections since ancient times. The utilization of plant-derived products and compounds is favoured because these products and compounds exhibit fewer side effects, have improved efficacy and have reduced chances of developing resistance (1–5). The bioactivity of plant extracts are as a result of secondary metabolites, also called phytochemicals (3). These phytochemicals are produced for normal plant defences. However, they inadvertently work against microbial systems and thus are often tapped for therapeutic interventions.

Here we extend the finding of our previous publications (4, 5) by looking at the safety of *Aspilia pluriseta* Schweinf. (*Asteraceae*) extracts in an *in vitro* system. *A. pluriseta* is a common herb in Kenya (5, 6), as well as in East, Central, and Southern Africa, (7, 8). *A. pluriseta* is traditionally used to manage and treat wounds, cough, stomach illness, burn wounds, pimples, ears-, eye-, nose infections, kwashiorkor, fever, worms disorders, and diabetes mellitus with little or no scientific validation (6, 8–13). Recently we have reported *A. pluriseta* selective antitubercular activity (5). Other scientists have reported *A. pluriseta* antiviral (8), antihelmintic (14), antimalarial, hypoglycaemic (6, 13, 15), molluscicidal (16) and complement modulating activity (17). However, the scientific evidence of its pharmacological activity is not fortified by data on its cytotoxicity. We therefore aimed to fill this scientific information-gap using an *in vitro* cytotoxicity system. We report that, the *A. pluriseta* extracts (except methanol solvent fraction) have $CC_{50} > 20$ µg/mL, and $SI > 1.0$, which indicates that, *A. pluriseta* extracts are safe for use in drug discovery and that the reported bioactivity is not as a result general toxicity..

Methodology

Plant material collection

Ethnopharmacological approach was used to identify the plant under study (*A. pluriseta*) from Mbeere community of Embu County, Kenya. The gleaned information was further confirmed from documentation by Riley and Brokensha (1988) in *The Mbeere in Kenya (ii), Botanical identity and use* (18). *A. pluriseta* root samples were collected in an open community field, and the plant is not among the endangered plant species. Therefore, no prior permission was sought before the plant samples were collected. We collected the plant samples within GPS co-ordinates 0°46'27.0"S 37°40'54.9"E; -0.774156, 37.681908. Further authentication of plant sample identity was undertaken by Prof. S. T. Kariuki, a botanist at Egerton University, Kenya. A voucher specimen (number NSN2) was given and deposited at the same institutions herbalium.

Processing of plant samples

The plant materials were processed, extracted and finally fractionated as described in Njeru and Muema (5). Briefly, root materials were cut into small pieces and allowed to air-dry in the dark at 23 ± 2˚C until they attained a constant weight. They were thereafter ground with an electric miller (Retsch SR 200, Haan, Germany). Fifty grams of ground material was macerated in 200 mL methanol (Sigma Aldrich, St. Louis, USA) for 48 hours. This extract was filtered out using Whatmann 1 filter paper, and the process repeated once more. Both filtrates were pooled together, and methanol evaporated from the filtrate by a rotor evaporator (Laborota 4000 efficient, Heidolph, Germany). The resulting dry extract was stored at -20˚C until use. To fractionate *A. pluriseta* root samples, we used organic solvents of increasing polarity. Root powder (50 g) was macerated in 200 mL of petro ether solvent with intermittent shaking for 48 hours. Thereafter, the extract was filtered out. Another 200 mL petro ether (PE) was added into plant material, and the process repeated after which the two filtrates were pooled together. The resulting marc was air-dried, after which it was further extracted with solvents of increasing polarity (namely dichloromethane (DCM), ethyl acetate (EA), and finally methanol (MeOH) solvent. The organic solvent fractions were concentrated with rotor evaporator as described before (5).

In vitro cytotoxicity test

An MTT assay previously described by Njeru, Obonyo (19) was followed to evaluate the toxicity of the *A. pluriseta* extracts on Vero cells (from African green monkey kidney cells (*Cercopithecus aethiops* epithelial cell line; ATCC CCL-81)). MTT assay is a colourimetric assay pegged on the ability of mitochondrial enzyme (succinate dehydrogenase) to reduce yellow water-soluble MTT to an insoluble coloured substance (formazan) that is spectrophotometrically measurable. The amount of the formazan formed is directly proportional to the measure of cell viability. This is because only metabolically active cells can reduce MTT into formazan. The cell line grown to 70–80% confluency in a medium (containing 100 mL DMEM, 10 mL fetal bovine serum (FBS), 1 mL penicillin-streptomycin, 1 mL amphotericin B, 1 mL L-glutamine and 0.1 mL gentamycin) was incubated in the presence of sample extracts at standard conditions (37˚C in 5% CO_2) at 1.0×10^5 cells/mL in a 96-well microtiter plate. The cells were exposed to decreasing concentrations of respective plant extracts (250 – 0.24 µg/mL for petroleum ether and dichloromethane fractions; 500 – 0.49 µg/mL for ethyl acetate and methanolic fraction). Each sample

concentration was tested in duplicates for 48 hours. A post-exposure incubation of 4 hours in 10 µL of 5 mg/mL MTT solution followed the addition of 100 µL acidified isopropanol (0.04 N HCl in isopropanol). The well plates were gently shaken for 5 minutes to dissolve the formazan, and then optical density measured using ELISA Scanning Multiwell Spectrophotometer (LabSystems - Multiskan EX) at 562 nm using 690 nm as the reference wavelengths. The last column of microtiter well plate containing medium without plant extracts were included as the negative control. The percentage cell viability (%) was calculated at each concentration using the formula provided below (1, 19, 20).

$$\text{Cell viability (\%)} = \frac{\text{OD of sample}_{562} - \text{OD}_{690}}{\text{OD of control}_{562} - \text{OD}_{690}} * 100$$

Cytotoxic concentration values which represented the plant extract concentration that kills 50% of the Vero cells (CC_{50}), was determined by regression analysis. A particular plant extract was considered cytotoxic if it had CC_{50} of less than 20 µg/mL and selectivity index (SI) of less than 1.0 (1, 21, 22).

Results

The cytotoxicity test was performed against Vero cells (from monkey kidney fibroblast cells) to ascertain the safety of *A. pluriseta* fractions. We chose the Vero cell line as an ideal *in vitro* model for the study because of its sensitivity to toxicity, ease to culture and readily available in our test facility. Additionally, it is recommended as a model to detect basal cytotoxicity (1, 23, 24). In this study, we set a threshold of the cytotoxic concentration (CC_{50}) below 20 µg/mL to be toxic, and above 20 µg/mL to be non-toxic as previously reported (1, 21, 22). Our initial test for the cytotoxicity of the methanolic crude (cMeOH) extract revealed that the CC_{50} was within the acceptable toxicity limit (CC_{50} of 24.51) (25). Therefore, we hypothesized that fractionation could help us identify active fractions that would not only maintain a strong bioactivity (5) but also be within the acceptable toxicity limits (CC_{50} > 20 µg/ml), and selectivity limits (SI > 1.0). Solvent extract fractionation gave us one fraction (MeOH at CC_{50} 14.36 µg/ml), which was cytotoxic. The PE fraction at CC_{50} 78.6 µg/ml, DCM fraction, at CC_{50} of 191.7 µg/ml, and EA fraction at CC_{50} > 500 µg/ml were all within the acceptable toxicity limit according to the set criteria (Table 1).

Table 1
Cytotoxicity of *A. pluriseta* solvent crude and fraction extracts

	$_C$MeOH	PE	DCM	EA	MeOH
CC_{50} (µg/mL)	24.51	78.6	191.7	> 500	14.36

$_C$MeOH: crude methanolic extract; PE: Petroleum ether fraction extract; DCM: Dichloromethane fraction extract; E.A.: Ethyl acetate fraction extract; MeOH: Methanol fraction extract; CC_{50}: Concentration that kills 50% of the cells.

To determine the selectivity index (SI) of the solvent fraction extracts, we divided their CC_{50} with their antitubercular MIC (all in µg/mL) (data published in (5)) as previously done by others (1, 21, 22). The SI ranged from 1.1488 to 80 (Table 2), which according to Afagnigni, Nyegue (1) and Mongalo, McGaw (21), suggested that the extract fractions were not toxic, or in case of MeOH fraction that it exhibited cytotoxicity and antitubercular activity almost equally (1, 21, 22).

Table 2
Selectivity index of *A. pluriseta* solvent extract fractions

	PE	DCM	EA	MeOH
Selectivity index	3.144	7.668	80	1.1488

PE: Petroleum ether fraction extract; DCM: Dichloromethane fraction extract; EA: Ethyl acetate fraction extract; MeOH: Methanol fraction extract

Discussion

Although plants contribution to new and novel leads for therapeutic drug development has been accepted for a long time now, it is currently a known fact that plant extracts are not always safe (19, 26). The cytotoxicity of many herbal-derived extracts is a potential source of more deleterious side effects to subjects. It is, therefore, imperative to determine whether plant extract showing potential drug activities are active within the acceptable toxicity and selectivity index limits (1, 21, 22, 27, 28). Interestingly, we found that the crude extract and solvent extract fractions (except methanolic solvent extract fraction) demonstrated activity within the acceptable cytotoxicity limit (Table 1). Furthermore, all fractions had selectivity index of > 1.0, which further confirms that the extract fractions are not toxic and hence the reported bioactivity in (5) was not due to basal metabolic toxicity or in case of MeOH solvent extract fraction, the bioactivity and cytotoxicity are almost the same (21).

Conclusion

Our findings demonstrate that *A. pluriseta* root extracts previously reported bioactivity is within acceptable cytotoxicity and selectivity index limit, and thus provide a potential source for safe drug candidate(s).

Limitation

It is important to note that the *in vitro* cytotoxicity results do not always equate to *in vivo* toxicity. This may be attributed to physiological, anatomical pharmacodynamic and pharmacokinetic considerations in living animals and cell culture (1, 21, 27). Therefore, there is a need for further *in vivo* toxicity assessment of the extract fractions.

Abbreviations

PE
petroleum ether; DCM:dichloromethane; EA:ethyl acetate; MeOH:methanol; cMeOH:crude methanolic extract; MTT:3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; CC_{50}:Cytotoxic concentration; SI; selective index

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

SNN conceived the idea, sought, and was awarded the funding and worked through the final draft. JMM partly performed the experimental studies, wrote the initial draft, and both authors approved the final manuscript.

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