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QUALITATIVE AND QUANTITATIVE EVALUATION OF MYCOTOXIN LEVELS IN EXTENSIVELY IMPORTANT MEDICINAL PLANTS USED IN FORMULATION OF HERBAL DRUGS AND OTHER EDIBLES

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ABSTRACT

The investigation was outlined to interpret on the mycotoxic noticeable quality of the herbs materials utilized in home grown planning. Dried organic product tests of *Terminalia bellir*ica (Baheda), *Terminalia chebula* (Harada), *Asparagus racemosus* (Shatavari), *Withania somnifera* (Ashwagandha), *Aegle marmelos* (Bael), *Synzygium cumini* (Jamun), *Solanum nigrum (*Makoi), *Emblica officinalis* and *Tinospora cordifolia* (Giloy) were gathered from arbitrary market sources. They were under given to Mycological examination for the acumen and identification of organisms utilizing credible extraction processes. Extracts coding AC0904, SAM 01, SAM 03, SAM 05, SAM 07 were subjected to qualitative and Analytic analysis presence of Aflatoxin A1 was found to be predominant and exceeding permissible count. Study affirmed two subjects oe the extraction procedure for aflatoxin was effective as all sample showed qualitative presence of Aflatoxin however wasn't found in analytical study because of Limit of detection and second that the extracts of *S.nigrum* and *E. officinalis* were highly contaminated by Aflatoxin reaching 1.19 ng/ml.

Keywords: Qualitative and Quantitative Evaluation, Mycotoxin, Medicinal Plants

INTRODUCTION

Natural drug is one of the most established type of social insurance known to the humanity. Consequently, herbal Medicine has been utilized restoratively all around the globe, being a significant part of different conventional drug framework. The utilization

of Ayurveda is one of the most established, most extravagant and most various convention related with the utilization of restorative plants in India **[1]**. In this arrangement of medication, various pieces of the restorative plants are utilized in unrefined just as powdered structure. More than 8000 plant species have been accounted for the arrangement of nearly 25,000 details that are utilized to treat different diseases **[2]**. As indicated by Ayurveda, immaculate processing is the premise of all wellbeing and 'triphala beat' is a superb ayurvedic medicate that goes about as an ideal tonic for appropriate absorption **[3]**.

Asparagus racemosus (family-Asparagaceae) otherwise called "Shatavari, signifies "she who has a hundred spouses" shows that this herb is exceptionally compelling in issues related with female conceptive framework. Charak Samhita composed by Charak and Ashtang Hridyam composed by Vagbhata, the two primary messages on Ayurvedic prescriptions, records Asparagus racemosus as a feature of the recipes to treat issue influencing women "swell being" *Syzygium cumini* (Family Myrtaceae) is otherwise called *Syzygium jamunum and Eugenia cumini*. Other normal names are Jambul, Black Plum, Java Plum, Indian Blackberry, Jamblang, Jamun and so forth. The tree organic products once in a year and the berries are sweetish harsh to taste **[4]**. The ready organic products are utilized for wellbeing drinks, making jelly, squashes, jams and wine also they possess antidiabetic properties therefore the seeds powder is generally dried consumed **[5]**. Bael has a significant spot in the customary frameworks of medication the Ayurveda **[6]**. The leaves, roots, bark, leafy foods seeds are accounted for to have different therapeutic properties and to fix different human infirmities and illnesses **[7, 8]**. The leaves should be the most helpful in the treatment of fever, to stop stomach torment discontinuous fever, ease urinary issues, palpitation of the heart, looseness of the bowels, dyspepsia, stomachalgia, original shortcoming, heaving, fever and swellings **[8]**. The restorative estimation of Kalmegh is because of its instrument of activity by chemical enlistment. It is a significant cold property herb utilized in fever and to scatter poisons from body. It is utilized to treat gastrointestinal tract and upper respiratory tract contaminations, fever, herpes, sore throat, hepatitis and an assortment of other constant irresistible infection. It shows cardiovascular impacts, fruitfulness impacts and security of Liver and Gallbladder **[9]**. The constituents of natural mix that is, dried

organic product powder of *Aegle marmelos, Terminalia chebula, Terminalia bellirica* are exposed to contagious defilement at different strides of its readiness. The natural products get polluted during development (while the organic products are on tree), in the wake of reaping (when natural products are dried), preparing and during capacity. Post-collect decay by filamentous organisms is one of the most widely recognized dangers related with handled and put away natural items. The informal techniques for reaping, accumulation, stockpiling of crude materials, preparing and poor stockpiling of home grown medications, retailed in the business sectors frequently straightforwardly in the unhygienic conditions are the primary driver of contagious contaminations **[10]**.

Mycotoxins are auxiliary abiotic peril metabolite delivered by organisms in the polluted nourishments **[11]**. The poisons may frame on fields or during the capacity or notwithstanding preparing of the food sources **[12]**.

What's more, mycotoxins created by them in these home grown medications can likewise cause a few illnesses of liver, kidney, sensory system, strong, skin, respiratory organs, stomach related tract, genital organs, and so forth **[13]**. Event of mycotoxin tainting in dried products of *E. officinalis, T. bellirica* and *T. chebula* appear to be very unavoidable. The warm and sticky climatic states of significant regions of J&K, alongside the poor states of broadened stockpiling could likewise advance the germination and development of capacity organisms. Hence, the present examination was directed to explore the status of form and mycotoxin pollution related with these three significant parts of triphala so as to evaluate the conceivable introduction of neighborhood clients to the debased home grown medications.

MATERIALS AND METHODOLOGY

Isolation of mycoflora associated with the market samples: Surface mycoflora associated with the market samples of dried fruits of *Terminalia bellir*ica (Baheda), *Terminalia chebula* (Harada), *Asparagus racemosus* (Shatavari), *Withania somnifera* (Ashwagandha), *Aegle marmelos* (Bael), *Synzygium cumini* (Jamun), *Solanumnigrum (*Makoi), *Embalica officinalis* and *Tinospora cordifolia* (Giloy) was determined by using surface washing technique. In this method, 5g of sample broken into small pieces was taken in an Erlenmeyer flask of 250ml capacity containing 25ml sterilized distilled water and subjected to horizontal shaking for 30 minutes on a rotary shaker. The liquid was then decanted and centrifuged at 3000 r.p.m.,

for 15 minutes. The residue thus obtained was mixed with 15ml sterilized distilled water and shaken vigorously to obtain a homogenous suspension. This suspension was poured in sterilized Petri-plates at the rate of 1ml / plate with the help of sterilized pipette. For the recovery of maximum number of fungal propagules from the surface of each sample, three different media – Dichloran Rose Bengal Chloramphenicol Agar (DRBC), Dichloran 18% Glycerol Agar (DG 18) and Malt Salt Agar (MSA) were used. The medium was poured by making gentle rotational movements of the Petri-plates so as to ensure uniform spreading of the sample. Petri-plates thus prepared were incubated at 28+2ºC and after 7 days of incubation, observations for the development of different fungal species were made.

Extraction of mycotoxins from dried therapeutic plants:

Aflatoxins were extracted with the help of Soxhlet apparatus, the diseased sections of plants 10-15 gm mainly was dried in the shade and its moisture was completely evaporated then ground. With the help of reflux extraction using methanol and Acetonitrile as solvents in which Aflatoxin can be dissolved the mycotoxins were extracted at temperature around 70°C for 8 hours.

Subjective Detection of Aflatoxin B1, B2, G1, G2

Known measure of test concentrates and gauges of explored mycotoxins got from Sigma Aldrich Co. were spotted on actuated TLC (Thin Layer Chromatographic) plates and created with various dissolvable frameworks. Range of mycotoxins present in therapeutic herbs the concentrates which were set up by the previously mentioned strategies were stacked 10 µl were stacked on TLC plate. 10 µl of test which contained 100ng/ml convergence of Aflatoxin B1, B2, and G1.G2 were stacked in the principal position of TLC plate and the rest tests were stacked neighboring to the standards. The retention time 0.6 and slight green fluorescence corresponded to Aflatoxin B 1, For Aflatoxin B2 0.4 while G1 and G2 showed appearance at around 0.8 and 0.9 **[14]**. For TLC, 10 µL of extracts were loaded in plates, together with specific standards, developed in benzene: methanol: acetic acid (24:2:1) and toluene: ethyl acetate: formic acid (6:3:1) solvents and then observed under long wavelength UV- light at 365 nm **[15]**.

Run in HPLC to quantitate Aflatoxin and Patulin

A well-equipped HPLC system Shimadzu class VP.V6.10 Chiral Pak-IB column eluting solvent used was Acetonitrile: Methanol in

the ratio of 70:30, flow rate kept for the elution of Aflatoxin were 1 mL/min column temperature: 25°C detection wavelength: 350 nm.

Preparation of Standard:

In a 4 mL salinized vial (total AF 400 ng/mL in acetonitrile: AFB_1 200 ng/mL, AFB_2 50 ng/mL, AFG_1 100 ng/mL, AFG_2 50 ng/mL).

Patulin standard stock solution preparation - In a 4 mL salinized vial (PAT 200 ng/mL in methanol). Preparation of Sample: The samples which were implemented for the Evaluation of Aflatoxin B1, B2, G1, G2 and Patulin were AC0904, SAM 01, SAM03, and SAM 04 as the showed qualitative presence of these toxins when were run on TLC plate.

RESULT AND DISCUSSIONS

HPLC Plot of Aflatoxin B1, B2, G1, G2

Aflatoxin B 1

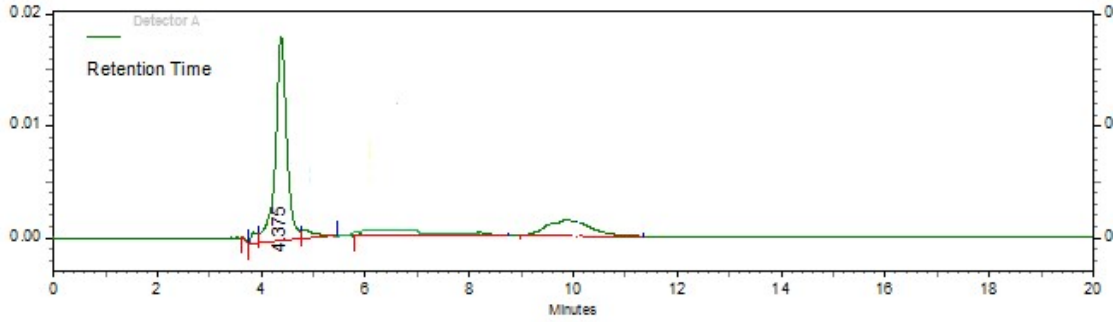

Figure 1: The above graph represents the peak at 4.375 minute retention factor and this was obtained when 100 % pure aflatoxin B1 was run in HPLC

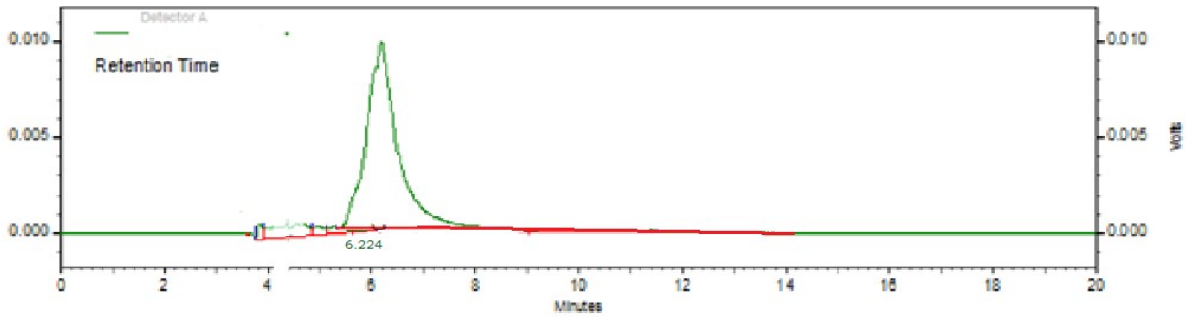

Figure 2: The above graph represents the peak at 6.224 minute retention factor and this was obtained when 100 % pure aflatoxin B2 was run in HPLC

Aflatoxin G1

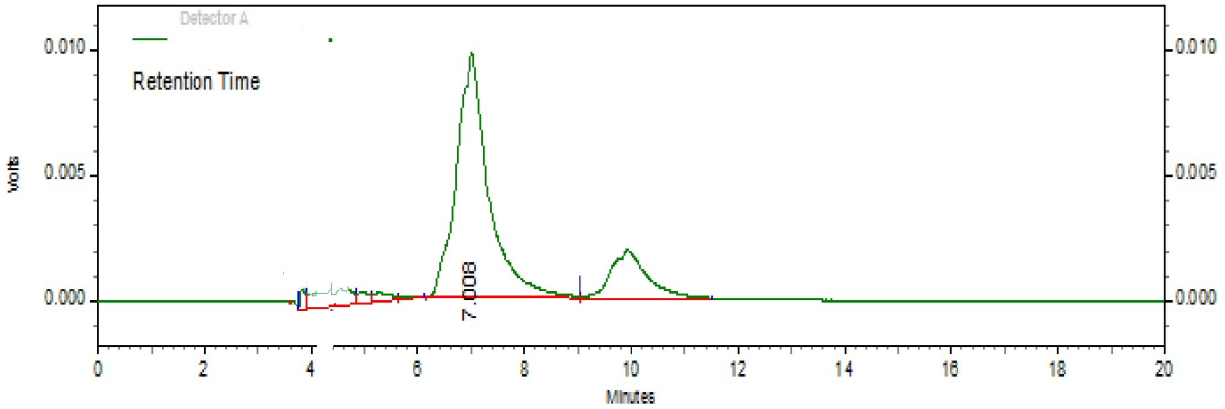

Figure 3: The above graph represents the peak at around 7 min retention factor and this was obtained with 96% pure Aflatoxin G1

Aflatoxin G2

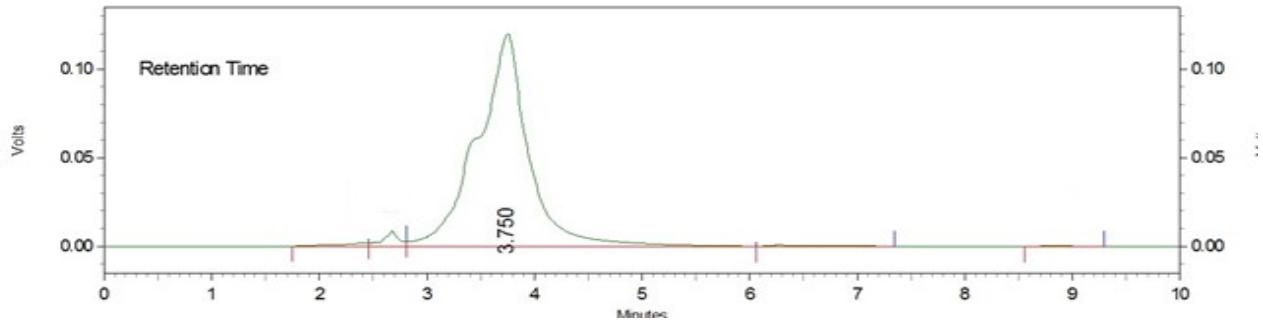

Figure 4: The above graph represents the peak at around 3.7 min retention factor and this was obtained with 96% pure Aflatoxin G2

Graphs of Samples

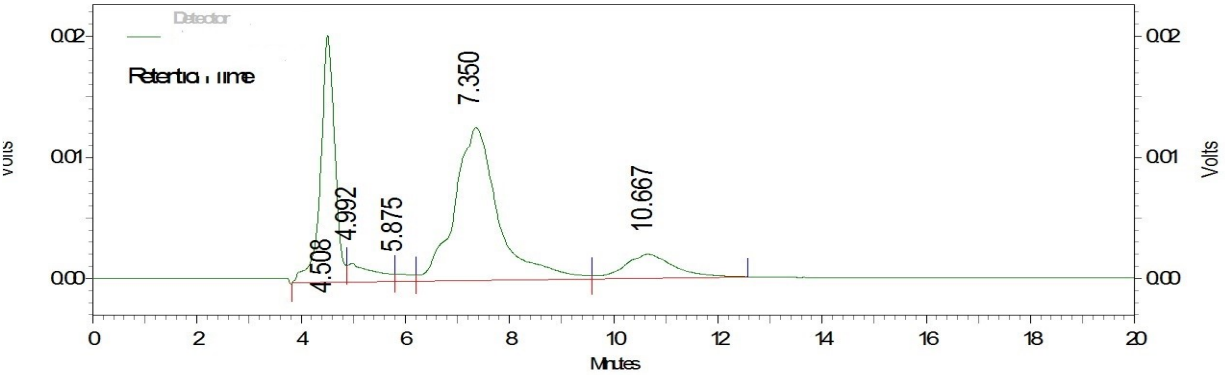

Figure 5 : The above graph represents different peaks which were run at the wavelength of Aflatoxin the above graph is of AC0904 here the peak at 7.360

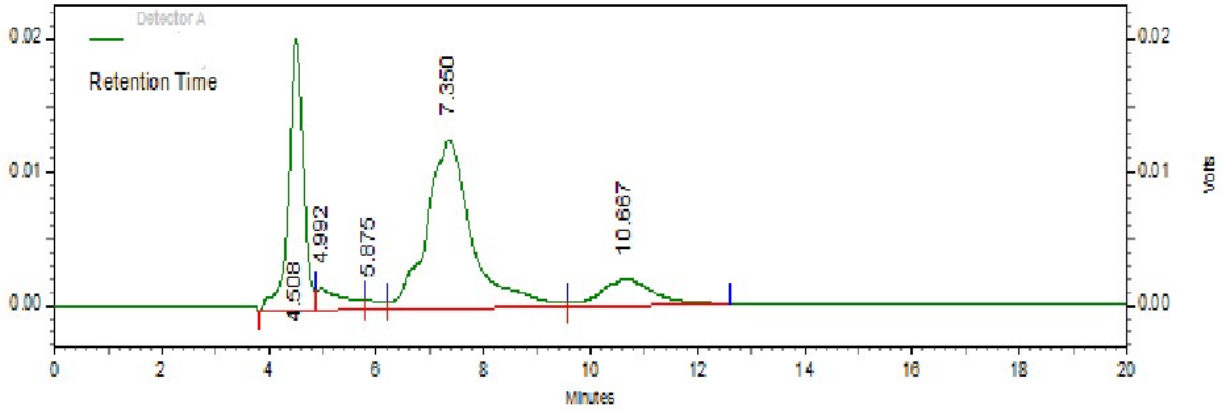

Figure 6: The figure above represents chromatogram of sample SAM 01 here there comes the presence of Aflatoxin B1 and Aflatoxin G1 has been observed predominantly

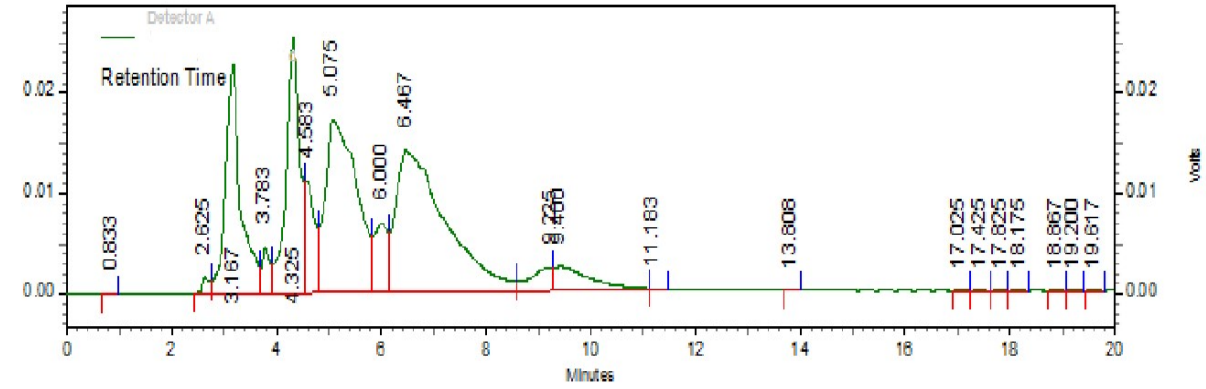

Figure 7 : The above figure resembles several peaks and here it could be observed that all the four Aflatoxins are present in the sample SAM 03

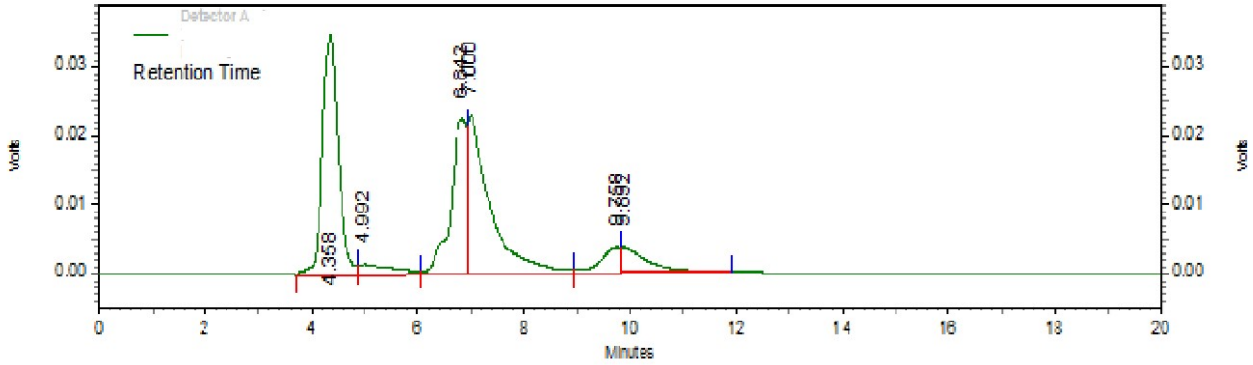

Figure 8: The above figure represents the presence of Aflatoxin B1 and Aflatoxin G1 which are present in slight enormous amount and rest others could be present but in very small amounts in SAM 05 .

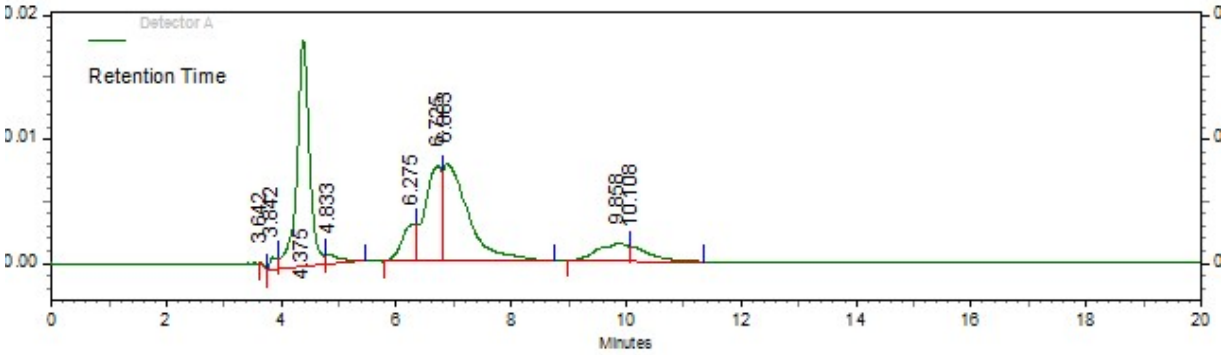

Figure 9: The above figure represents the sample SAM 07 purely extracted Aflatoxin B1

To quantify the results of thin layer Chromatography of Aflatoxin B1, B2, G1, G2 and Patulin the distance traveled by the toxin present in plant extract being considered was divided by the total distance traveled by the mobile phase. The ratio of the result also known as retardation factor called the retardation factor (R_f). In general, it is well known that a substance whose structure resembles the stationary phase will have low R_f, while one that has a similar structure to the mobile phase will have high retardation factor. Retardation factors are characteristic, but will change depending on the exact condition of the mobile and stationary phase. For this reason, in this research standard was applied to the plate just before the samples. The table mentioned below shows the retardation factors of it (29.4% of whole samples). The analysis of herbal plants when its diseased part was extracted in order to procure mycotoxins samples showed no aflatoxin levels over the permissible limits (15 ngg-1 for aflatoxin). In sample AC0904 (680 ng/ml was obtained by system), AFB1 was seen over the permissible limits. The lofty levels possessed in samples for total aflatoxin and AFB1 , AFB2 , AFG1 , and AFG2 were 9.08 ngg -1 , 7.41 ngg-1 , 0.28 ngg-1 , 1.54 ngg-1 and 0.13 ngg-1 , respectively. The mean levels for AFB1, AFB2, AFG1, AFG2 and total aflatoxins in all samples for SAM 01 the mean Aflatoxin was observed to be 1.32228±0.4021 ngg-1, subsequently for SAM 03 the observed amount was 0.0871±0.03030 ngg-1, a mediocre or feeble amount was observed in the sample SAM 07 0.27928±0.0920 ngg-1, the amount least found was observed in SAM 05, 0.04426±0.0151 ngg-1 and 1.8321±0.677 ngg-1, respectively. In samples contained at least one type of aflatoxin, the mean of levels for AFB1 , AFB2 , AFG1 , AFG2 and total aflatoxins are 2.33774±1.599 ngg1 , 0.12473±0.1030 ngg-1 , 0.4573±0.3130 ngg-1 , 0.716±0.0511 ngg-1 and 2.6600±2.41710

ngg-1 , respectively. Out of all studied 18 samples these samples AC0904, SAM 01 SAM 03, SAM 05 and SAM 07 were found to be contaminated by AFB1, and ninety percent of samples contained at least one type of aflatoxins showed contamination by AFB1. Amounts of humidity in samples contained at least one type of aflatoxins ranged from 6.3 to 12.40 percent.

CONCLUSION

Therapeutic plants and herbs are normally utilized in many family units as culinary for flavor and fragrance, as natural teas or to mitigate sickness. This investigation infers that restorative herbs and flavors can be sullied with mycotoxins-delivering parasites. The outcomes demonstrated that the crude materials as well as the prepared herbs also are vulnerable to toxigenic parasites. Defilement of these items with toxigenic growths presents genuine wellbeing dangers, since their essence can cause sick impacts as opposed to improving the personal satisfaction. Legitimate stockpiling conditions and quality control at each phase of handling, bundling or promoting are essential for the wellbeing of the purchasers. This investigation gives a premise in evaluating the level of contagious and potential mycotoxin tainting in therapeutic plants, herbs, and flavors, accordingly giving quality control. Therefore a valid quality control is required.

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