



## Fungal load evaluation of the leave and fruit extracts of *Vitex Doniana* (black plum) using three basic phyto-medicinal preparatory protocols

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### Abstract

The studies were carried out to determine the phytochemical content and fungi using different solvents and drying methods of leaves and fruits sample of black plum (*V. doniana*). The research was conducted at laboratory of plant biology department, Bayero University Kano. The results shows that, alkaloid ( $1.31 \pm 0.02$ ), Flavonoid ( $0.40 \pm 0.01$ ) saponin ( $0.139 \pm 0.02$ ), tannin ( $0.80 \pm 0.01$ ) phenol ( $0.09 \pm 0.001$ ) quinone, glycoside and terpenoid were present in the leave extract of black plum (*vitex doniane*). The leaves of this plant contain highest percentage of alkaloid ( $1.31 \pm 0.02$ ) when compared with rest. Higher number of phytochemicals were found present in the shade dried extract followed by oven dried and sun dried extracts. Fungi such as *Penicillium* spp (66.7%) and *Madurella* spp. (33.3%) were found present in shade dried but *Fusarium* spp (25%), *Penicillium* spp (25%), *Aspergillus* spp (25%) and *Rhizoctonia* spp. (25%) were presence in oven dried sample. The sun dried leaf sample shows the presence of *Mucor* spp. (40%), *Fusarium* spp (20%) and *Penicillium* spp (40%). The highest percentage of contaminant fungi such as *Penicillium* (66.7%) was observed in the shade dried sample. But the isolated fungi from fresh fruits sample, shows the presence of *Aspergillus niger* (33.3%), *Alternaria brassica* (25%), *Mucor Racemosa* (8.3%), *Aspergillus flavus* (16.7%) and *Rhizopus stolonifer* (16.7%) fruits of this plant contain high percentage of *Aspergillus Niger* (33.3%). It is lastly concluded that, the shade dried and sun dried methods should be used with caution in Phyto-medicines as there is a clear risk of causing further health problems, but rather the oven dried method as it contains the less percentage of toxic fungi.

**Keywords:** *V. doniana*, fungal contamination, fungi, Phyto-medicine

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### Introduction

Phytochemicals are chemicals from which plants drugs are obtained, and plants are the success of raw materials for both pharmaceutical and aromatic industries (Hassan *et al.*, 2021a) [5]. United States foods and drugs administration (2007) reported that phytochemicals are chemical compound which occur naturally in plants. The name phytochemicals is actually derived from the Greek word: phyto meaning “plants” and chemical meaning “substance”. Some chemicals are responsible for color and others for organoleptic properties, such as the deep purple of blueberries and they are responsible for smelling garlic, but the term is generally used to refer to chemical that have biological significance (Sathiamoorthy *et al.*, 2007) [19]. Fruits usually, incorporate high levels of nutrients element and sugars and their low pH values make them exceptionally desirable to fungal decay (Abdullah *et al.*, 2016) [1].

Phytochemicals are non-nutritive plants chemicals that have diseases preventive properties and they are non-essential nutrients, meaning that they are not required in human body for suitable life and many chemicals having potential properties to affect disease such as cancer, stroke, or metabolic syndrome.

It is also served as anti-biotic, anti-fungal, anti-inflammatory, diabetes control and higher blood pressure (Mir *et al.*, 2013)<sup>[10]</sup>. Fungi are considered as an essential post-harvest losses agent of different fruits, based on cultivar, season and production area amid other factors (Valiuskaite *et al.*, 2006)<sup>[21]</sup>.

Anti-bacterial activities in this context explain the means of phytochemicals that have an effect on infectious micro-organisms and macro-organisms such as fungal, bacterial, insects, stomach disorder and plants disease. Phytochemicals are called secondary metabolite. In Nigeria, *V. doniana* is used in treating different diseases, such as diarrhea, diabetes (Tiwari *et al.*, 2007). A fruit plant (*V. doniana*) is extremely widespread in tropical Africa occurring from Senegal east to Somalia and South Africa, also in Comoros and Seychelles. It is occasionally cultivated elsewhere e.g. Mauritius (Dharmasiri *et al.*, 2003)<sup>[3]</sup>.

## Materials and Methods

### Collection and Handling of Plant Materials

Fresh leaves of Black plum (*V. doniana*) were collected, identified and authenticated from Biological sciences department, Bayero University Kano, Nigeria. The leaves were drugged into three (3) each was washed thoroughly with distilled H<sub>2</sub>O. First sample was placed into oven at 60<sup>0</sup>c of temperature for three (3) hours to obtained oven dried sample. Second sample was placed. Under sun for two (2) days to obtained sun dried sample. Thirds sample was placed in well ventilated room for four days to obtain air shed (cure) dried sample. All three (3) different dried leaves were separately grounded into powder by using pestle and mortar and then sieved with a 3mm siever. Each sample was stored in separate clean containers (Lakache *et al.*, 2016)<sup>[8]</sup>.

### Extraction Preparation

Four (4) different solvents were used in the percolation process; these are acetone, methanol, distilled water and petroleum ether. The extraction process was adopted from the standard procedure described by Sofowara, (1993). 25g of fine powder of leave of Black plum from each three different drying methods were weighed and suspended into a 250ml capacity comical flask. These were percolated with 250ml of (acetone, methanol, Distilled water and petroleum ether), then shaken vigorously for one week. The extract was filtered through a whatman filter paper. The filtrates were then concentrated using a water bath set at 40<sup>0</sup>c till evaporated (Sahira and Catherine, 2015)<sup>[18]</sup>.

### Phytochemicals Analysis

Qualitative and quantitative chemical test for the screening of the phytochemical constituents in the local plants under study were carried out in extracts using the standard procedures as described by Harborne, (1998) and Sofowara, (1993).

### Isolation of Fungi from Fresh Fruits of *V. doniana*

204 fruits of Black Plum were collected at random from *vitex* tree of which, 147 were healthy fruits without symptoms of disease. 57 of the fruits were diseased fruits having symptoms of disease. Only two types of symptoms were identified on all disease fruits. The symptoms were: Blight and rot. Fruit sample were surface sterilized with 1% sodium hypochloride solution and rinsed with distilled water. The sample were cut with sharp sterilized razor blade and forceps and then cultured on the labeled potato dextrose agar (P.D.A) plates

(Moran *et al.*, 2012)<sup>[11]</sup>. The plate were prepared in triplicate. The P.D.A plates were inverted and incubated at room temperature, then the plates were observed after five (5) days and the different growing organisms were sub-cultured onto S.D.A media in separate petri-dishes and incubated at room temperature in inverted form. They were observed macroscopically and microscopically by characteristics features and identified, using the methods of (Hassan *et al.*, 2021a)<sup>[5]</sup>.

The fungal isolate were characterized based on macroscopic and microscopic appearance which comprises pigmentation, colour of aerial and substrate hypae, shape and kind of sexual spore presence of special structures such as foot cell, sporangiophore or conidiophores and the characteristic of spore. A small portion of the mycelia growth were picked carefully with the aid of a pair sterile dissecting needle and placed on a drop of lacto-phonol cotton blue on a slide and covered with a covered slip. The slide were examined under microscope with x10 and x40 objectives lens respectively, (Oyeleke and Manga, 2008)<sup>[17]</sup>.

### Isolation of Fungi from Different Dried Leafs Sample of *V. doniana*

Fresh leaves of Black plum (*V. doniana*) were collected identified and authenticated at herbarium unit of plant Biology department Bayero University Kano. The leaves were drugged into three (3) each was washed thoroughly with distilled H<sub>2</sub>O. First sample was placed into oven at 60<sup>0</sup>c of temperature for three (3) hours to obtained oven dried sample. Second sample was placed. Under sun for two (2) days to obtained sun dried sample. Thirds sample was placed in well ventilated room for four days to obtain air shed (cure) dried sample. All the three different dried leaves were used for the isolation of fungi (Dharmasiri *et al.*, 2003)<sup>[3]</sup>. Leaf sample were surface sterilized with 1% sodium hypochloride solution and rinsed with distilled water. The sample were cut with sharp sterilized razor blade and forceps and then cultured on the labeled potato dextrose agar (P.D.A) plates. The plate were prepared in triplicate. The P.D.A plates were inverted and incubated at room temperature, then the plates were observed after five (5) days and the different growing organisms were sub-cultured onto S.D.A media in separate petri-dishes and incubated at room temperature in inverted form. They were observed macroscopically and microscopically by characteristics features and identified, using the methods of (Moran *et al.*, 2012)<sup>[11]</sup>.

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## Result

### Result for Qualitative Phytochemicals Screening of Shed Dried Leafs Extract of Black Plum

The result shows the presence of alkaloid, saponin tannin, phenol, terpenoid, quinone, flavonoid and glycoside. As shown in table 1 below;

**Table 1:** Qualitative phytochemicals screening of shed dried leaf extract of black plum

Phytochemical	Distilled water	Acetone	Methanol	Petroleum ether
Alkaloids	+	+	+	+
Flavonoids	+	-	-	-
Saponin	+	-	-	+
Tannin	+	+	-	-
Phenol	+	-	+	+
Terpenoid	+	+	+	+
Quinone	-	-	-	+
Glycoside	+	-	-	-

Keys: + = presence, - = absent

### Result for Qualitative Phytochemicals Screening of Sundried Leaf Extracts of Black Plum

The result indicate the presence of alkaloid, saponin, tannin, phenol, terpenoid, flavonoid and glycoside in the sun dried leaf sample of black plum but quinone was absent in this extract (table 2).

**Table 2:** Qualitative phytochemicals screening of sundried leaf extracts of black plum

Phytochemical	Distilled water	Acetone	Methanol	Petroleum ether
Alkaloids	+	+	+	-
Flavonoids	-	-	-	-
Saponin	+	+	+	-
Tannin	+	-	+	-
Phenol	+	+	-	+
Terpenoid	+	+	+	+
Quinone	-	-	-	-
Glycoside	+	+	+	+

Keys: + = presence, - = absent

### Result for Qualitative Phytochemicals Screening of Oven Dried Leaf Extract of Black Plum

The result indicated the presence of alkaloid, saponin, tannin,

phenol, terpenoid and quinone in the oven dried leaf extract sample of black plum while flavonoid and glycoside were absence in this extract (Table 3).

**Table 3:** Qualitative phytochemicals screening of oven dried leaf extract of black plum

Phytochemical	Distilled water	Acetone	Methanol	Petroleum ether
Alkaloids	+	+	+	+
Flavonoids	-	-	-	-
Saponin	+	-	-	+
Tannin	+	+	-	-
Phenol	+	-	+	+
Terpenoid	+	+	+	+
Quinone	-	-	-	+
Glycoside	+	-	-	-

Keys: + = presence, - = absent

### Result for Quantitative Phytochemicals Screening of Leave Extract of Black Plum

The result shows the percentages of five tested phytochemicals in the leaf extract sample of black plum, such phytochemicals are alkaloid, flavonoid, tannin, saponin and phenol (table 4).

**Table 4:** Quantitative phytochemicals screening of leave extract of black plum

Phytochemical	Quantity presence in plate
Alkaloids	1.31 ± 0.02
Flavonoids	0.40 ± 0.01
Saponin	0.139 ± 0.02
Tannin	0.80 ± 0.02
Phenol	0.09 ± 0.001

### Result for the Cultural Characteristics of Fungal Isolate of Leaves and Fruits of *V. doniana*

The table below shows the macroscopic characteristics, colour, hypha type and spore type of each isolated fungi.

**Table 5:** Cultural characteristics of fungal isolate of leaves and fruits of *V. doniana*

Macroscopic Characteristics	Colour	Hypha type	Spore type	fungi
Cottony dense white with black dots covering the whole plates.	Dense white	Non septate	Roughly spherical in shape	<i>Mucor spp</i>
Dense grey with black dots.	Grey with black dots.	<i>Non septate</i>	<i>Roughly spherical in shape</i>	<i>Rhizopus spp</i>
Cottony and dark brown.	Dark brown	<i>Non septate</i>	<i>Roughly spherical in shape</i>	<i>Aspergillus spp</i>
Colony become dark to reddish brown and acquired a brownish black reverse	Dark to reddish brown.	Septate	Flask shaped phialides that bear rounded conidia	<i>Madurella spp</i>
Black, velvet, hairy, with white surrounding.	Black and green	<i>Septate</i>	<i>Roughly spherical in shape</i>	<i>Penicillium spp</i>
Cottony wide spread	White	<i>Septate</i>	<i>Club- shape</i>	<i>Fusarium spp</i>
Hairy, white to deep brown	White to deep brown mycelium	<i>Septate</i>	<i>They do not produce spores</i>	<i>Rhizoctonia spp</i>
Hairy, brown with white colony in circles	Pale brown to olive brown	Septate	<i>Club-shape</i>	<i>Alternaria spp</i>

### Result for Frequency of Occurrence of the Isolated Fungi from of Leaves *Vitex Diniana*

The table below shows, the frequency and percentage

occurrence of the isolated fungi sundried leaf sample, shade leaf sample and oven dried leaf sample of *V. doniana*.

**Table 6:** frequency occurrence of the isolated fungi from of leaves *V. doniana*

Sample	Isolated fungi	Frequency	Percentage of occurrence %
Shade dried	<i>Penicillium spp.</i>	2	66.7
		1	33.3
Oven dried	<i>Madurella spp</i>	Total =3	Total =100
	<i>Fusarium spp</i>	1	25
	<i>Penicillium spp</i>	1	25
	<i>Aspergilla us spp</i>	1	25
	<i>Rhizectonia spp</i>	1	25
		Total = 4	Total =100
Sundried	<i>Mucor spp</i>	2	40
	<i>Fusarium spp</i>	1	20
	<i>Penicillium spp</i>	2	40
		Total =5	Total =100

**Result for the Frequency of Occurrence of the Fungal Isolate of the Fresh Fruit of Black Plum: (*V. doniana*)**

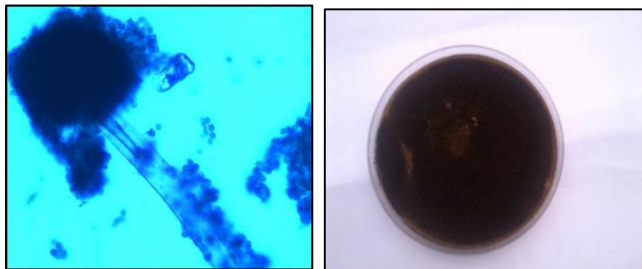
The table below shows, the frequency and percentage

occurrence of isolated fungi from fresh fruits sample of *V. doniana* based on the two visible symptom appeared on fruits (Rot and blight).

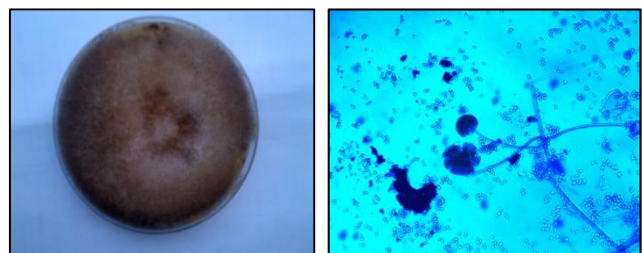
**Table 7:** frequency of occurrence of the fungal isolate of the fresh fruit of black plum: (*V. doniana*)

Sample and Symptom	Isolated fungi	Frequency	Percentage of occurrence %
Fresh fruits with symptom of blight	<i>A. niger</i>	4	33.3
	<i>Alternaria brassica</i>	3	25
	<i>Mucor racemosa</i>	1	8.3
Fresh fruits with symptom of rot	<i>Aspergillus flavus</i>	2	16.7
	<i>Rhizopus stolonifer</i>	2	16.7
		Total =12	Total =100%

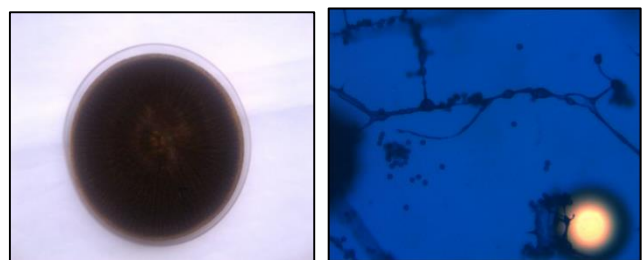
**Pictures of the Isolated Fungi**



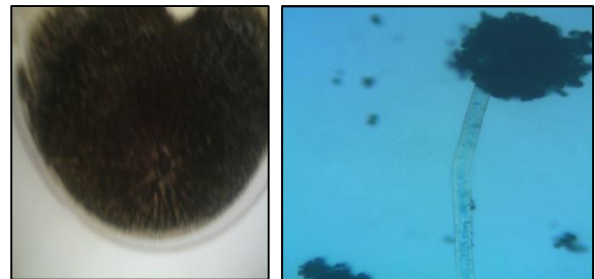
*Aspergillus niger*



*Rhizopus stolonifer*



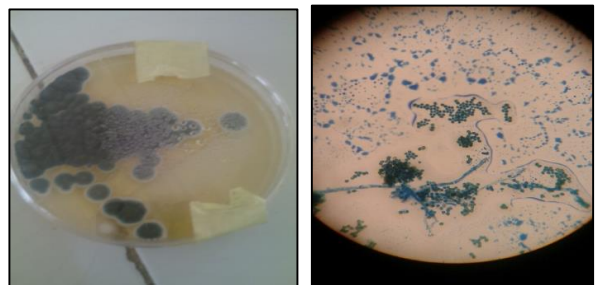
*Alternaria brassica*



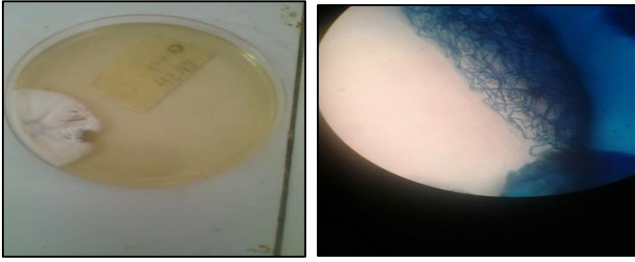
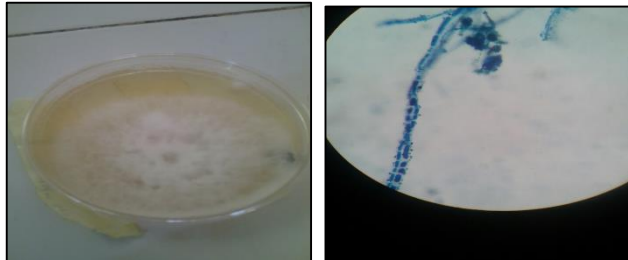
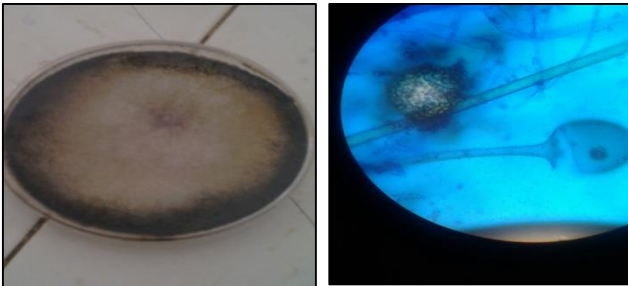
*Aspergillus flavus*



*Madurella spp*



*Penicillium spp*

*Rhizoctonia spp**Fusarium spp**Mucor racemosa*

### Discussion

Plants contained many phytochemicals useful to human health system and also the plants in protecting themselves against pathogenic agents (Hassan *et al.*, 2020) [6]. In this study different phytochemicals content analysis and isolation of fungi associated with different drying methods of leaf sample and fruits sample of *V. doniana* was done in order to ascertain somewhat the best method of preparing plant material for certain medicinal uses. The results of phytochemical screening of the three preparation dried methods of leaf extract sample of Black plum (*V. doniana*), as shown in table 1-4 shows that in shed drying method (table 1), percolation with distilled water shows the presence of alkaloid, saponin, tannin, phenol, terpenoid, flavonoid and but quinone, was absent, while in acetone alkaloid, tannin, terpenoid are present while flavonoid, saponin, phenol, quinone and glycoside were absent in this extract. However methanolic extracts shows the presence of alkaloid, phenol, and terpenoid while flavonoid, saponin, tannin, quinone and glycoside were absent. While petroleum ether extract indicates the presence of alkaloid, saponin, phenol, terpenoid and quinone while flavonoid tannin and glycoside were absent. The above results shows that distilled water extract sample has highest phytochemicals content when compare with petroleum ether extract acetone extract, and methanol extract sample. Table 2 (sundried) shows the presence of saponin, tannin, phenol, terpenoid and glycoside in the aqueous extract sample while flavonoid and quinone were

absent, while alkaloid, saponin, phenol terpenoid and glycoside are presence in acetone extract. However flavonoid, tannin and quinone were absent. Alkaloid, saponin, tannin terpenoid and glycoside are present in the methanol extract while flavonoid saponin, tannin and quinone were absent. This result also shows that aqueous extract has the highest phytochemical content. But the oven dried (table 3) extraction shows the presence of alkaloid, saponin, tannin, phenol, terpenol and glycoside in aqueous, alkaloid, tannin and terpenoid in acetone extract, alkaloid, phenol and terpenoid in methanol extract and glycoside alkaloid, saponin, phenol, terpenoid, and quinone presence in petroleum ether extract. Quantitative phytochemical analysis of the plan leave and fruit done shows that the leave extract sample of black plum contained heights percentage of alkaloid ( $1.31 \pm 0.02$ ) follow by tannin, flavonoid saponin and phenol respectively.

This study reveals that shade dried leaf sample has higher number phytochemicals, as it contain the highest number of phytochemicals especially in aqueous extract sample, and follow by oven dried leaf sample and sundried leaf sample respectively. The results of this work agreed with finding of Joon *et al.* (2013) which revealed the presence of flavonoid, tannin, saponin, glycoside and terpenoid in the extract of mango leafs aqueous extract. Hassan *et al.* (2021a) [5], also report the presence of alkaloid, glycoside, tannin, flavonoid, in both the ethanolic and the aqueous extract of mango leaf. The presence of these phytochemicals had also observed in other plant research by Osuagwa *et al.* 2007 and Magaji *et al.* 2020.

The result of isolated fungi of the three different drying methods of leaf sample of *V. doniana*, revealed the presence of *Penicillium spp* (66.7%), and *Madurella spp.* (33.3), in shade dried leaf sample. *Fusarium spp.* (25%), *Penicillium spp.* (25%), *Aspergillus niger* (25%) and *Rhizoctonia spp.* (25%) were presence in oven-dried leaf sample while *Mucor racemosa* (40%), *fusarium spp.* (20%), and *penicillium spp.* (40%) were found presence in sun dried leaf sample. *Penicillium* species has the highest percentage of occurrence in shade dried sample (66.7%) sun dried sample the other two methods, followed by mucor with (40%) in sun dried leaf sample. The least one is the fusarium species with (20%) in sun dried leaf sample. This research findings are in line with the results reported from the research done by Abdullah *et al.*, 2016 [1].

In line with this report, the shade dried and sun dried methods should be used with caution in *phytomedicines* as there is a clear risk of causing further health problems, but rather the oven dried methods as it contains the less percentage of toxic fungi, as seen in table 5. However the result of fungi isolate from fresh fruits sample of *V. doniana*, shows the highest percentage of *A. niger* (33.3%), followed by *Alternaria brassica* with (25%) then *Aspergillus flavus* and *Rhizopus stolonifer* with equal percentage (16.7%). Some *Aspergillus species* such as *A. flavus*, *A. niger*, and *A. fumigatus*, are ubiquitous *saprophyties* which use to cause diseases broadly known as *Aspergillosis*. But species of mucor spp. and *Rhizopus* are human pathogenic fungi responsible for causing common infections collectively known as *zygomycosis* as reported by Muhammad and Muhammad, (2019). Frequency of occurrence of both the fungal species from both the leaves and fruit indicates *Penicillium spp* and *A. niger* has the highest occurrence as shown in table 6 & 7.

The presence of *mycotoxins* in plant material can caused both

acute and chronic risk of health (Dharmasiri *et al.*, 2003)<sup>[3]</sup>. Because *mycotoxins* are secondary *metabolic* products which are non-motile have a relative low molecular weight (Nath *et al.*, 2015) and may be secreted into the medicinal plants materials (Hassan *et al.*, 2021b)<sup>[7]</sup>. They are thought to play a dual role coupled with other microorganisms in the same environment. This aids parasitic fungi (*pathogenic*) in invading host tissue (Lakache *et al.*, 2016)<sup>[8]</sup>.

### Conclusion

The methods; Shade drying and sun drying should always be used with caution in phyto-medicine, as the research has revealed a clear high risk of causing further health problems, but rather the oven dried method of preparation should be adopted, as it extract product has been observed to contains less percentage of toxic fungal contaminants in this research.

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