**Novel Research in Microbiology Journal (2018), 2(5): 75-84**
***Research article***

**(Print) (ISSN 2537-0286)**
**(Online) (ISSN 2537-0294)**
**https://nrmj.journals.ekb.eg/**
**DOI: 10.21608/NRMJ.2018.17863**

# Microbiological analysis and total aflatoxins levels from shoot powder of *Phyllanthus amarus* (Schum. and Thonn) from Tororo, Uganda

Odda, J.[1,2]; Aliero, A.A.[3]*; Waako, P.[1,4]; Obua, C.[5]; Kabasa, J.D.[6]

[1]Department of Pharmacology and Therapeutics, College of Health Sciences, Makerere University, P O Box 7072, Kampala, Uganda; [2]Department of Pharmacology and Toxicology, School of Pharmacy, Kampala, International University, Western Campus, P O Box 71, Ishaka, Uganda; [3]Department of Microbiology and Immunology, Faculty of Biomedical Sciences, Kampala International University, Uganda; [4]Faculty of Health Sciences, Mbale campus, Busitema University, Mbale, Uganda; [5]Mbarara University of Science and Technology, P O Box 1410, Mbarara, Uganda; [6]Department of Physiological Sciences, College of Veterinary Medicine and Biosecurity, Makerere University, P O Box 7062, Kamala, Uganda

*Corresponding author E-mail: aimustapha.adamu@kiu.ac.ug, adamualieroa@gmail.com

Received: 9 October, 2018; Accepted: 21 October, 2018; Published online: 29 October, 2018

## Abstract

*Phyllanthus amarus* (Schum. and Thonn) has been used traditionally for treating over 50 diseases globally including malaria in Tororo, Uganda. Although efficacy and acute toxicity studies of this plant have been determined, safety regarding microbiological analysis and total aflatoxins levels in this Ugandan shoot powder of *P. amarus* is still lacking. The microbiological analysis was determined according to the procedures recommended in WHO guidelines. Total aflatoxins were determined using Afla Test Kit, VICAM Series (South Africa). In terms of microbial analysis, the herbs were prepared based on indigenous knowledge according to the WHO criteria. Although this herbal shoot powder had microbial safety requirements with key microbial contaminants (i.e. mold fungi, *Salmonella typhi*, and *Escherichia coli*) within acceptable ranges, however, aflatoxins in all the investigated samples were beyond acceptable levels. Current results are useful in developing and establishing public health standards for the production and safe handling of herbal products of the Ugandan *P. amarus*.

**Keywords**: *Phyllanthus amarus*, herbs, Microbial analysis, aflatoxins levels, Uganda

## 1. Introduction

Currently, the use of botanicals and traditional medicines is widespread around the world (Marcelo and Taís, 2012). The cost and poor access to allopathic medicines have served to a great extent as a stepping stone to the popularity of traditional medicines. According to the National Centre for Complementary and Alternative Medicine (NCCAM), the United States of America reported that 40% of its population depends on complementary and alternative medicines (NCCAM, 2013). Botanical dietary supplements represents distinctively one of the major contributors of complementary and alternative medicines in the USA (Kessler, 2001), with previous estimated returns of $5.1 billion annually towards the close of 1990s (Eisenberg, 1998).

According to Wu *et al*., (2014), about 18% of the US populations (over 38 million US citizens) tested were using herbal preparations and dietary supplements in 2007. It is estimated that the majority of people in developing countries as well as a vast number of the rural communities globally depend on botanicals as their primary sources of health care in controlling infectious diseases such as malaria (Birdi *et al*., 2006). Wu *et al*., (2014) added that a number of populations were also reported using high rates of herbs and supplements, including women (Schaffer *et al*., 2003; Kennedy, 2005), middle aged adults (Schaffer *et al*., 2003), Caucasians (Mackenzie *et al*., 2003; Kelly *et al*., 2006), Asians (Bair *et al*., 2002; Tanaka *et al*., 2008) and Latinos (Howell *et al*., 2006). Previously Waako, (2003) stated that in many communities, people were linked to culture and hence strongly believed that traditional medicines were readily accessible, safe and affordable. According to Oudhia, (2008), *P. amarus* and *Plumbago zylenica* were traditionally used globally for treating over 50 and 35 diseases and disease conditions, respectively.

Medicinal plants constituted very valuable aspect of pharmaceutical, pharmacological as well as medical developmental studies, which provided raw materials for drug synthesis and for pharmacologically active compounds used for treatment of infectious diseases such as malaria (Si-Yuan *et al*., 2013). A previous few examples reported by Sebisubi, (2006) supported this idea including; acetyl salicylic acid derived from the willow bark and morphine, which were sources of many active compounds exhibiting narcotic and analgesic properties. Moreover, procaine derived from cocaine and quinine from cinchona bark, both served as precursors of antimalarial compounds such as chloroquine and primaquine. MoH, (1987) in an earlier study stated that in African countries such as Uganda, it is recommended to include ethno medicine in local health teams and in Primary Health Care delivery.

Unfortunately, points of weakness against use of herbal medicines were lack of documented standard procedures for their collection, preparation, administration as well as storage of their products (OAU/STRC, 1991). Accordingly, there is an urgent need to evaluate the traditional procedures of processing these selected medicinal plants according to international recognized requirements (WHO. 1998, 2003).

Parts of the current study regarding the extent of knowledge, attitude and practices (KAP) of herbs of malaria, and standard use of *P. amarus* have already been published (Odda *et al*., 2013). Recently, efficacies of *P. amarus*, antimalarial activity and acute toxicity have also been published (Odda *et al*., 2018). However, safety aspects related to processing and storage practices based on indigenous knowledge (IK) of microbial analysis and aflatoxins levels of this important Ugandan herb have not yet been documented.

Current study is therefore a pioneer effort based on use of locally available antimalarial medicinal plants or their natural products with internationally acceptable levels of safety, efficacy, and good quality. This is the basis for herbal products that contain consistent levels of active ingredients, predictable pharmacological and physiological effects. Therefore, the aims of our study were to determine the microbiological analysis and total aflatoxins levels in this *P. amarus* antimalarial herb when used singly in Tororo, Uganda.

## 2. Materials and methods

### 2.1. Study design and study area

These experimental studies were carried out at the Department of Microbiology, Makerere University, college of Veterinary Medicine, Animal Resources and Biosecurity in liaison, with the Department of Food Technology and Nutrition, Makerere University, College of Agriculture and Environmental Sciences, Kampala, Uganda at December, 2012.

According to the Uganda Bureau of Statistics at 2017, Tororo District has an approximate population of 517,080 with a sex ratio of 94.2 males per every100 females (UBOS, 2017). Kamuli is the village where the studied herb was used as mono-herb in the treatment of malaria fever based on indigenous knowledge (IK) of the herbalists (Fig. 1).

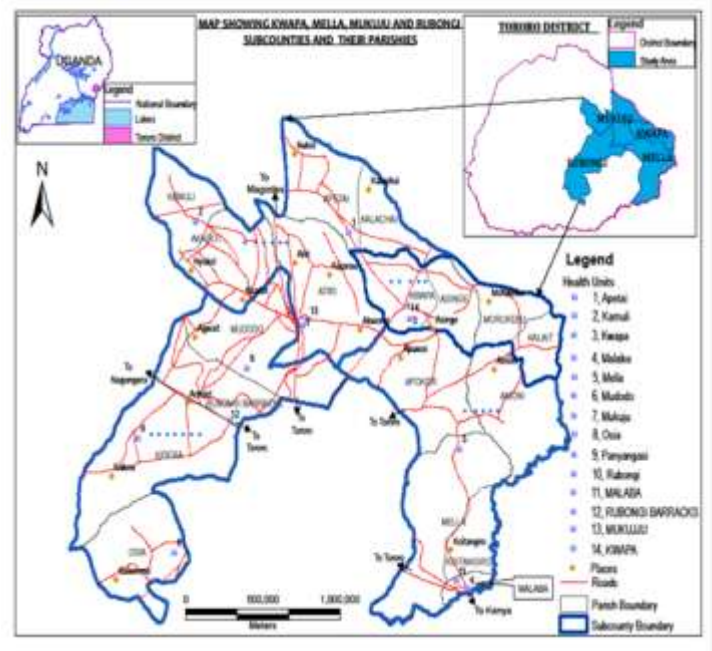


**Fig. 1**: Map of Tororo District in Eastern Uganda showing Kamuli village in Mukujju sub-country, which was the source of *P. amarus* herb

### 2.2. Microbiological studies

*P. amarus* shoots were collected from Kamuli Village, Pagoya LCI zone approximately 10 Km from Tororo town in Eastern Uganda, during December, 2012. Samples were identified at the Department of Biological Sciences Herbarium, Makerere University prior to air-drying at room temperature under shade, and then were ground into powder using an electric mill. Some of the powder was used for microbiological analysis, whereas the rest was stored for total aflatoxins levels determination. The microbiological analysis was carried out according to the procedures recommended in WHO. (1998) guidelines.

One gram (1g) proportion of each sample was aseptically taken after thorough mixing into a sterile flask containing 99 ml of sterile dist. water. These samples were soaked for 2-3 min. with occasional stirring. Each of these produced suspensions was shaken for 10 min. to obtain a homogenous suspension of microorganisms. These suspensions were diluted serially by taking 1 ml of the stock suspension and then adding it to 99 ml of sterile dist. water to obtain a dilution of $10^{-1}$; this step was repeated serially to obtain $10^{-2}$-$10^{-6}$ dilutions. 0.1 ml of each of these dilutions was plated separately on Nutrient agar, Xylose Lysine Deoxycholate agar (XLD) and Potato dextrose agar (PDA) media using a glass spreader.

A pair of Nutrient agar plates were inoculated and incubated in 2 sets for each dilution; one set of plates used for the isolation of Coli forms was incubated in a Griffin incubator (UK) at 37°C for 24 h., whereas the second set of plates used to confirm if the coli form was *E. coli* incubated using a Memmert incubator (Germany) at 45°C for 24 h. The XLD plates enriched with Selenite F were also incubated at 37°C for 24 h., while PDA plates used to isolate mold fungi were incubated at 28°C for 7 days. After incubation, separate bacterial and fungal colonies were counted. On the other hand, *E .coli* (ATCC 25922) was used as a reference culture.

### 2.3. Determination of Aflatoxins levels in *P. amarus* shoot powder

The toxicological aspect of this study was carried out at the Department of Food Science, Makerere University (VICAM Afla Test® WBSR, 2008); the total aflatoxins of interest were $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$. Aflatoxins levels determination were carried out using VICAM Afla Test, because it has the advantage of measuring these key aflatoxins without using toxic solvents such as chloroform, and can be used in any laboratory with a High performance liquid chromatography (HPLC) system. Before screening of dried *P. amarus* shoot powder for estimation of aflatoxins levels, calibration were performed according to manufacturer's instructions i.e. performing the test using low, high calibrator and yellow mycotoxin standard, whose results are presented here with the test sample result (02-1C). Having satisfied the calibration requirements, samples for aflatoxins determination passed through the following steps:

#### 2.3.1. Sample extraction

25 g of each ground *P. amarus* sample was mixed with 5 g NaCl and then placed in a blender to which 100 ml of methanol: water (80:20) was added. The mixture was blended at high speed for 1 min.; the extract was then filtered through filter paper.

#### 2.3.2. Extract dilution

Five milliliter of the filtrate was diluted with 20 ml of 10% Tween 20 solution and mixed well. The diluted extract was passed through glass micro-fiber filter into glass syringe barrel to measure 4 ml, thus become ready for extraction with column chromatography.

### 2.3.3. Column chromatography

4 ml of the diluted extract (equivalent to 0.2 g sample) was passed through Afla Test®-P affinity column at the rate of 1-2 drops/second until air comes through the column. This was followed by passing 10 ml of purified water through the column at a rate of about 2 drops/second, this process was repeated twice. Elution of the affinity column was carried out by passing 1.0 ml of HPLC grade methanol through the column at the rate of 1-2 drops/second, 1 ml of this elute was then collected in a glass cuvette. Finally, 1.0 ml of the Afla Test® Developer was added to collected elute in the cuvette and were mixed well, then the cuvette was placed in a calibrated fluorometer. The readout of total aflatoxins concentrations was carried out after 60 seconds.

## 3. Results

### 3.1. Microbiological analysis of the *P. amarus* shoot powder

Results of the microbiological analysis of *P. amarus* herbal powder included *E. coli*, *S. typhi*, yeasts and mold fungi as the key microbes of herbal products specified by WHO. (1998). Among the coliforms, *E. coli* is responsible for most of herbal toxic effects and hence it has been cut off here. *E. coli* ATCC 25922 was used as a reference organism to demonstrate if the culture media was still capable of supporting *E. coli* growth or not, hence minimize false negative results regarding this bacterium. On day 7, one of the dilutions which was processed and stored according to indigenous knowledge (IK) coded (02-1C), had molds (greenish gray colonies) growing on PDA medium, whereas the samples processed and stored according to WHO guidelines coded (02-1D) had no such growth. The overall results of this microbiological analysis are demonstrated in Table 1.

Following 24 h. incubation at 37$^{o}$C, IK (02-1C) processed herbal powder had 620 cfu/g coli forms (pink colonies) indicating that poor unhygienic method of preparation was used. Whereas the second set of plates of the same sample incubated at 45$^{o}$C had no growth (absence of *E. coli*) indicating absence of fecal contamination. It was also noticed that IK (02-1C) processed samples had 120 cfu/g of yeasts/molds, whereas the samples who's processing met WHO criteria (02-1D) had 20 cfu\g only. In terms of microbial analysis, current results recorded that prepared herbs based on indigenous knowledge conformed to specifications just as those handled according to the WHO criteria.

### 3.2. Screening of aflatoxins levels of *P. amarus* powder

According to Bbosa *et al*., (2013), exposure to high levels of aflatoxins especially $AFB_1$, $AFB_2$, $AFG_1$ and $AFG_2$, are associated with Hepatocellular carcinoma (HCC). However, the WHO and other regulatory bodies like European Medicines Agencies (EMEA) now demanded that raw materials for making drugs including herbal preparations should be screened for total aflatoxins to ensure that they are safe and of acceptable qualities (WHO, 2013; EMEA, 2005).

Results of total aflatoxins in herbal products presented in this work were only assessed at baseline level because aflatoxins would still be there for six months later. Unfortunately, after testing for these aflatoxins in IK processed samples, there was a technical problem with the

HPLC equipment before processing according to WHO guidelines; hence tests for the (02-1D) could not be carried out, accordingly were not described in Table (2).

**Table 1**: Microbiological analysis of *P. amarus* shoot powder processed according to Indigenous Knowledge (02-1C) and WHO requirements (02-1D)

| Lab code | Sample ID | Total Coli form Count (cfu/g) | *E. coli* count (cfu/g) | *Salmonella* detection | Yeasts & mold Count (cfu/g) | *Remarks/ Comments Based on the cut off below |
|---|---|---|---|---|---|---|
| 01 | Plain water | 0 | 0 | Absent | 260 | Acceptable |
| 02 | *E. coli* ATCC 25922 | 0 | Growth | N/A | N/A | Acceptable |
| 03 | 02-1C | 620 | 0 | Absent | 120 | Acceptable |
| 04 | 02-1D | 0 | 0 | Absent | 20 | Acceptable |

*Acceptance criteria for herbal preparations and powders;

Note: To determine whether the preparations had acceptable or unacceptable levels (too contaminated for human use) according to United States Pharmacopeia (USP), Vol. 12 (Updated as of 2015) as follows: *Salmonella* should be absent in at least 25 g of the powder or preparations; yeasts and molds must have minimum acceptable level of $10^5$ cfu/g; maximum is $10^6$ cfu/g, whereas *E. coli* acceptable level is $10^2$/g; maximum limit is $10^3$/g of the sample.

**Table 2**: Total aflatoxins levels in the shoot powder of *P. amarus* processed according to Indigenous knowledge (02-1C)

| ID Code/ Description | Specification | Test Method | Time elapsed (Months) | | |
|---|---|---|---|---|---|
| | | | 0 | 3 | 6 |
| **High Calibrator** | 22PPB | VICAM *[1] | 22PPB | 22PPB | 22PPB |
| **Low Calibrator** | -1.0PPB | VICAM *[1] | -1.0PPB | -1.0PPB | -1.0PPB |
| **Yellow Mycotoxin STD** | 54±5 | VICAM *[1] | 50 | 50 | 50 |
| **02-1C** | 10PPB | VICAM *[1] | 23 PPB | 23 PPB | 23 PPB |

PPB = parts per billion

Note: For the system results to be valid, all the high, low calibrators and yellow mycotoxin standard (STD) readings were within specification indicated in the table based on USP, Vol. 38. Maximum acceptable aflatoxins levels in herbal powder were 20 PPB (USP, Vol. 38).

## 4. Discussion

There is a growing concern about the quality and safety of herbal drugs due to microbial contamination caused by many environmental factors such as; temperature, humidity, extent of rainfall during pre-harvesting and post-harvesting periods, handling practices and the storage conditions of medicinal plant materials (Marcelo and Taís, 2012).

Results of the presence study on microbial analysis of shoot powder of *P. amarus* processed by IK showed the presence of total coliform count of 620 cfu/g sample which indicated poor handling processes. This was in accordance with previous studies of Kaume *et al*., (2012); Noor *et al*., (2013) who reported the presence of coliform from herbal medicines available in Kenya and Bangladesh, respectively. Although total coliform bacteria are unlikely to cause disease but their presence in this product indicates that the disease causing pathogens may also be present. There were no any common fecal contaminating isolates detected in the studied samples. This was in contrary to the findings of Abba *et al*., (2009) who reported the presence of fecal coliform contaminant from herbal medicinal products marketed in Kaduna Metropolis, Nigeria. Moreover, previous studies of Alwakeel, (2008); Idu *et al*., (2011) reported the presence of pathogenic bacteria such as; *B. cereus*, *B. subtilis*, *Aeromonas hydrophila*, *Shigella* spp., *Enterobacter agglomerans*, *E. cloacae*, *Vibrio fluvialis*, *Pasteurella multocida*, *S. epidermidis*, *Klebsiella* spp. and *Pseudomonas aeruginosa* in the analyzed plant samples.

Currently, we observed the presence of yeasts and mold fungi in all the collected samples. This was in accordance with previous studies of Bugno *et al*., (2005); Bugno *et al*., (2006); Anyanwu, (2010) who reported the presence of yeast fungi as *Candida* spp., and mold fungi such as; *Aspergillus* spp., *Penicillium* spp., *Mucor* spp., and *Trichosporon* spp. Some of these fungal species are extremely important from the mycotoxicological point of view. Kumar *et al*., (2009) stated that fungal deterioration adversely affects the chemical composition of raw materials and thereby decreases the medicinal potency of herbal drugs. Marcelo and Taís, (2012) added that although high fungal loads may be acceptable due to the natural origin of those products, however, they indicated the possibility of their spoilage and mycotoxigenesis.

The shoot powder of *P. amarus* processed by IK coded (02-1C) underwent aflatoxins screening. The common molds notorious for aflatoxins production are *A. flavus*, *A. parasticus*, *A. nomius*, and *A. bombyicis*. Given that the studied herbs were collected in the way mimicking how collection is done based on IK, however, it is possible that the recommended good collection procedures were not followed. This is attributable to the fact that these molds can survive on plant debris, tree leaves, decaying wood, animal excreta, dead insects, stored grains, and even immunocompromised humans and animals. The other factors commonly linked to aflatoxins production are incomplete drying and poor storage practices which led to molds growth (Ogiehor, 2002). Subroto, (2011) reported that optimum conditions for aflatoxins production were temperatures in range of 24-35°C, and substrate moisture content exceeding 7% (10% with ventilation). Previous studies by Kitya *et al*., (2010) confirmed that traditional methods of food processing were contributing to risk factors of HCC if safety is not considered to these products.

Therefore, guidelines for proper processing procedures must be available and utilizable at this level.

## Conclusion

Although antimalarial shoot powder of *P. amarus* subjected to microbial safety analysis had key herbal contaminants such as; yeasts, molds, *S. typhi*, and *E. coli* within acceptable ranges, however, aflatoxins in all the samples investigated were beyond these acceptable levels. Current results are useful in developing and establishing public health standards for the production and safe handling of herbal products from the Ugandan *P. amarus*.

## Conflict of interests

The authors declare that there is no conflict of interest regarding publication of this article.

## Funding

This article is a result of the Ph.D. study of John Odda, and was partly funded by The Carnegie Regional Initiative in Science and Education-African Natural Products Training Network (RISE-AFNNET)-2010-2013, Makerere University, Kampala, Uganda, and Carnegie Next Generation of Academics Competitive Ph.D. Grants.

## Acknowledgments

Sincere appreciation to The Carnegie Regional Initiative in Science and Education-African Natural Products Training Network (RISE-AFNNET)-Makerere University, Kampala, Uganda, and The Carnegie Next Generation of Academics Competitive Ph.D. Grants who funded the study that contributed to this article.

## 5. References

**Abba, D.; Inabo, H.I.; Yakubu, S.E. and Olonitola, O.S. (2009).** Contamination of herbal medicinal products marketed in Kaduna Metropolis with selected pathogenic bacteria. African Journal of Traditional, Complementary and Alternative Medicines. 6: 70-77.

**Alwakeel, S.S. (2008).** Microbial and heavy metals contamination of herbal medicines. Research Journal of Microbiology. 3(12): 683-691.

**Anyanwu, C.U. (2010).** Fungal contaminants of powdered herbal drugs sold in parts of Enugu State, Southeast, Nigeria. Plant Product Research Journal. 14: 46-50.

**Bair, Y.A.; Gold, E.B.; Greendale, G.A.; Sternfeld, B.; Adler, S.R.; Azari, R. and Harkey, M. (2002).** Ethnic differences in use of complementary and alternative medicine at midlife: longitudinal results from SWAN participants. American Journal of Public Health. 92(11): 1832-1840.

**Bbosa, G.S.; Kitya, D.; Lubega, A.; Ogwal-Okeng, J.; Anokbonggo, W.W. and Kyegombe, D.B. (2013).** "Review of the biological and health effects of aflatoxins on body organs and body systems," in Aflatoxins – Recent Advances and Future Prospects, ed. M. Razzaghi-Abyaneh (Rijeka: InTech), pp. 239-265.

**Birdi, T.J.; Brijesh, S. and Poonam, D. (2006).** Approaches towards Preclinical Testing and Standardization of Medicinal Plants. Foundation for Medical Research, India.

**Bugno, A.; Almodovar, A.A.B.; Pereira, T.C.; Pinto, T.J.A. and Sabino, M. (2006).** Occurrence of toxigenic fungi in herbal drugs. Brazilian Journal of Microbiology. 37: 47-51.

**Bugno, A.; Buzzo, A.A.; Nakamura, C.T.; Pereira, T.C.; Matos, D. and Pinto, T.J.A. (2005).** Avaliação da contaminação microbiana em drogas vegetais. Revista Brasileira de Ciências Farmacêuticas. 41(4): 491-497.

**Eisenberg, D.M.; Davis, R.B.; Ettner, S.L.; Appel, S.; Wilkey, S.; Van Rompy, M. and Kessler. R.C. (1998).** Trend in alternative medicine in the United States, 1990-1997: Results of a follow up national survey. Journal of American Medical Association. 280: 1569-1570.

**EMEA. (2005).** ICH Topic Q 6 A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances.

**Howell, L.; Kochhar; K.; Saywell, R.; Zollinger, T.; Koehler, J.; Mandzuk, C.; Sutton, B.; Sevilla-Martir, J. and Allen, D. (2006).** Use of herbal remedies by Hispanic patients: do they inform their physician?. The Journal of the American Board of Family Medicine. 19(6): 566-578.

**Idu, M.; Erhabor, J.O. and Idele, S.O. (2011).** Microbial load of some medicinal plants sold in local markets of Benin City, Nigeria. International Journal of Medicinal and Aromatic Plants. 1(3): 272-277.

**Kaume, L.; Foote, J.C. and Gbur, E.E. (2012).** Microbial contamination of herbs marketed to HIV-infected people in Nairobi (Kenya). South African Journal of Science. 108: 9-10.

**Kelly, J.P.; Kaufman, D.W.; Kelley, K.; Rosenberg, L. and Mitchell, A.A. (2006).** Use of herbal/natural supplements according to racial/ethnic group. Journal of Alternative and Complementary Medicine. 12(6): 555-561.

**Kennedy, J. (2005).** Herb and supplement use in the US adult population. Clinical therapeutics. 27(11): 1847-1858.

**Kessler, R.C.; Davis, R.B.; Foster, D.F.; Van Rompay, M.I.; Walters, E.E.; Wilkey, S.A.; Kaptchuk, T.J. and Eisenberg, D.M. (2001).** Long-term trends in the use of complementary and alternative medical therapies in the United States. Annals of Internal Medicine. 135(4): 262-268.

**Kitya, D.; Bbosa, G.S. and Mulogo, E. (2010).** Aflatoxin levels in common foods of South Western Uganda: a risk factor to hepatocellular carcinoma. European Journal of Cancer Care. 19(4): 516-521.

**Kumar, A.; Shukla, R.; Singh, P. and Dubey, N.K. (2009).** Biodeterioration of some herbal raw materials by storage fungi and aflatoxin and assessment of *Cymbopogon flexuosus* essential oil and its components as antifungal. International Biodeterioration and Biodegradation. 63: 712-716.

**Mackenzie, E.R.; Taylor, L.; Bloom, B.S.; Hufford, D.J. and Johnson, J.C. (2003).** Ethnic minority use of complementary and alternative medicine (CAM): a national probability survey of CAM utilizers. Alternative therapies in health and medicine. 9(4): 50-57.

**Marcelo, G.F.A. and Taís, M.B. (2012).** Microbial Quality of Medicinal Plant Materials. License InTech. pp. 67-81.

**MoH, (1987).** Uganda Policy on Ethno Medicine.Uganda, Ministry of Health.

**NCCAM. (2013).** Complementary, Alternative, or Integrative Health: What's In a Name?

**Noor, R.; Huda, N.; Rahman, F.; Bashar, T. and Munshi, S.K. (2013).** Microbial contamination in herbal medicines available in

Bangladesh. Bangladesh Medical Research Council Bulletin. 39(3): 124-129.

**OAU/STRC. (1991).** In 4th OAU/STRC Inter-African Symposium on Traditional Pharmacoppeia and African Medicinal Plants. Abuja - Nigeria: OAU.

**Odda, J.; Waako, P.; Obua, C. and Kabasa, J.D. (2018).** *In vivo* antiplasmodial activity and safety of the aqueous ethanolic extract of *Phyllanthus amarus* (Schum. and Thonn). International Journal of Pharmacy and Pharmaceutical Research. 12(3): 265-280.

**Odda, J. et al. (2013).** Local Indigeneous Knowledge, Attitudes and Practices in Processing Antimalarial Herbs: An Assessment Based on WHO Requirements. Open Science Repository Anthropology.

**Ogiehor, I.S. (2002).** Extension of Shelf Life of Garri by Combination of Preservative Factors. University of Benin, Benin city, Nigeria.

**Oudhia, (2008).** *Phyllanthus fraternus* G.L. Webster. Record from PROTA4U. Schmelzer, G.H. and Gurib-Fakim, A. PROTA, Wageningen, Netherlands.

**Schaffer, D.M.; Gordon, N.P.; Jensen, C.D. and Avins, A.L. (2003).** Non-vitamin, non-mineral supplement use over a 12-month period by adult members of a large health maintenance organization. Journal of the American Dietetic Association. 103(11): 1500-1505.

**Sebisubi, F.M. (2006).** Extraction and Characterization of Possible Antiplasmodial Compounds from Selected Medicinal Plant Species in Uganda. Makerere University, Kampala.

**Si-Yuan, P.; Shu-Feng, Z.; Si-Hua, G.; Zhi-Ling, Yu.; Shuo-Feng, Z.; Min-Ke, T.; Jian-Ning, S.; Dik-Lung, M.; Yi-Fan, H.; Wang-Fun, F. and Kam-Ming, K. (2013).** New Perspectives on How to Discover Drugs from Herbal Medicines: CAM's Outstanding Contribution to Modern Therapeutics. Evidence Based Complementary and Alternative Medicine, 627375.

**Subroto, M. (2011).** Aflatoxin Effect on Health.

**Tanaka, M.J.; Gryzlak, B.M.; Bridget, Z.M.; Nisly, N.L. and Wallace, R.B. (2008).** Patterns of natural herb use by Asian and Pacific Islanders. Ethnicity and Health. 13(2): 93-108.

**UBOS. (2017).** National Population and Housing Census, 2014 – Area Specific Profile Series, Kampala, Uganda.

**VICAM. (2008).** Afla TestR WBSR. Instructional Manual No.715001720 Rev. A. AM, USA.

**Waako, P. (2003).** The Antimalarial Potential of Uganda's Traditional Medicines: A Study of Six Plants Used to Treat Malaria Symptoms. Makerere University, Uganda.

**WHO. (2013).** World health statistics. World Health Organization, Geneva.

**WHO. (2003).** WHO Guidelines on Good Agricultural and Collection Practices (GACP). World Health Organization, Geneva.

**WHO. (1998).** Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva.

**Wu, C.H.; Wang, C.C.; Tsai, M.T.; Huang, W.T. and Kennedy, J. (2014).** Trend and pattern of herb and supplement use in the United States: results from the 2002, 2007, and 2012 national health interview surveys. Evidence Based Complementary and Alternative Medicine.