Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 202017(2):881-887. **OPEN ACCESS**

Effects of drying strategies, altitude and habitat on determination of aflatoxin-B1 levels in eight *Artemisia* species

Shah Zaman[1,2*], Barkat Ulllah[2], Syed Wadood Ali Shah[3], Gul Rahim[1] and Ali Hazrat[1]

[1]Department of Botany, University of Malakand KPK, **Pakistan**
[2]Department of Botany, Islamia College University Peshawar, KPK, **Pakistan**
[3]Department of Pharmacy, University of Malakand KPK, **Pakistan**

***Correspondence: shahzaman@uom.edu.pk** **Received 18-03-2020, Revised: 17-05-2020, Accepted: 20-05-2020 e-Published: 07-06-2020**

Aflatoxins are known to be ubiquitous contaminants of food all over the developing countries of the world. Aflatoxin B-1 is the chief toxin of the majority of medicinal plants. The study was carried out with an aim to detect and check the effects of different drying methods, altitude and habitat on the level of aflatoxins B-1 in eight selected species of *Artemisia*. Determination and quantification of AFB1 in the samples was carried out using AFB1 Elab science ELISA Kit (Catalog No: E-TO-E01696T, USA); assay was repeated three times for each treatment. To know the significance of values ANOVA Tuky's HSD post hoc test was used. Each species showed significant differences of AFB1 in each treatment at $p<0.05$. The evaluation of *Artemisia* species against different drying strategies suggested that COD is more efficient treatment for plant drying with lowest value (6.66± 0.33 ng/kg) and higher value (20± 0.57 ng/kg) of AFB1 at F= 154.2, Df= 16, $p<0.05$ as compare to CSD with lowest value (11.66 ± 0.33 ng/kg) and higher value (26.66 ± 0.88 ng/kg) of AFB1 at F= 88.44, Df= 16, $p<0.05$ and UCSD with lowest value (21.33 ± 0.88 ng/kg) and higher value (41 ± 0.57 ng/kg) of AFB1 at F= 71.88, Df= 16, $p<0.05$. However none of the value exceeds maximum approved limit of 5 mg/kg or 5000 ng/kg for AFB1. The AFB1 level showed consistency with altitude recorded and habitat of the collected medicinal plants. This study provides details about the variation of AFB1 with different drying conditions, altitude and habitat recorded.

Keywords: Genus *Artemisia*; Aflatoxins B-1; Drying methods; Altitude; Habitat

INTRODUCTION

Among the angiosperm Asteraceae is a largest family which includes over 20,000 species which are cosmopolitan in nature. Asteraceae include one of the economically important genus Artemisia which is distributed along North America, Europe and Asia (Shehata et al., 2015). In Pakistan, this genus comprises of 25 well known species (Zeb et al., 2019). Artemisia L. is included in the tribe Anthemideae (Nigam et al. 2019). The genus Artemisia of Anthemideae tribe contains essential oil and possess high chemical and biological diversity therefore currently most evaluations studies focused on phytochemical attentions of this genus (Abad et al., 2012).

Aflatoxins are renowned to be ubiquitous contaminants of food throughout the developing world (MozaffariNejad et al., 2014). Aflatoxins group of metabolites contain structurally similar structures and form a unique set naturally occurring heterocyclic highly oxygenated compounds with specific forms designated as AFB1 (6-methoxydifurocoumarone), AFB2, AFG1, AFG2, AFM1 and AFM2 (Sewram et al., 2006).

Main producers of such toxins are Aspergillus flavus section and Alternaria alternata section, as well as Penicillia and Scopulariopsis etc (Migahed et al., 2017). High relative humidity and high ambient temperatures are the favourable factors for such fungus development (Razzazi-Fazeli et al., 2004).

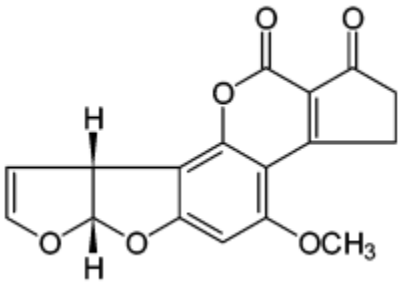

Figure 1: Chemical structure of Aflatoxin-B1.

The post-harvesting process directly influences the quantity and quality of active constituents in the sold products of medicinal plants and has large significance in the production chain (Rocha and Melo 2011). Drying of medicinal plants in the sun or shade are traditional drying methods which have many disadvantages moreover relative air humidity and high ambient air temperature promote mold and insect development in the harvested crops during the harvesting season however hot air-convective drying is universally used to minimize these problems (Rocha and Melo 2011). The aim of this study was to investigate the afltoxin B-1 level in highly medicinal plant of genus *Artemisia* and compare the values of afltoxin B-1 in each drying methods to evaluate effective method for reducing afltoxin B-1 level.

MATERIALS AND METHODS

Plant Materials

Fresh plant materials of genus *Artemisia* were collected from the different localities of District Swat Pakistan (Figure 2). The exact altitude and localities of plants were recorded using GPS (table 1). The plants were identified by Professor Dr. Ali Hazrat in the Department of Botany University of Malakand. Voucher specimens were deposited in the herbarium of Islamia College University Peshawar KP Pakistan. Plant materials were processed for the formation of coarse powder in three separate procedures:

a) Plant materials was chopped and oven dried (COD)

b) Plant material was chopped and shade dried on room temperature (CSD)

c) Plant material left un-chopped and shade dried on room temperature (UCSD)

The dried material obtained and subjected to grinder for formation of coarse powder drug. Powder drug was stored in light resistant and air tight bottle.

Determination and quantification of AFB1 using ELISA

Determination and quantification of AFB1 in the samples was carried out based on a competitive enzyme immunoassay by using AFB1 (Aflatoxin B1) ELISA Kit (Catalog No: E-TO-E01696T, USA) test kit.

Preparation of samples

Following the standard procedure of test kit manual of Elab science ELISA Kit (Catalog No: E-TO-E016 size 96T, USA) test kit samples were prepared and separation with aflatoxin column were performed. Toxin unevenly distributes in the sample therefore 2 gm of sample were taken in triplicate from each sample after thoroughly shaking and mixing of the crushed powder form sample. Sample of 2 gm were mixed with 10 ml of 70% methanol and centrifuge at 4000 rpm at room temperature for 10 minutes. The supernatant of 0.5mL was taken and mixed with deionized water. About 50µL was taken from 0.5 mL for detection and analysis.

Procedure of ELISA test

Records were kept for sample wells and standard wells in the form of numbers. Sample of 50 µL of sample and standard were added per well. Then 50µL of HRP conjugate and 50µL of antibody working solution were loaded to each well. The mixture was gently mixed for 5 sec and incubated at 25 °C for 30 minutes. The sealer were uncovered the liquid was removed carefully in each well and 300 µL wash buffer were added immediately. This process of washing was repeated at interval of 30sec for 5 times. The plates were inverted and pat it against thick clean absorbent paper. For color development 50 µL of substrate reagent A and then Substrate reagent B was added to each well. The mixture was gently oscillate for 5 seconds and then incubated at 25 °C for 15 minutes under shading light. To stop the reaction stop solution of 50 µL was added to each well and gently oscillate to mix thoroughly.

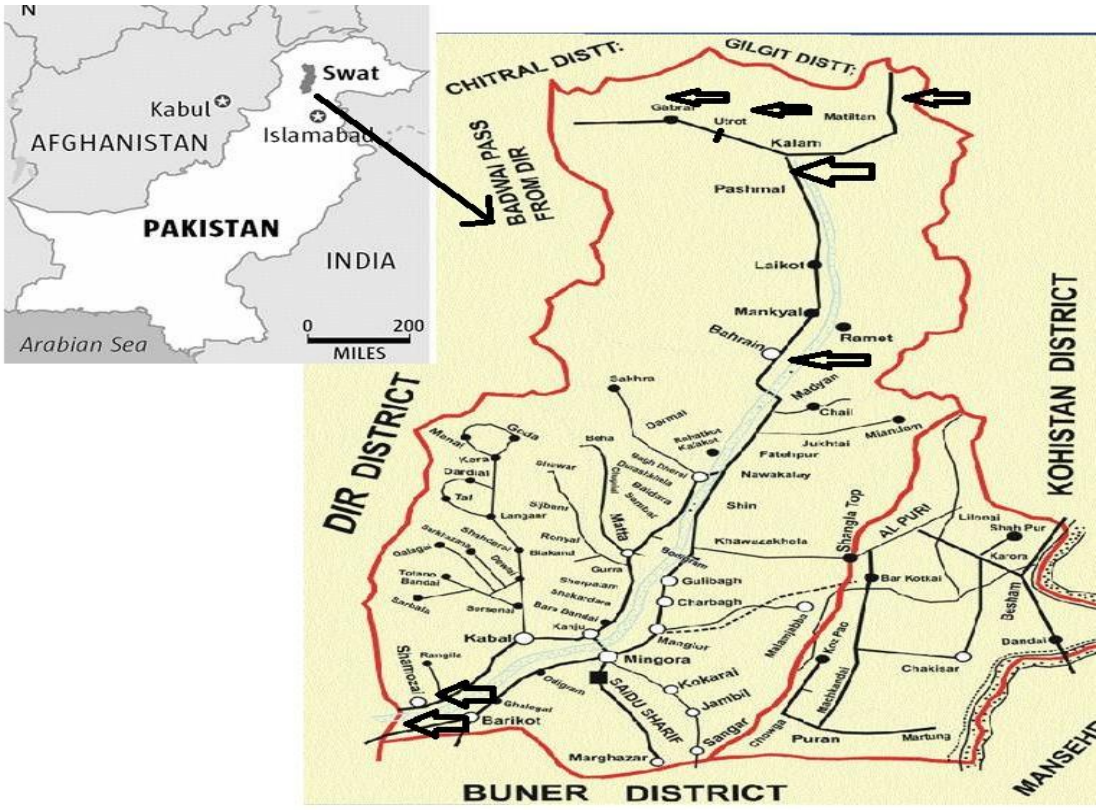

Figure 2: Map of District Swat, Collection sites represented with bold arrows

Table 1: Selected *Artemisia* L. species habitat and altitude.

Species	Habitat	Altitude recorded (m)	Altitude range(m)	Location
Artemisia maritima	Dry	693	700-1700	Gora ghat
Artemisia scoparia	Arid	760	400-2200	Shamozia
Artemisia vulgaris	Moist	1387	1500-3800	Bahrain
Artemisia persica	Moist	1430	2500-3500	Bahrain
Artemisia brevifolia	Low humidity	1954	2700-4500	Kalam
Artemisia annua	Moist	2225	2000-3700	Utror
Artemisia santolinifolia	Moist	2479	2000-4000	Mahodand
Artemisia absinthium	Moist	2550	1000-3500	Gabral

The OD (optical density) measurement of each well was determined in ELISA plate reader at 450 nm using formula:

Absorbance (%) = A/A_0 x 100%

Statistical analysis

The data from each variable was obtained independently in the form of three replicates. Using Microsoft excel 2007 descriptive statistics was performed to calculate mean, standard deviation and standard error for each treatment. One way ANOVA was performed by using SPSS

16.0 version. To know the significance of ANOVA Tuky's HSD (honestly significant difference) post hoc test was used.

RESULTS

The results of occurrence of aflatoxins-B1 level in collected medicinal plants of current study are presented in the table 2 and 3. The comparative evaluation of each *Artemisia* L. species against different strategies i.e., COD, CSD and UCSD suggested that for each *Artemisia* L. species lower value of aflatoxins-B1 found in COD method, higher value was recorded in UCSD method and medium value of aflatoxins-B1 recorded in CSD method. The species on the basis of aflatoxin-B1 level significantly different in each strategy as they do not share any alphabet for mean in common. Moreover the statistical value of F, Df and p are also given in table 2.

The statistical evaluation of eight *Artemisia* L. species against different drying strategies suggested that species such as *A.maritima*, *A.vulgaris*, *A.persica*, *A.santolinifolia* and *A.absinthium* showed significant differences while other species showed non-significant differences at f, Df and p value in COD method as given in table 3. In case of CSD *A.scoparia*, *A.annua* are significantly different while all other species are significantly non-different. In case of UCSD *Artemisia maritima* is significantly different from all the species while the other species were non-significantly different.

The outcomes of these findings showed that aflatoxins-B1 level in each *Artemisia* species significantly higher in UCSD method and lower in COD method at $p<0.05$. This indicates that increasing temperature (COD method) during drying of medicinal plants can reduce the chances of fungal contamination.

The results from comparative evaluation of *Artemisia* L. species against different strategies in each treatment indicate that a gradual increase of aflatoxin B-1 is observed in each treatment from *A.maritima* to *A.absinthium* as given in table 2 and 3. The altitudes recorded for specie through GPS also increases gradually from *Artemisia maritima* to *A.absinthium* as given in Fig 3.

Moreover comparatively higher value of aflatoxins-B1 level in *A.brevifolia*, *A.annua, A.santolinifolia* and *A.absinthium* in each treatment may be due to higher moist habitat as given (table 1 and Figure 4).

Table 2: Evaluation of each *Artemisia* specie against each method for aflatoxins-B1 level

Methods	Plants							
	A. maritima	***A. Scoparia***	***A. vulgaris***	***A. persica***	***A. brevifolia***	***A. annua***	***A. santolinifolia***	***A. absinthium***
COD	6.7 ± 0.33^{a}	7.6 ± 0.33^{a}	9.6 ± 0.33^{a}	12.6 ± 0.33^{a}	13.3 ± 0.33^{a}	14.6 ± 0.33^{a}	17.3 ± 0.33^{a}	20.0 ± 0.57^{a}
CSD	13 ± 0.57^{b}	11.7 ± 0.33^{b}	16 ± 0.57^{b}	16.6 ± 0.33^{b}	19.3 ± 0.33^{b}	23 ± 0.58^{b}	25.6 ± 0.88^{b}	26.7 ± 0.88^{b}
UCSD	21 ± 0.88^{c}	25 ± 1.2^{c}	27 ± 1.52^{c}	29.3 ± 0.88^{c}	32.3 ± 0.58^{c}	36 ± 0.58^{c}	39.6 ± 0.88^{c}	41.0 ± 0.57^{c}
Statistics	F= 132.8 , Df= 6, p<0.05	F= 158.8 , Df= 6, p<0.05	F= 204.6, Df= 6, p< 0.05	F= 227.1, Df= 6, p< 0.05	F= 490.4, Df= 6, p<0.05	F= 445.8 , Df= 6, p< 0.05	F= 229.2, Df= 6, p< 0.05	F= 239.1 , Df= 6, p< 0.05

The data mention in the each column with dissimilar later are significantly different while with similar letter are non- significant, the p value was kept (<0.05).

Table 3: Evaluation of *Artemisia* species against each method for aflatoxins-B1 level

Plant Species	Drying strategies		
	Mean±SD COD	**Mean±SD CSD**	**Mean±SD UCSD**
Artemisia maritima	$6.66^{a}\pm0.33$	$13^{ab}\pm0.57$	$21.33^{a}\pm0.88$
Artemisia scoparia	$7.66^{ab}\pm0.33$	$11.66^{a}\pm0.33$	$25^{ab}\pm1.15$
Artemisia vulgaris	$9.67^{c}\pm0.33$	$16^{c}\pm0.57$	$27.66^{bc}\pm0.88$
Artemisia persica	$12.66^{d}\pm0.33$	$16.66^{c}\pm0.33$	$29.33^{cd}\pm0.88$
Artemisia brevifolia	$13.33^{de}\pm0.33$	$19.33^{cd}\pm0.33$	$32^{de}\pm0.57$
Artemisia annua	$14.66^{ef}\pm0.33$	$23^{e}\pm0.57$	$36^{ef}\pm0.57$
Artemisia santolinifolia	$17.33^{g}\pm0.33$	$25.66^{ef}\pm0.88$	$39.66^{fg}\pm0.88$
Artemisia absinthium	$20^{h}\pm0.57$	$26.66^{fg}\pm0.88$	$41^{gh}\pm0.57$
Statistics	F= 154.2, Df= 16, p<0.05	F= 88.44, Df= 16, p<0.05	F= 71.88, Df= 16, p<0.05

The data mention in the each column with dissimilar later are significantly different while with similar letter are non- significant, the p value was kept (<0.05).

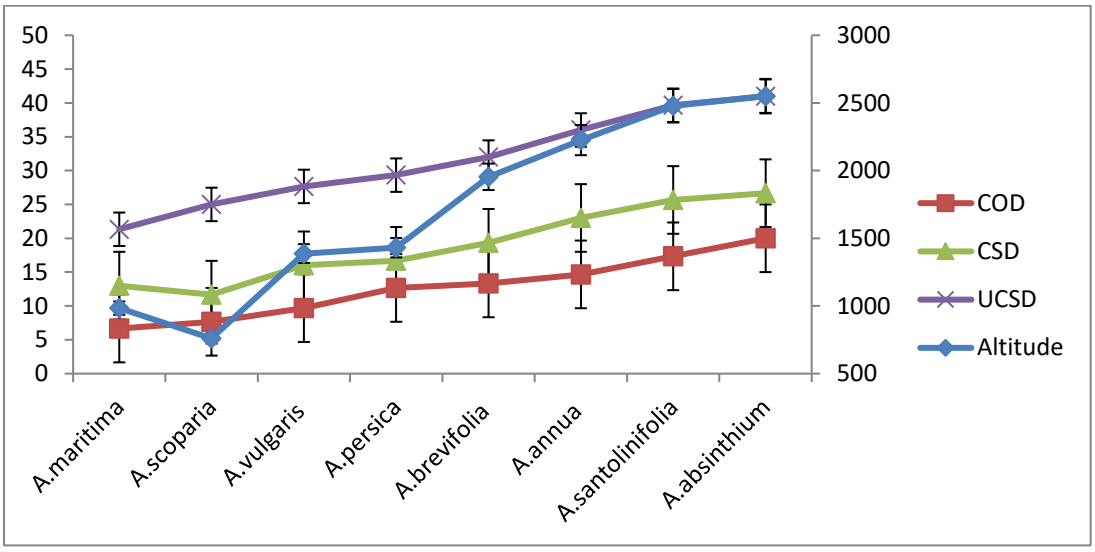

Figure 3: Aflatoxin B-1 frequency of *Artemisia* species along the altitude recorded.

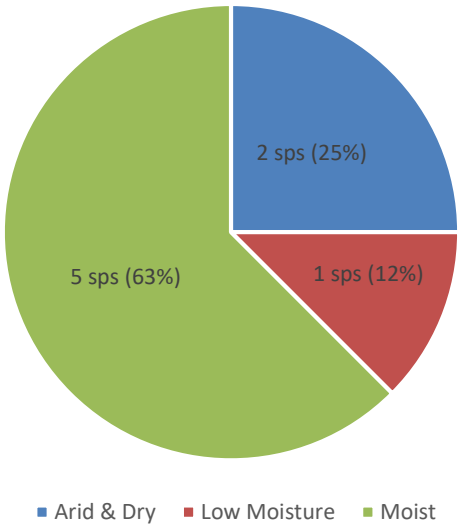

Figure 4: Aflatoxin B-1 frequency of *Artemisia* species along habitat.

The species distribution on the basis of habitat showed that two species *A.maritima* and *A.scoparia* belongs to dry habitat while five species *A.vulgaris*, *A.persica*, *A.annua* and *A.absinthium* belongs to moist habitat only *A.*brevifolia is low humidity loving specie. The results of table 2 and 3 showed consistency with high level of aflatoxin B-1 level in moist habitat loving species as compare to species of dry habitat.

DISCUSSION

Medicinal plants are often collected by the local who are mostly unaware about the scientific and standard procedure of collection which results in to the contamination of toxins. According to Rizzo et al. (2004) during harvesting, handling, storage and distribution, plants are subjected to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins. According to European Union regulations maximum approved limit for aflatoxin

B-1 level is 5 mg/kg or 5000 ng/kg for AFB1 (MozaffariNejad et al., 2014). In our current investigation none of the sample exceeds the prescribed limit of AFB1.

Drying time is also important for afltoxin level, methods i.e., COD, CSD and UCSD involves different drying times due to temperature and air fluctuations however COD is prefer due to saving of time and active constituents of medicinal plants. The studies conducted by Iqbal et al., (2011) showed that high temperatures possibly decrease mycotoxin concentrations due to lack of fungal growth.

A study conducted by Hansen et al., (1993) concluded that high temperature do not causes damages to active constituents of medicinal plants they reported very low amount of anti-cancerous drug taxol by keeping the temperature of drying air at 30ºC as too long drying time causes taxol degradation. They also reported that yield of taxol form *Taxus baccata* remained constant at 40, 50 and 60ºC. In our current study the drying temperature for COD was kept at 50ºC for drying medicinally important plants of *Artemisia*. Various authors stressed upon the choice of the correct drying temperature according to Rocha and Melo (2011) drying temperature for medicinal plants is highly important on the basis of ecological and economic value and research is need on this topic at urgent basis. For drying medicinal plants on large basis 50 and 60°C drying air temperatures appear to be reasonable. Increase temperature treatment causes reduction in the oil contents of medicinal plants due to existence of volatile compounds. A study conducted by Venskutonis (1997) observed a decrease amount of 43 and 31% from the total isolated compounds of *Thymus vulgaris* L. and *Salvia officinalis* under 60°C oven drying with comparable results as obtained from fresh plant.

Moist habitat and high altitude influence contamination of aflatoxins as recorded in our current scheme of studies. Similarly studies conducted by Ashiq et al., (2014) warm and moist conditions of tropical and subtropical countries are favorable for aflatoxin production. Mohamadi and Rajaei (2016) found significant effects on the oil yield of *Artemisia persica*, the effects of relative humidity and height were significant at $p<0.01$.

Country like Pakistan hosts diverse flora in which major portion comprises medicinal plants. However indigenous communities, hakeems and herbalists involve in the collection, drying and preservation of medicinal plants are unaware about the standard methods. A finding such as Iqbal et al., (2011) suggests that an increased ability to undertake aflatoxin analysis in Pakistan would be very beneficial, where there is currently limited information and capability. It is therefore this study will provide a conceptual approach for systematic processing of medicinal plants.

CONCLUSION

This study has carried out to check the effects of different drying methods, altitude and habitat on the level of aflatoxins B-1 in eight selected species of *Artemisia* L. which is of highly medicinally importance. The study has identified the importance of oven drying of medicinally important species moreover contents of aflatoxins B-1 gradually increases with increase of altitude; it was found that moisture habitat also favors the contamination of aflatoxin B-1 contents.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

We thank the poultry research institute (PRI) Rawalpindi Pakistan for providing the lab facility and Dr. Ghulam Farid for the technical assistance.

AUTHOR CONTRIBUTIONS

SZ designed and performed the experiments and also wrote the manuscript. BU and SWA supervised the study. GR and AH help in the collection and identification of plants.

Copyrights: © 2020@ author (s).

This is an open access article distributed under the terms of the **Creative Commons Attribution License (CC BY 4.0)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

REFERENCES

Abad MJ, Bedoya LM, Apaza L, Bermejo P. 2012. The *Artemisia* L. genus: a review of bioactive essential oils. *Molecules.*, *17*(3): 2542-2566.

Ashiq S, Hussain M, Ahmad B. 2014. Natural occurrence of mycotoxins in medicinal plants: a review. *Fungal Genet Biol*., *66*: 1-10.

Elabscience. 2018. Enzyme immunoassay for the quantitative analysis of aflatoxin B1. 8thEdition.USA.

Hansen RC, Keener HM, ElSohly HN. 1993. Thin-layer drying of cultivated Taxus clippings. *T ASABE.*, 36(5): 1387-1391.

Iqbal SZ. 2011. Aflatoxin contamination in chilies from Punjab Pakistan with reference to climate change. *Int J Agric Biol*., *13*(2): 261-265.

Migahed FF, Abdel-Gwad MM, Mohamed SR. 2017.Aflatoxigenic fungi associated with some medicinal plants. *Annu Res Rev Biol* , 1-20.

Mohamadi N, Rajaei P. 2016. Effects of Different Ecological Condition on the Quality and Quantity of Essential Oils of Artemisia persicaBoiss.Populations from Kerman, Iran. *J ESSENT OIL BEAR PL*, *19*(1), 200-207.

MozaffariNejad AS, Sabouri GM, Kamkar A. 2014. Determination of aflatoxin B1 levels in Iranian and Indian spices by ELISA method. *Toxin Rev*, *33*(4), 151-154.

Razzazi-Fazeli E, Noviandi CT, Porasuphatana S, Agus A, Böhm J. 2004. A survey of aflatoxin B 1 and total aflatoxin contamination in baby food, peanut and corn products sold at retail in Indonesia analysed by ELISA and HPLC. *MYCOTOXIN RES*, *20*(2), 51-58.

Rizzo I, Vedoya G, Maurutto S, Haidukowski M, Varsavsky E. 2004. Assessment of toxigenic fungi on Argentinean medicinal herbs. *Microbiol. Res*., *159*(2), 113-120.

Rocha RP, Melo EC. 2011. Influence of drying process on the quality of medicinal plants: A review. *JMPR*, *5*(33), 7076-7084.

Sewram V, Shephard GS, van der Merwe L, Jacobs TV. 2006. Mycotoxin contamination of dietary and medicinal wild plants in the Eastern Cape Province of South Africa. *J AGR FOOD CHEM*, *54*(15), 5688-5693.

Shehata, E, Grigorakis S, Loupassaki S, Makris DP. 2015. Extraction optimisation using water/glycerol for the efficient recovery of polyphenolic antioxidants from two Artemisia species. *Sep. Purif*, *149*, 462-469.

Venskutonis PR. 1997.Effect of drying on the volatile constituents of thyme (Thymus vulgaris L.) and sage (Salvia officinalis L.).*Food chem*, *59*(2), 219-227.