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Detoxification of ochratoxin A in rice and maize using ethanolic leaf extracts of *Moringa oleifera* and *Vernonia amygdalina*

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Abstract

The non-availability of research data in North-central Nigeria coupled with the toxic and prevalent nature of ochratoxin A makes it necessary to quantify and identify possible remediative techniques that are effective, less toxic, locally available, cost friendly and easy to deploy. This research was aimed at evaluating the potency of phytochemical remediation on ochratoxin A (OTA) in stored rice and maize using ethanolic leaf extracts of *Moringa oleifera* and *Vernonia amygdalina.* High Performance Liquid Chromatography coupled Ultraviolet/Visible Spectroscopy was employed in the determination of the detoxification levels of the OTA in the samples. Phytochemical constituents in the plant extracts were quantified using standard methods and ranged from 27.83 ± 1.53 – 184.73 ± 0.06 mg/g and 23.00 ± 1.00 – 171.67 ± 3.06 mg/g in the leaf extracts of *Moringa oleifera and Vernonia amygdalina* respectively. The levels of phytochemicals in *Moringa oleifera* extract were statistically ($p \leq 0.05$) higher than in *Vernonia amygdalina* extract. OTA residues were found to be above acceptable limits. Detoxification using 1 – 5 mg/cm^3 of *Vernonia amygdalina* leaf extract ranged from 34.80 (21.47 ± 1.76 µg/kg) to 56.67 % (14.27 ± 1.09 µg/kg) in rice and 38.87 (23.98 ± 0.95 µg/kg) to 58.45 % (16.30 ± 3.18 µg/kg) in maize while, detoxification using 1 – 5 mg/cm^3 of *Moringa oleifera* leaf extract were ranged from 57.18 (14.10 ± 0.85 µg/kg) to 72.12 % (9.18 ± 0.30 µg/kg) in rice and 53.28 (18.33 ± 0.58 µg/kg) to 70.89 % (11.42 ± 1.52 µg/kg) in maize respectively. Phytochemical detoxification of OTA in the stored rice and maize samples were observed to be efficient and promising with a detoxification order *Moringa oleifera* > *Vernonia amygdalina*.

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1. Introduction

Ochratoxin A (OTA) is a low molecular weight toxic compound (mycotoxin) produced in the mycelia structure by secondary metabolism of many filamentous species belonging to the genera *Aspergillus* and *Penicillium* [1–3]. It is a non-polar, weak organic acid that is slightly soluble in water, soluble in polar organic solvents (like alcohols, ketones, dimethylformamide, dimethylsulfoxide and chloroform), insoluble in petroleum ethers and saturated hydrocarbons [4]. OTA is chemically stable and this makes them generally heat-stable molecules [5, 6].

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There are presently over 500 identified mycotoxins [3, 7], however, international regulations have focused on the group of the most prevalent and toxic mycotoxins which include aflatoxins (AFs), zearalenone (ZEN), ochratoxin A (OTA), fumonisins (FBs), the trichothecenes group (deoxynivalenol (DON), nivalenol (NIV), T-2, and HT-2 toxins) and patulin (PAT). Biosynthetically, OTA is derived from the coupling of β-phenylalanine to dihydrocoumarins family. It is a pentaketide and its IUPAC name is: *N*-([(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1*H*-isochromen-7-yl]carbonyl)-L-phenylalanine [1, 8].

OTA is one of the most harmful mycotoxins majorly produced by genus *Aspergillus* (primarily by *Aspergillus ochraceus*) and the *genus Penicillium (P. verrucosum, P. viridicatum, P. nordicum* among others) [9, 10]. OTA is a member of the ochratoxin homologue; a family encompassed by more than 20 different metabolites among which are, ochratoxin A, B, and C with ochratoxin A being the most toxic and abundant while, ochratoxin B and ochratoxin C are the non-chlorinated and ethyl ester from of ochratoxin A [11].

OTA is a class 2B carcinogen. It was classified in 1993 by The International Agency for Research on Cancer because of its extensive occurrence on an immense range of agricultural produce and its danger to life of humans and animals [12]. Regulatory limits as introduced by the European Union (Amended Regulation EC No 123/2005) for the levels of OTA in some processed and unprocessed food products are; cereal grains (5 $\mu g \cdot kg^{-1}$), cereals derived products (3 $\mu g \cdot kg^{-1}$), processed coffee and coffee products (5 $\mu g \cdot kg^{-1}$), grape juice (2 $\mu g \cdot kg^{-1}$), all types of wine (2 $\mu g \cdot kg^{-1}$) and dried fruits (10 $\mu g \cdot kg^{-1}$) [13].

OTA has been identified in an array of animals to be hepatotoxic, nephrotoxic, neurotoxic, teratogenic, embryotoxic, genotoxic, immunotoxic and carcinogenic [12]. It inhibits protein synthesis, interferes with metabolic processes involving phenylalanine, promotes membrane lipid oxidative degradation (peroxidation), disrupts calcium equilibrium and inhibits oxidative phosphorylation (mitochondrial respiration) and mutagenicity/genetic damage [14]. OTA has been associated to kidney failure in farm animals like poultry and pigs. OTA has also been connected to renal diseases including tumors and chronic intestinal nephritis among other diseases.

Although, several remediation techniques such as physical, chemical and biological methods exists, they are limited by several factors like cost, chemical contamination, nutrient loss and the inability to deploy in large quantity. There is need to identify alternative, effective, less toxic, locally available, cost friendly techniques that is easy to deploy that will help remediate this toxin.

Moringa oleifera and *Vernonia amygdalina* are widely popular, nutrient-rich and medicinal plants [15]. The leaves of both plant species contain over 100 secondary metabolites (phytochemicals) which have been identified as promising chemopreventive/detoxifying agents and they include alkaloids, saponins, tannins, steroids, phenolic acids/compounds, phytostererols, carotenoids, tocols, aromatic acids, essential oils, chlorophylls, proteases inhibitors, organic acids, glucosinolates, flavonoids, and terpenoids [16–20].

This research is aimed at evaluating the efficacy of phytochemical remediation on ochratoxin A (OTA) in stored rice and maize obtained from Keffi, Nasarawa state, Nigeria.

2. Materials and methods

2.1. Sample collection

Samples of shelled maize and parboiled/dehusked rice (800 g each) were collected from four villages within keffi, Nasarawa state, Nigeria. The samples were collected after 6 months of storage using sterile paper bags and transported to the laboratory. Samples were then stored at 6 ∘C in a refrigerator until the next day before pre-treatment were carried out.

Plants under consideration (*Moringa oleifera* and *Vernonia amygdalina*) were collected from the wild. Plant stems were collected by hand and transported to the laboratory using pyrex glass jars (uncorked).

2.2. Sample pre–treatment

The maize sample (800 g) was grounded to a fine powder using a food grade high speed blender. The food particles were passed through a food grade sieve (particle ≤ 1 mm) and then mixed thoroughly to ensure that the matrix was homogeneous. This process was repeated for the rice sample.

Leaves of both plants were stripped from stalk/stems, washed under running water and air-dried at room temperature until they were completely dried. The dried samples were grounded to a fine powder using a food grade high speed blender. The resulting grounded leaves were passed through a food grade sieve (particle ≤ 1 mm) to ensure a high surface area for the extractions.

2.3. Extraction process for Ochratoxin

Ochratoxin A extraction was carried out using modified QuEChERS extraction technique as described by Paiga *et al.* [21]. The ground and homogenized maize sample (1.0 g) was weighed into a stoppered 250 cm^3 conical flask. The sample was then extracted with 10 cm^3 acetonitrile/water/formic acid (80/19.9/0.1, in v/v/v) for 30 minutes on a shaker. $MgSO_4$ (4 g) and NaCl (1 g) were then added and shaken for 30 seconds in vortex and then centrifuged further for 15 minutes at 5 °C and 4000 rpm. The supernatant was filtered through a developed set up of filter paper and celite (filter paper-celite-filter paper). The resulting filtrate was then stored in an amber autosampler vial at below 4 °C for further analysis.

2.4. Phytochemical extraction

A robust phytochemical extraction was carried out using cold maceration extraction as described by Kengne *et al.* [22], Banu and Cathrine [23] and Das *et al*. [24].The air-dried and powdered leaf material (250 g) was soaked in a stoppered containers using hexane/methanol 1:1 mixture (1/4 w/v) for 48 hours at room temperature and vigorously shaking for 5 minutes, five times per day. After 72 hours, the mixture was filtered through a whatman No. 1 filter paper and the filtrate was concentrated by evaporation at 45 °C using a rotatory evaporator (Buchi R-200) to obtain the crude extract that was subsequently dried in an oven at 40 °C. The extract was finally stored at 4 °C until further use.

2.5. Quantitative phytochemical analysis of the extracts

Using techniques outlined in AOAC standard procedures [25], phytochemical concentrations were determined using UV/Vis Spectrophotometer (UV752N) with digital display and wavelength range of 195 – 1020 nm. Target analytes which include tannins, flavonoids, terpenoids/triterpenes, glycosides, cardiac glycosides, saponins, alkaloids, anthraquinone and steroids were determined using standard methods. All analysis was carried out in triplicates.

2.6. Phytochemical treatment of the extract

Treatment of the ochratoxin extract was carried out using a modification of the technique as described Kengne *et al.* [22]. To 5 cm^3 mycotoxin extract in a test tubes, 1 mg/cm^3 of ethanolic extract of *Vernonia amygdalina* leaf was added. The resulting mixture was shaken vigorously for 30 seconds in vortex and allowed to stand for 72 hours at room temperature. The mixture was filtered using whatman No. 1 filter paper. This process was carried out in triplicate and repeated for 2 – 5 mg/cm^3 concentrations of the leaf extract. This process was repeated using ethanolic extract of *Moringa oleifera* leaf.

2.7. Clean-up of the treated mycotoxin extract

The clean-up of the treated mycotoxin extract was done using Immunoaffinity column (IAC) clean-up as described in the European standard [26] and by Stroka *et al.* [27]. An aliquot of 4 cm^3 of the filtrate was diluted to 20 mL with phosphate-buffered saline (PBS) solution pH 7.4. After conditioning the immunoaffinity column (IAC) with 8 cm^3 PBS, 20 cm^3 of the diluted filtrate was passed through it at a flow-rate of 2 cm^3/min. The column was washed with 50 % methanol (4 cm^3) and dried by pressing air. In the next step, retained mycotoxin extracts were eluted at a flow rate of 0.5 cm^3/min using 1 cm^3 of methanol and 1 cm^3 of distilled water and collected in a glass vial. 10 cm^3 of eluate was used for HPLC analysis.

2.8. HPLC properties/conditions

High performance liquid chromatography analysis was performed on a Biobase machine with reversed phase HPLC column that consists of a microprocessor-controlled 200 eluent delivery pump and a fixed wavelength Biobase model UV–Vis spectrophotometer detection system.The mobile phase consisted of water, acetonitrile and methanol (60:20:20, v/v/v) which was filtered, degassed, and pumped at a flow rate of 1 cm^3/min. The injection volume was 20 μL and the eluent was monitored using UV detection at 400 nm for ochratoxin under isocratic condition. Separation was achieved on a 4.6 mm×250 mm C18 reversed phase column with 5 μm ultra-pure silica gel packing material (vertex). All the system was maintained at room temperature. Data collection and handling were carried out by Biobase software (N2000 Chromatographic Data System).

2.9. Statistical analysis

The IBM SPSS statistical software version 27 and MSExcel version 2010 were used to process all quantitative data obtained. Results are expressed as mean ± standard deviation (SD).

3. Results and discussions

3.1. Phytochemical analysis

The presence of major phytochemical constituents like alkaloids, phenols, tannins, flavonoids, saponins, terpenoids/triterpenes, glycosides and steroids was identified in both leaf extracts with an exception of anthraquinone in *Vernonia amygdalina.* Quantitative results (Table 1) show that the highest phytochemical concentration in *Moringa oleifera* extract was total saponins content at 184.73 ± 0.06 mg/g while; the lowest constituent was total cardiac glycoside at 27.83 ± 1.53 mg/g. The highest phytochemical concentration in *Vernonia amygdalina* extract was total phenols/phenolic compounds content at 171.67 ± 3.06 mg/g while, the lowest constituent was total cardiac glycoside at 23.00 ± 1.00 mg/g. The levels of phytochemicals in *Moringa oleifera* extract were statistically ($p \leq 0.05$) higher than in *Vernonia amygdalina* extract. The results are similar to phytochemical components investigations reported by Adekanmi *et al*. [28] and Syahputra *et al.* [29], who investigated the phytochemical compositions of the ethanolic extracts of both plants at Ekiti state, Nigeria and Utara, Indonesia respectively.

Table 1. Phytochemical contents of ethanolic extracts for *Moringa oleifera* and *Vernonia amygdalina* leaf.

Phytochemicals	Mean concentrations (mg/g) of phytochemicals in ethanolic extracts of plant leaves	
	Moringa oleifera leaf extract	*Vernonia amygdalina* leaf extract
Flavonoids	116.67 ± 0.76^a	82.33 ± 1.25^b
Phenols/Phenolic compounds	152.17 ± 1.04^b	171.67 ± 3.06^a
Total Tannins	49.68 ± 2.84^a	47.84 ± 1.53^a
Alkaloids	67.67 ± 3.06^a	30.67 ± 2.52^b
Saponins	184.73 ± 0.06^a	58.99 ± 0.10^b
Anthraquinone	70.07 ± 1.45^a	-
Terpenoids/Triterpenes	66.10 ± 0.23^a	37.75 ± 1.39^b
Steroids	79.22 ± 0.84^a	40.89 ± 0.77^b
Glycosides	31.67 ± 0.76^a	27.37 ± 0.56^b
Cardiac glycosides	27.83 ± 1.53^a	23.00 ± 1.00^b

Values are mean ± standard deviation, n = 3. Values with the same superscript across the same row are not significantly different ($p \leq 0.05$)

Table 2. Residual concentrations (µg/kg) of ochratoxin A in stored rice and maize samples.

Samples	Residual concentration (µg/kg)
Rice	32.93 ± 2.02^b
Maize	39.23 ± 5.68^a

Values are mean ± standard deviation, n=3. Values with different superscript down the column are significantly different ($p \leq 0.05$)

Table 3. Residual concentrations (µg/kg) and detoxification levels (%) of ochratoxin A in rice and maize samples treated with *Vernonia amygdalina* extract.

Extract concentration (mg/cm^3)	Rice	Detoxification	Maize	Detoxification
1.0	21.47 ± 1.76^{bc}	34.80	23.98 ± 0.95^c	38.87
2.0	21.36 ± 0.35^{bc}	35.14	23.97 ± 1.84^c	38.90
3.0	19.66 ± 2.91^b	40.30	21.81 ± 1.76^{bc}	44.40
4.0	14.39 ± 2.56^a	56.30	18.86 ± 1.31^{ab}	51.93
5.0	14.27 ± 1.09^a	56.67	16.30 ± 3.18^a	58.45

Values are mean ± standard deviation, n=3. Means with the same superscripts down the column are not significantly different ($p \leq 0.05$).

3.2. Ochratoxin A in stored samples

Ochratoxin A was present in the stored rice and maize samples at residual levels higher than the 5 µg/kg FDA and EU maximum levels for ochratoxin A in foodstuffs [13]. Mean residual level of OTA (Table 2) in maize was 39.23 ± 5.68 µg/kg and was observed to be statistically ($p \leq 0.05$) higher than the mean level in rice 32.93 ± 2.02 µg/kg.

3.3. Detoxification of Ochratoxin A

The detoxification results in Table 3 show a reduction in residual concentrations of ochratoxin A in rice and maize as concentration of *Vernonia amygdalina* extract increased from 1 – 5 mg/cm^3. The highest detoxification levels obtained were 56.67 % (14.27 ± 1.09 µg/kg) and 58.45 % (16.30 ± 3.18 µg/kg) while the lowest were 34.80 % (21.47 ± 1.76 µg/kg) and 38.87 % (23.98 ± 0.95 µg/kg) for the rice and maize samples respectively.

The detoxification results in Table 4 show a reduction in residual concentrations of ochratoxin A in rice and maize as concentration of *Moringa oleifera* extract increased from 1 – 5 mg/cm^3. The highest detoxification levels obtained were 72.12 % (9.18 ± 0.30 µg/kg) and 70.89 % (11.42 ± 1.52 µg/kg) while the lowest were 57.18 % (14.10 ± 0.85 µg/kg) and 53.28 % (18.33 ± 0.58 µg/kg) for the rice and maize samples respectively.

3.4. Regression models

The regression models in Table 5 were developed from the detoxification results obtained after treatment of the samples with ethanolic extracts of *Vernonia amygdalina* and *Moringa oleifera* leaves respectively. These models provide insights into the detoxi-

Table 4. Residual concentrations (μg/kg) and detoxification levels (%) of ochratoxin A in rice and maize samples treated with *Moringa oleifera* extract.

Extract concentration (mg/cm^3)	Rice	Detoxification	Maize	Detoxification
1.0	14.10 ± 0.85^{b}	57.18	18.33 ± 0.58^{c}	53.28
2.0	13.70 ± 0.54^{b}	58.40	15.71 ± 0.54^{bc}	59.95
3.0	13.17 ± 0.78^{b}	60.01	13.85 ± 0.79^{ab}	64.70
4.0	11.57 ± 0.93^{ab}	64.86	12.99 ± 0.96^{ab}	66.89
5.0	9.18 ± 0.30^{a}	72.12	11.42 ± 1.52^{a}	70.89

Values are mean ± standard deviation, n=3. Means with the same superscripts down the same column are not significantly different ($p \leq 0.05$).

Table 5. Regression models for residual ochratoxin A levels in rice and maize treated with ethanolic extracts of *Vernonia amygdalina* and *Moringa oleifera* leaves.

Samples	Treatment Extract	Models	R^2 values
Rice	*Vernonia amygdalina*	$y = -0.405x + 10.38$	0.865
Maize	*Vernonia amygdalina*	$y = -0.432x + 12.16$	0.886
Rice	*Moringa oleifera*	$y = -0.738x + 12.11$	0.884
Maize	*Moringa oleifera*	$y = -0.584x + 11.44$	0.966

fication of the ochratoxin A in rice and maize hence, enabling the estimation of their respective residue concentrations/detoxification percentage after treatment with extracts of known concentrations.

The generated R^2 values indicate the strength and accuracy of each model. All R^2 values between 0.5 – 1.0 summarizes a positive relationship for both variables (extract concentration and expected residual levels of ochratoxin A) in the models. The R^2 values generated were in the range of 0.865 - 0.966 and indicates that the developed models can estimate accurately ($p \leq 0.05$) the expected residual levels after treatment with a known extract concentration.

4. Conclusion

The residual levels of ochratoxin A in the stored rice and maize samples revealed the presence of the toxin in levels above the FDA and EU maximum residual limits and indicated that the stored produce were potential exposure sources of ochratoxin A. The detoxification efficiency of the ethanolic leaf extract of *Moringa oleifera* was greater than for *Vernonia amygdalina*. The use of *Vernonia amygdalina* and *Moringa oleifera* leaf extracts proved to be effective in the phytoremediation of ochratoxin A in stored rice and maize samples and hence, will enhance food safety.

Data availability

The data that support the findings of this study are available on request from the principal author. The data are not publicly available due to ethical restrictions.

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References

[1] A. El Khoury & A. Atoui, "Ochratoxin A: General overview and actual molecular status", Toxins (Basel) **2** (2010) 461. https://doi.org/10.3390/toxins2040461.
[2] R. Bhat, R. V. Rai & A. A. Karim, "Mycotoxins in food and feed: Present status and future concerns", Comprehensive Reviews in Food Science and Food Safety **9** (2010) 57. https://doi.org/10.1111/j.1541-4337.2009.00094.x.
[3] World Health Organisation, "*Mycotoxins: Key facts"*, 2018. [Online]. https://www.who.int/news-room/fact-sheets/detail/mycotoxins.
[4] E. Janik, M. Niemcewicz, M. Ceremuga, M. Stela, J. Saluk-Bijak, A. Siadkowski & M. Bijak, "Molecular aspects of mycotoxins - a serious problem for human health", International Journal of Molecular Sciences **21** (2020) 8187. https://doi.org/10.3390/ijms21218187.
[5] World Health Organisation, "*Mycotoxins: Key facts"*, 2020. [Online]. https://www.who.int/news-room/fact-sheets/detail/mycotoxins.
[6] B. Kabak, "The fate of mycotoxins during thermal food processing", Journal of the Science of Food and Agriculture **89** (2009) 549. https://doi.org/10.1002/jsfa.3491.
[7] M. A. Haque, Y. Wang, Z. Shen, X. Li, M. K. Saleemi & C. He, "Mycotoxin contamination and control strategy in human, domestic animal and poultry: A review", Microbial Pathogenesis **142** (2020) 104095. https://doi.org/10.1016/j.micpath.2020.104095.
[8] T. Koszegi & M. Poor, "Ochratoxin A: Molecular interactions, mechanisms of toxicity and prevention at the molecular level", Toxins **8** (2016) 111. https://doi.org/10.3390/toxins8040111.

[9] A. Alshannaq & J. H. Yu, "Occurrence, toxicity, and analysis of major mycotoxins in food", International Journal of Environmental Research and Public Health **14** (2017) 632. https://doi.org/10.3390/ijerph14060632.
[10] K. Toregeani-Mendes, C. Arroteia, C. Kemmelmeier, V. Dalpasquale, E. Bando, A. Alves, O. Marques, P. Nishiyama, S. A. G. Mossini & M. Machinsk, Jr., "Application of hazard analysis critical control points system for the control of aflatoxins in the Brazilian groundnut-based food industry", International Journal of Food Science & Technology **46** (2011) 2611. http://doi.org/10.1111/j.1365-2621.2011.02791.x.
[11] A. A. Ismaiel & J. Papenbrock, "Mycotoxins: producing fungi and mechanisms of phytotoxicity", Agriculture **5** (2015) 492. https://doi.org/10.3390/agriculture5030492.
[12] R. A. El-Sayed, A. B. Jebur, W. Kang & F. M. El-Demerdash, "An overview on the major mycotoxins in food products: characteristics, toxicity, and analysis", Journal of Future Foods **2** (2022) 91. https://doi.org/10.1016/j.jfutfo.2022.03.002.
[13] The Commission of the European Communities, "Commission regulation (EC) No 1881/2006: Setting maximum levels for certain contaminants in foodstuffs", Official Journal of the European Union **364** (2006) 1. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF.
[14] D. Ringot, A. Chango, Y. Schneider & Y. Larondelle, "Toxicokinetics and toxicodynamics of ochratoxin A, an update", Chemico-Biological Interactions **159** (2006) 18. https://doi.org/10.1016/j.cbi.2005.10.106.
[15] A. M. Alqasim, "Ethnomedicinal studies of medicinal plants with antifungal activities in Keffi local government, Nasarawa state, Nigeria", Asian Journal of Plant Science and Research **3** (2013) 109. https://www.pelagiaresearchlibrary.com.
[16] K. K. Mishra, C. D. Kaur, A. K. Sahu, R. Panik, P. Kashyap, S. P. Mishra & S. Dutta, "Medicinal Plants Having Antifungal Properties", Intechopen Limited, London, United Kingdom, 2020, pp. 1-14. http://dx.doi.org/10.5772/intechopen.90674.
[17] C. D. Monteiro & J. R. A. dos Santos, "Phytochemicals and their antifungal potential against pathogenic yeasts", In Phytochemicals in Human Health, IntechOpen Limited, London, United Kingdom, 2020, pp. 1-31. http://dx.doi.org/10.5772/intechopen.87302.
[18] N. Z. Abd Rani, K. Husain & E. Kumolosasi, "Moringa Genus: A review of phytochemistry and pharmacology", Frontiers in Pharmacology 9**9** (2018) 108. https://doi.org/10.3389/fphar.2018.00108.
[19] I. A. Muraina, A. O. Adaudi, M. Mamman, H. M. Kazeem, J. Picard, L. J. McGaw & J. N. Eloff, "Antimycoplasmal activity of some plant species from northern Nigeria compared to the currently used therapeutic agent", Pharmaceutical Biology **48** (2010) 1103. https://doi.org/10.3109/13880200903505633.
[20] R. K. Cimanga, L. Tona, K. Mesia, C. T. Musuamba, T. De Bruyne, S. Apers, N. Hernan, V. S. Miert, L. Pieters, J. Totte & A. J. Vlietink, "In vitro antiplasmodialacivity of extracts and fractions from seven medicinal plants used in the democratic republic of Congo", Journal of Ethnopharmacology **93** (2004) 27. https://doi.org/10.1016/j.jep.2004.02.022.
[21] P. Paíga, S. Morais, T. M. Oliva-Teles, M. Correia, C. Delerue-Matos, S. Duarte, A. Pena & C. Lino, "Extraction of ochratoxin A in bread samples by the QuEChERS methodology", Food chemistry **135** (2012) 2522. http://dx.doi.org/10.1016/j.foodchem.2012.06.045.
[22] I. C. Kengne, A, G. Fankam, E. K. Yamako & J. Tamokou, "Phytochemical analysis, antifungal, and antioxidant properties of two herbs (*Tristemma mauritianum* and *Crassocephalum bougheyanum*) and one tree (*Lavigeria macrocarpa*) species", Advances in Pharmacological and Pharmaceutical Sciences **54** (2023) 2565857. https://doi.org/10.1155/2023/2565857
[23] K. S. Banu, & L Cathrine, "General techniques involved in phytochemical analysis", International Journal of Advanced Research in Chemical Science **2** (2015) 25. https://www.arcjournals.org/pdfs/ijarcs/v2-i4/5.pdf.
[24] K. Das, R. K. S. Tiwari & D. K. Shrivastava, "Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends", Journal of Medicinal Plants Research **4** (2010) 104. https://doi.org/10.5897/JMPR09.030.
[25] AOAC (Association of Official Analytical Chemists), "The official methods of analysis: association of official analytical chemists", 18th Edition, USA, Washington DC, 2005. https://www.sciepub.com/Portal/AZJournals.
[26] European Standard, "Determination of aflatoxin B1, B2, G1 and G2 in pistachios by high performance liquid chromatography with post-column derivatization and immunoaffinity column clean-up", Institute of Sciences of Food Production, National Research Council of Italy (ISPA-CNR), Italy, 2019, pp. 1-9. https://nucleus.iaea.org.
[27] J. Stroka, E. Anklam, U. Jörissen & J. Gilbert, "Immunoaffinity column cleanup with liquid chromatography using post-column bromination for determination of aflatoxins in peanut butter, pistachio paste, fig paste, and paprika powder: collaborative study", Journal of Association of Official Analytical Chemists International **83** (2000) 320. https://www.researchgate.net/publication/12544814.
[28] A. A. Adekanmi, U. T. Adekanmi, A. S. Adekanmi & H. Oyekanmi, "Assessment of proximate composition and phytochemical properties of bitter leaf (*Vernonia amygdalina*) and water leaf (*Talinum triangular*)", United International Journal for Research & Technology **1** (2020) 13. https://www.researchgate.net/publication/347628922.
[29] R. A. Syahputra, A. Sutiani, P. M. Silitonga, R. Zulmai, & A. Kudadiri, "Extraction and phytochemical screening of ethanol extract and simplicia of moringa leaf (*Moringa oleifera lam.*) from Sidikalang, North Sumatera", International Journal of Science, Technology & Management 2 (2021) 2072. https://doi.org/10.46729/ijstm.v2i6.381.