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Original Article

Toxicity Assessment of *Vachellia karro* (Hayne) Banfi and Galasso Pods using Brine Shrimp Assay

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ABSTRACT

Background and Objective: *Vachellia karroo* is widely used in folk medicine in the Eastern Cape Province South Africa, however, the pods are usually discarded as waste. This study evaluated the toxicity of acetone, hexane, aqueous and methanol extracts of *Vachellia karroo* pods using brine shrimp model. **Materials and Methods:** Different concentrations (0.0625-1 mg/mL) of the extracts were used to incubate the cysts and nauplii of brine shrimp (*Artemia salina*) to evaluate their effects on the hatching of the cyst and mortality of the nauplii respectively. The percentage of hatched cysts and Lethal Concentration (LC_{50}) needed to kill 50% of the nauplii were recorded. **Results**: Successful hatching of the cysts was in order: Aqueous extract> methanol extract> hexane extract> acetone extract. The hatching of nauplii was in a concentration dependent fashion, with hatching success decreasing with increase in concentration of extracts. **Conclusion**: Lethality of extracts determined based on Meyers' index of toxicity, revealed that acetone and hexane extracts of *V. karroo* were moderately toxic.
Key words: Vachellia karroo, Toxicity, Brine shrimp, Lethality, Nauplii, Cyst.

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INTRODUCTION

The importance of medicinal plants and traditional health systems in solving the heath care problems of the word is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at international level, often to the detriment of natural habitats and mother population in the countries of origin. Most developing countries have adopted traditional medical practice as an integral part of their culture.[1] Bacterial and fungal infections are widespread throughout the world. The situation is more critical especially in the third world countries where inadequate sanitation and primary health care programs make it difficult and expensive to combat diseases.[2] Many plants have been used for centuries as remedies for human diseases, this has encouraged scientists to screen higher plants for various biological activities.[3,4] Although many plants have valuable therapeutic properties, some could be toxic as well. Numerous research studies have recently focused on both pharmacology and toxicity of medicinal plants used by humans. This is of high importance in order to achieve a safe treatment with plant products.[5]

The evaluation of various plant products according to their traditional uses and medicinal value based on their therapeutic efficacy have led to the discovery of newer and more potent drugs for treating various ailments.[6] One of such plants of medicinal value is *Vachellia karroo* commonly known as *Acacia karroo* a member of the genus *Acacia* Miller that belongs to the family Fabaceae. The species can grow under different climatic conditions but its limiting factors are water availability and intense cold.[7] *V. karroo* is the most widespread *Acacia* in Southern Africa, occupying a diverse range of habitats including dry thornveld, river valley scrub, bushveld, woodland, grassland, river banks and coastal dunes.[8] *A. karroo* shows a huge variety in terms of its growth form, with plants from different areas in the species geographical range often having a different appearance. The main part used is usually the leaves, while the pods that are currently used in this study are often discarded as waste. This study therefore aimed at evaluating the toxicity profile of the pods using *Artemia salina* brine shrimp.

MATERIALS AND METHODS

Collection of plant material

The pods of *V. karroo* were collected from Alice in the Central Eastern Cape Province of South Africa, situated at 32.79 01° S, 26.83 30° E. The plant was identified and validated by Professor Cupido of the Botany Department, University of Fort Hare and voucher specimen (MAP/003/2018) was deposited at the Giffen Herbarium.

Preparation of extracts

Pods of *V. karroo* were air dried, then ground to a fine powder. Sixty g each of the ground plant materials were extracted separately in distilled water, methanol, acetone and hexane for 48 h on an orbital shaker. Extracts of the plant sample were filtered using a Buckner funnel and filter paper (Whatman No. 1). A rotary evaporator was used to concentrate the acetone, hexane and methanol extracts to dryness under reduced pressure at 57°C, while the aqueous filtrate obtained was concentrated using a freeze dryer (Vir Tis benchtop K, Vir Tis Co., Gardiner, NY).

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Hatching assay

This assay was evaluated as described by Otang *et al.*[9] Ten (10) *A. salina* cysts was stocked in each of the petri dishes containing 30 mL of the prepared extracts and positive controls. The petri dishes were partly covered, incubated at 30°C under constant illumination for 72 h. The number of nauplii in each petri dish was counted every 12 hours for 72 h. The percentage of hatched cysts was calculated by subtracting the number of nauplii from the total number of cysts stocked.

Lethality assay

This assay was evaluated as described by Otang *et al.*[9] *A. salina* cysts were hatched in sea water, then 10 nauplii were pipetted into each petri dish containing the *V. karroo* extracts and controls as in the hatching assay. The petri dishes were then examined and the number of living nauplii (that exhibited movement during several sec of observation) was counted after every 12 h for 72 h under constant illumination. The percentage mortality (M %) was calculated as: Mortality (%) = (Total nauplii – living nauplii) / Total nauplii × 100.

Data analysis

The hatching success and mortality data obtained from the 5 different concentrations of each fraction and control experiments were used to construct the dose-response curves. From which the corresponding LC50 values were derived. The LC50 was taken as the concentration required for 50% death of the nauplii. The statistical analysis was performed on MINITAB version 17 for windows. One-way analysis of variance (ANOVA) followed by Fischer's Least Significant Difference (for means separation) was used to test the effect of concentration and time of exposure of the plant extracts on the hatching of cysts and mortality of nauplii respectively.

RESULTS AND DISCUSSION

The hatching success of *A. salina* cysts incubated with different *V. karroo* pod extracts and control is shown in Figure 1. Sea water had a significantly higher hatching success (73%) compared to the solvent extracts and positive control (8 .4%) ($p< 0.05$). The hatching of the cysts in acetone (15.2%) and hexane (18.4%) extracts were not significantly different from each other ($p < 0.05$). Aqueous and methanol extracts gave the highest hatching success of the cysts compared to the other extracts. The hatching success was in the order sea water>aqueous>methanol>hexane>acetone>potassium dichromate

The effect of extracts of *V.karroo* pods at varying concentrations and controls on the hatching of *A. salina* cysts are shown in Figure 2. Hatching of *A. salina* cysts significantly decreased with increasing concentrations of the acetone and hexane extract while methanol and aqueous extracts had almost the same pattern of hatching with the highest hatching observed at 0.25 mg/mL. The percentage hatching success of cysts incubated with the acetone extract showed significant differences at varying concentrations. The aqueous and methanol extracts showed equal but significantly higher hatching success of the cysts at (0.625 mg/mL to 0.25 mg/ml), compared to other extracts and control. In addition, hexane extract gave zero percent hatchability at all the concentrations tested. With increasing concentration, fewer cysts incubated in acetone and hexane extracts were hatched. The observed decrease in hatching success of *A. salina* cysts as the concentrations of extract increased could be as a result of relative concentration of toxic metabolites present as the concentration increased.[10]

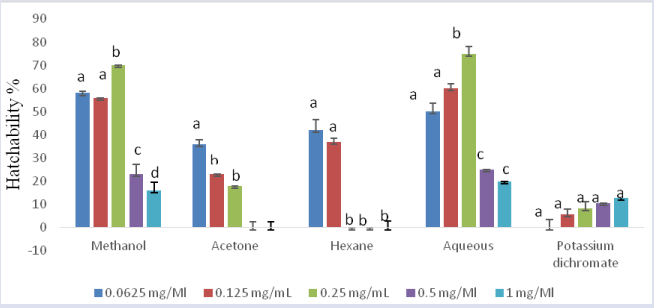

Figure 2: Percentage of hatched *A. salina* cysts incubated in different concentrations of *V. karroo* pod extracts and control.

Values are Means ± SD of three replicates of the concentrations for each plant extract/control. Set of bars with different letters are significantly different at $p > 0.05$.

The effect of exposure time on hatching of *A. salina* cyst is shown in Figure 3. In all the extracts, hatching of the cysts increased with exposure time. There was no hatching at 12 h and the lowest hatching was observed at 24 h for all the extracts and controls while the highest hatching was at 72 h.

This implies that *A. salina* was strongly sensitive to the extract and hatching was strongly dependent on exposure time. Hatching was more successful in hexane, acetone, methanol and aqueous extracts of *V. karroo* pods at 24 h exposure compared to the controls. Hatching of *A. salina* cysts into nauplii in methanol, acetone, hexane and aqueous extracts at 48 h of exposure were 60, 21, 24, 55 %, respectively. The optimal hatching of cysts to yield a large number of nauplii was achieved with 72 h of exposure. In all the extracts, hatching of the cyst was lower when compared with sea water at all the hours exposed. *Artemia salina* is highly vulnerable to toxins and chemical metabolites at the early developmental stages,[11] this could have led to the very low hatching of the cysts observed at 12 h of exposure.

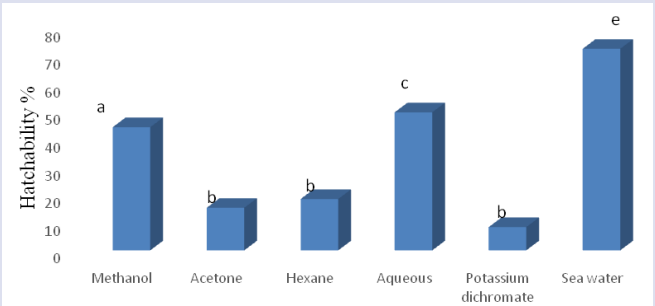

Figure 1: Percentage hatching success of *A. salina* cysts incubated in different solvent extracts of *Vachellia karroo* pods and controls.

Values are means of 5 concentrations for each plant extract/control ± SD of three replicates. Bars with different letters are significantly different at $p < 0.05$.

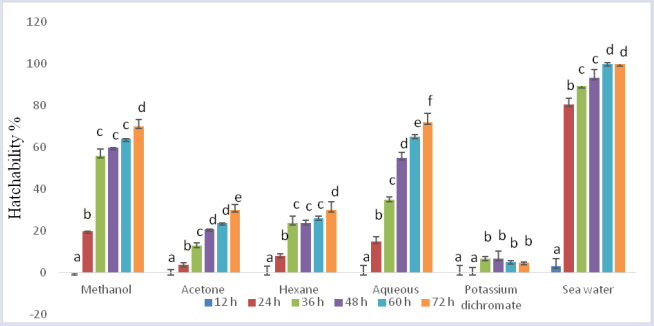

Figure 3: Percentage of hatched *A. salina* cysts incubated at different exposure times with *V. karroo* pod extracts/ controls.

Values are means of three replicates ± SD (at different h). Set of bars with different letters are significantly different at $p < 0.05$.

Brine shrimp lethality assay (BSLA)

The percentage lethality of *V. karroo* pod extracts against *A. salina* nauplii is shown in Figure 4. There was significantly higher ($p<0.05$) percentage mortality percentage (99%) of the nauplii incubated with the potassium dichromate control compared to the extracts with no significant difference between the acetone and hexane extracts. Hexane extract gave the highest mortality of 62.2% compared to the other extracts, while sea water gave the least mortality of 0%. Brine Shrimp Lethality Assay is a convenient method for monitoring biological activities of various plant species. Although this method does not provide any adequate information regarding the mechanism of toxic action, it is very useful for the assessment of toxic potential of various plant extracts[1,12] and provides preliminary screening data that can be backed up by more specific bioassays once the active compounds have been isolated.[13]

The effect of varying concentrations of the *V. karroo* pod extracts on mortality of nauplii is shown in Figure 5. The mortality of nauplii was in a concentration-dependent manner, increasing with concentration. The maximum mortality was observed for all the extracts and controls at 1 mg/ml. Hexane extract showed the highest degree of mortality while aqueous extract had lowest degree of mortality compared to other solvents. The percentage mortality of hexane, methanol, acetone and aqueous was significantly lower than the positive control. Maximum mortalities occurred at the highest concentrations of 1 mg/mL in incubations of all extracts, which was significantly higher than the positive control. It could be deduced that the extracts have both toxicological and pharmacological activities based on the dosage administered.

Mortality of nauplii incubated in *V. karroo* pod extracts increased with time (Figure 6). No mortality was observed with nauplii incubated

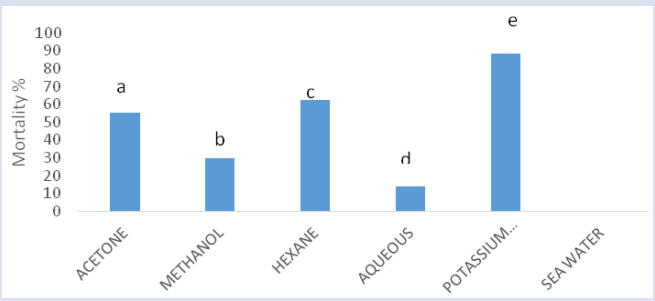

Figure 4: Percentage mortality of *A. salina* nauplii incubated in *V. karroo* pod extracts and controls.

Means are values of five concentrations for each plant fraction/control ± SD of three replicates, Bars with different letters are significantly different ($p < 0.05$).

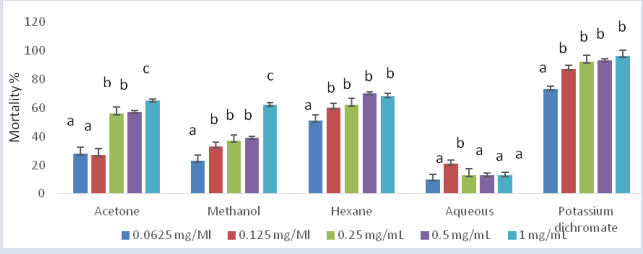

Figure 5: Percentage mortality of *A. salina* nauplii incubated in different concentration of the *V. karroo* extracts and control.

Values are means ± SD of 3 replicates (of all the concentrations) for each plant extract/control ±SD. Set of bars with different letters are significantly different at $p < 0.05$.

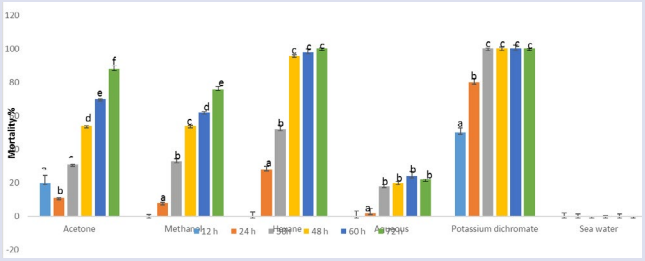

Figure 6: Percentage mortality of *A. salina* nauplii incubated at different exposure time to *V. karroo* pods and controls.

Values are means of three replicates ± SD (at different h). Set of bars with different letters are significantly different at $p < 0.05$.

in sea water throughout the duration of the experiment. Overall, mortality of nauplii in the various extracts and control followed the trend potassium dichromate >hexane >acetone >methanol >aqueous >seawater. Among all the extracts, mortality was highest in hexane extract while it was lowest in the aqueous extracts with the degree of mortality increasing with exposure time for all extracts. The essence of exposing the nauplii to plant extracts over a long period of time was to determine their threshold of withstanding toxic compounds present in the various extracts. According to Carballo *et al.*[14] maximum sensitivity of nauplii to test compounds is achieved at the second and third stage instar which occurs after 48 h of incubation. However, in this study it was not the case as maximum sensitivity was reached after 72 h of exposure.

Toxicity testing criteria

Table 1 presents the half-minimal lethal dose (LC50) of extracts of *V.karroo* pods against brine shrimp nauplii. Clarkson's *et al.*[15] toxicity criterion for the assessment of plant extracts classifies LC_{50} of extracts in the following order: extracts with LC_{50} above 1000 μg/ml are non-toxic, LC_{50} of 500 - 1000 µg/ml are low toxic, extracts with LC_{50} of 100 - 500 μg/ml are medium toxic, while extracts with LC_{50} of 0 - 100 μg/ml are highly toxic (Clarkson *et al.* 2004).

Table 1: Lethal dose concentration (LC_{50}) of *V. karroo* pod extracts against *A. salina* brine shrimp.

V. karroo Extracts	LC_{50} (mg/mL)	Toxicity status
Acetone	0.22	Medium toxic
Methanol	4.33	Non-toxic
Hexane	0.22	Medium toxic
Aqueous	1.76	Non-toxic

LC_{50} is the concentration (mg/mL) of the plant extracts sufficient to obtain 50% of inhibition of nauplii mortality of *A. salina,* respectively.

Acetone and hexane extracts showed medium level of toxicity while methanol and aqueous extracts were not toxic with LC_{50} above 1000 μg/ml. Therefore, the aqueous extract may be considered safe for consumption and since most usage of plants traditionally uses water either for cooking or as an extractive agent, the use of *Vachellia karroo* pods may be encouraged.

CONCLUSION

The results of this study indicate that aqueous and methanol extracts of *Vachellia karroo* pods are not toxic, therefore supporting its traditional therapeutic usage. This implies that rather than discard the pods as a waste as it being currently done, *Vachellia karroo* pods could be salvaged and processed along with the leaves, thus reducing environmental pollution.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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ETHICAL APPROVAL

Ethical approval was granted by the University of Fort Hare Animal and Plant Use Research Ethics Committee, South Africa with protocol number AFO091SMAPO1.

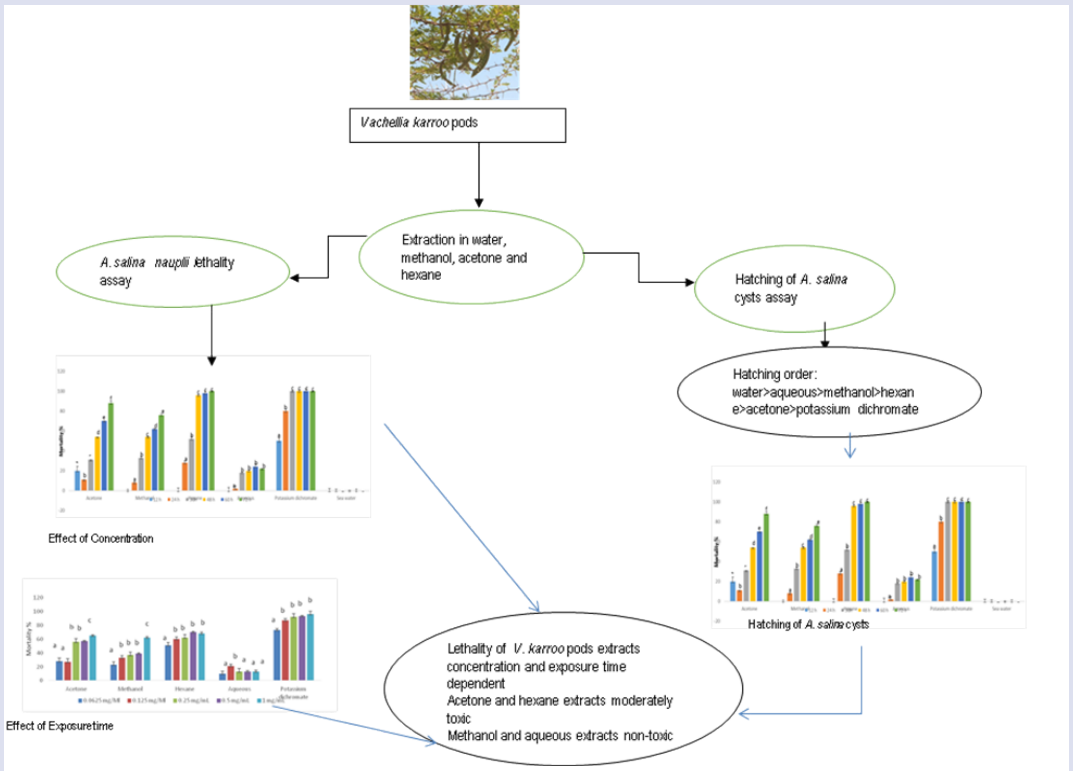
ABBREVIATIONS

V. karroo: *Vachellia karroo; A. salina*: Artemia salina; BSLA: Brine Shrimp Lethality Assay; LC50: Half Minimal Lethal Concentration.

REFERENCES

1. Gadir SA. Assessment of bioactivity of some Sudanese medicinal plants using brine shrimp (*Artemia salina*) lethality assay. J Chem Pharm Res. 2012;4(12):5145-8.
2. Abdel-Moneim AE, Dkhil MA, Al-Quraishy S. The redox status in rats treated with flaxseed oil and lead-induced hepatotoxicity. Biol Trace Element Res. 2011;143(1):457-67.
3. Saadabi AM. Antifungal activity of some Saudi plants used in traditional medicine. Asian J Plant Sci. 2006;5(5):907.
4. Hamidi M, Jovanova B, Panovska T. Toxic o logical evaluation of the plant products using Brine Shrimp (Artemia salina L.) model. Macedonian Pharm Bull. 2014;60(1):9-18.
5. Parra AL, Yhebra RS, Sardiñas IG, Buela LI. Comparative study of the assay of Artemia salina L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. Phytomedicine. 2001;8(5):395-400.
6. Mishra G, Singh P, Verma R, Kumar S, Srivastav S, Jha KK, Khosa RL. Traditional uses, phytochemistry and pharmacological properties of Moringa oleifera plant: An overview. Der Pharmacia Lettre. 2011;3(2):141-64.
7. Maroyi A. Acacia karroo Hayne: Ethnomedicinal uses, phytochemistry and pharmacology of an important medicinal plant in southern Africa. Asian Pacific J Trop Med. 2017;10(4):351-60.
8. Taylor CL, Barker NP. Species limits in Vachellia (Acacia) karroo (Mimosoideae: Leguminoseae): Evidence from automated ISSR DNA "fingerprinting". South Afri J Bot. 2012;83:36-43.
9. Otang WM, Grierson DS, Ndip N. Assessment of potential toxicity of three South African medicinal plants using the brine shrimp (Artemia salina) assay. Afr J Pharm Pharmacol. 2013;7(20):1272-9.
10. Famewo EB, Clarke AM, Afolayan AJ. Identification of bacterial contaminants in polyherbal medicines used for the treatment of tuberculosis in Amatole District of the Eastern Cape Province, South Africa, using rapid 16S rRNA technique. J Health Pop Nutr. 2016;35(1):27.
11. Krishnaraju AV, Rao TV, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Biological screening of medicinal plants collected from Eastern Ghats of India using Artemia salina (brine shrimp test). Int J Appl Sci Eng. 2006;4(2):115-25.
12. Naidu K, Mokhlis H, Bakar AA. Multiobjective optimization using weighted sum artificial bee colony algorithm for load frequency control. Int J Electric Power Energy Syst. 2014;55:657-67.
13. Pisutthanan S, Plianbangchang P, Pisutthanan N, Ruanruay S, Muanrit O. Brine shrimp lethality activity of Thai medicinal plants in the family *Meliaceae*. Naresuan Univ J: Sci Technol (NUJST). 2013;12(2):13-8.
14. Carballo JL, Hernández-Inda ZL, Pérez P, García-Grávalos MD. A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. BMC Biotechnol. 2002;2(1):17.
15. Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG, *et al. In vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. J Ethnopharmacol. 2004;92(2-3):177-91.

GRAPHICAL ABSTRACT



SUMMARY

- This study evaluated the toxicity profile of extracts of *Vachellia karroo* pods using brine shrimp model.
- Lethality of extracts was concentration and exposure time dependent.
- Acetone and hexane extracts were moderately toxic, while methanol and aqueous extracts non-toxic.
- Aqueous extracts of the pods are considered safe at the concentrations evaluated.

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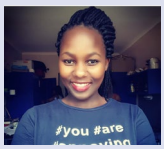

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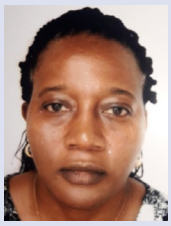

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