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Anti-aflatoxigenic of Agave Extracts to Increase Their Food Safety Applications

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Abstract

Agave is a broad wild plant growing in arid soil, with medicinal and cosmetic applications. This study aimed to evaluate antifungal activities of normal and treated Agave extracts. Fourier transmitted infrared apparatus (FTIR) analyzed changes in the chemical composition of several extracts. The gas chromatography-mass spectrum (GC-MS) was utilized to explore each extract component. Aflatoxin reduction due to extract-treatment in fungal media was investigated by high-performance liquid chromatography (HPLC). The juice of the Agave reflected the best antifungal compared to eleven plants. The GC spectrum showed various components that mainly possess antifungal potency, components with antibacterial were also present. Water extraction manifested high antifungal activities. Minimal inhibitory concentration varied between 124.5 to 199.9 mg/Kg against pathogenic bacteria, while minimal fungicidal concentration was between 8.7 to 31.3 mg/Kg. Boiled extract reflected changes in chemical composition by FTIR, noted an enhancement of its antimicrobial activity. The best antifungal efficiency recorded against Aspergillus flavus ITEM 698, which produces aflatoxins. The extract application in fungal media gave a suppression of aflatoxin production. These results recommended their applications for food safety amelioration, particularly to control mycotoxigenic fungi contamination.

Keywords:Aflatoxins; *Agave ferox;* Antifungal activity; Boiled Agave-extract; Aflatoxins; Mycotoxigenic fungi.

1. Introduction

While, the global situation is going worse for safe antimicrobials inaccessibility, and of the microorganism resistance of current utilized material. The side effect associated with antibiotics and drug resistance was manifested increase during the past decades [1]. Plant extracts are rich in molecules with antimicrobial activity, which inhibit harmful microorganisms without health risk. Their components are playing bioactive functions in living cells [2]. Antibacterial and antifungal activities occurred as the result of bioactive component applications [3]. The impact of these compounds is related to their phytochemical contents, antioxidant activity, and volatile compounds [4].

Effective molecules are included in leaves, stems, seeds, fruits, vegetables, and grains [5]. These molecules worked against cellular damage through oxidative stress, maintained public health, and stopped the occurrence of chronic disease [6]. Agave represents special parameters due to its originality and is affluent by various natural molecules. Agave varieties contain up to 20% non-structural carbohydrate [7], considerable polysaccharide amounts [8], fibers [9], and considerable phenolic, flavonoids, homo-isoflavonoids [10], which joined to broad biological activities.

Mycotoxigenic fungi are great hazards exercised menace of food safety and cause contamination, which diverse due to changes in environmental factors [11]. Their related risks included mycotoxin and biological

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contamination. Mycotoxins were classified as pre-carcinogenesis, mutagenic, and teratogenic compounds for cell systems [12]. Previous studies refer to a better mycotoxin reduction using plant extracts [13, 14]. Likewise, plant oils and minor components were inhibited the fungal growth [15, 16].

Increment in the request of food safety and the decline of synthetic antifungals turned over the attention to natural components rich in active molecules. These molecules possessed pharmaceutical and therapy functions against fungus infection. The study aimed to ameliorate the antifungal efficiency of agave extracts, these solutions, managed using various extraction methods. Juices or extracts, which inhibit mycotoxigenic fungi, were possessed a novel characteristic, recommended its wide applications for food safety.

2. Materials and methods

2.1. Plants and Microorganisms

Fresh leaves of two agave species (*A. ferox* and *A. Americana*) besides other plant leaves were collected from the research garden of the University. The strains of toxigenic fungi included *Aspergillus flavus* ITEM 698, Aspergillus *niger* ATCC 1015, *Penicillium chrysogenum* ATCC 48271, *Fusarium oxysporum CCMF* 358, and *Candida albicans* ATCC 10231, obtained from Agro-food microbial culture collection, Institute of Sciences of Food Production (ISPA), Italy. Bacterial strains were *Pseudomonas aeruginosa* NCIB 950, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* NCIB 3610, *Klebsiella pneumoniae* NCIB 418, and *Escherichia coli* NCIB 86, which obtained from TCS bioscience LTD, Botolph Claydon. Buckingham, MK1821 R.

2.2. Microorganisms' growth media

For bacteria, nutrient agar (NA) and trypticase soy broth (TSB) were applied in the reactivation and the enrichment of the utilized bacterial strains, while trypticase soy agar (TSA) was applied in the experimental study. For fungi, potato dextrose agar (PDA) and Sabouraud Dextrose agar (SDA) were utilized for fungal strains reactivating, yeast extract sucrose broth (YESb) was applied in the mycelial growth inhibition evaluation of Agave. The fungal strains reactivated using the SDA media with chloramphenicol. It was maintained using PDA media with chloramphenicol. For bacterial strains, it was cultured and activated use Tryptic soy broth media at 37 °C/36 hr and was maintained using NA media at 4°C until the antimicrobial test.

2.3. Plant juice and extracts Preparation

Leaves juice was prepared as follows: about 500g of each plant were frozen in a plastic sealed bag, then crushed by a mechanical press without solvent, collected in sealed vials after it was freeze-dried (Dura-Dry MP freeze-dryer; FTS System, USA).

For extracts, 500g of each plant was dried using a hot-air oven (*JP-Selecta*, model No., 2000208, Spain) at 40°C /48h. Dried leaves milled using a Model TR-20 mill, *JP-Selecta*, Spain. Into a conical flask stoppered with rubber corks, water extract (WE) prepared using de-ionized water, and Dimethyl sulphoxide (DMSO) utilized to prepare DMSO extract. The extracting flasks occasional shaking for 24hr., then centrifuged (8385Xg/15min), and filtered using sterilized 0.45µm pyrogen-free membrane before evaluations. The effect of heat-treatment was measured at various temperatures (25, 40, 70, 100, and 121°C).

2.4. Preparation of the conidial suspension of fungi

Conidia harvested from 7-day-old cultures by pouring a sterile 0.01% aqueous solution of Tween 80 onto the culture plates and scraping the plate surface with a bent glass rod to facilitate the release of conidia. The number of conidia adjusted to approximately 10^6 conidia /mL using a Burker-Turk counting chamber (Heamocytometer).

2.5. Determination of antifungal and antibacterial activity

Antimicrobial activity was set using agar diffusion assay according to [13]. The incubation condition was at 37°C/ 24hr for bacteria and yeast but was 25°C/96hr for fungi. The test susceptibility was determined by measuring the zone inhibition diameter around the well. Antibiotic Novobiocin (30µg/ mL) and antifungal Nystatin (30µg/ mL) were applied as references of antibacterial and antifungal, respectively.

2.6. Determine the Minimal Concentration of Inhibitory and antifungal

The minimal inhibitory concentration (MIC) was determined as described by Espinel -Ingroff et al. [17].

Minimal antifungal concentration (MFC) was evaluated according to the method described by Badr et al. [16].

2.7. Determination of antifungal activity using broth media method

Antimicrobial activity was measured according to the Committee for Clinical Laboratory Standard (NCCLS), 1999. Briefly, 100mL of YESb media in conical flasks (500mL) were utilized for the growth of each fungal strain. Fungi spores dissolved in autoclaved Tween-water (5mL/tube). Applied strains were *A. flavus*, *A. niger*, *P. chrysogenum*, and *F. oxysporum*. A loop of suspension for each fungal type seeded on the YESb media. The flasks were incubated for 5 days/28ºC then the fungal mycelia was filtrated on a known weighted filter paper. Compared to the control; the decreases in mycelia weight due to the presence of Agave components were calculated and expressed as reduction ratio. The averages of three replicates for each extract or juice and the combination were calculated.

The media contained *A. flavus* fungi evaluated for aflatoxins concentrations in the presence and absence of *A. forex* extract. Aflatoxin amounts extracted from the media, cleaned up by immune-affinity column (Aflatest™) and determined using HPLC system, Waters 1525 as the conditions described by Abdel-Fatah et al., [13].

2.8. Determination of Agave components using GC-MS

The extracts of the two agave plants were analyzed by GC–MS using an Agilent 7890 gas chromatography apparatus (Agilent Technologies, Santa Clara, CA), controlled by Agilent GC/MSMS Hunter Acquisition software. The conditions were the same and congruent with that described by Shehata et al., [18].

2.9. FTIR spectroscopy analysis

Fourier transforms infrared spectroscopy used for determination of the bonds starching vibration present in the extract component. The FTIR spectrum of dried samples was detected by the Perkin Elmer instrument in the range of 450 to 4000 cm^{-1} at a resolution of 4 cm^{-1}.

2.10. Statistical analysis

The data were treated statistically using analysis of variance (ANOVA) as described by Snedecor and Cochran (1969), standard divisions and means compared by LSD at 5% using SPSS program Ver. 16.

3. Results

As a pre-step of this study, Agave extracts for the two types of leaves (*A. ferox and A. americana*) were compared with extracts of other plant leaves. Data in **Table (1)**, showed the antimicrobial impact of these extracts using agar well diffusion assay. Inhibition zone increment of each extract indicates more antifungal efficacy. *Ficus benjamina* and *Morus nigra* extracts showed antifungal activity against *Aspergillus* strain but did not show any antibacterial activity against bacterial strain tested. The extracts of *A. ferox* followed by *A. americana* possess a better antifungal and antibacterial activity. This experiment showed good inhibition for both bacterial and fungal strains related to the application of *A. ferox* in the growth media. It represents the best result of all applied material included in this investigation. Forward to this point, *A. ferox* has been selected for the rest part of the experiment.

3.1. The evaluation of several treatments on antimicrobial activity

The factors that affect Agave antimicrobial efficacy were studied, these included temperature, solvent system, plant part, and leaves-age (**Table 2**). The impact of these factors was evaluated against investigating bacteria and fungi strains. Data recorded using the well diffusion assay for evaluating the inhibition that occurred. The effect of drying for juice and extracts was investigated. The diameters of inhibition zones were recorded with non-significant differences for antimicrobial activity against all strains except for the *A. niger* ATCC 1015 strain.

a- Plant-part source of the extract

The leaf is divided into two parts, the head (the inner part that is close to the core) and the tail (the outer part far from the core). The effect of each part on antimicrobial characteristics was studied. According to the results presented in **Table (2)**, the plant-part where the juice was sourced from was recorded with

significant differences on tested fungi except *A. favus* and *P. chrysogenum*. While not all pathogenic bacteria were registered have significant differences.

b- Maturation degree impact

The maturation degree of leaves has clear impacts on both antibacterial and antifungal characteristics. Both the MP juice and water extract collected from leaves of different ages (young, middle age, and mature), have different antibacterial and antifungal efficacy (**Table 2)**. The antimicrobial potency showed cumulatively rise by the increment of leaf age from young to mature.

c- Heat treatment effect of the Agave extract

The temperatures of both juice and the WE have exhibited a variety of impacts on antibacterial and antifungal activities. Data represented in **Tables (2)** present great significant differences between the cold and hot extraction. Results showed an ameliorative effect for the antimicrobial potency of juice or extract produced using heating up to 100°C (boiling), where the antimicrobial values recorded by the best result. Raise the heating condition to autoclaving temperature (121°C) inverse the efficacy. Both of antibacterial and antifungal impacts of the solutions at this temperature exhibited worse compared to the cold solutions.

3.2. Effect of the solvent system of extraction

The WE manifested the best system in bacterial and fungal inhibition. The antimicrobial efficacy of extracts was ordered as WE > DMSO > MP, which is demonstrated in **Fig. 1**. Using water for extraction was improved the solubility of the active component. The impact of extracts was noted highly for *A. flavus* compared to other fungal strains applied. The effect of extracts on *A. niger*, *P. chrysogenum*, *F. oxysporum*, and *C. albicans* seems approximate, and it was so close using the DMSO, also in the case of the MP juice. Moreover, these extracts exhibited a high efficacy for inhibiting the growth of pathogenic bacteria. Inhibition zone using extracts in an agar diffusion assay for *P. aeruginosa*, *K. pneumonia*, and *E. coli* strains have chiefly been nearby.

Table. 1 Antimicrobial activity of leaf juice of some plants

Scientific name	**Fungi strains**					**Bacterial strains**				
	A. flavus	*A. niger*	*P. chrysogenum*	*F .oxysporium*	*C. albicans*	*Staph. aureus*	*E. coli*	*P. aeruginosa*	*B. subtilis*	*K. pneumoniae*
Psidium guajava	--	-	+	+	-	-	-	+	-	+
Vitis vinifera	-	+	+	+	-	+	+	+	+	+
Citrus sinensis	-	-	-	-	-	-	-	+	-	+
Citrus tangerina	-	+	+	+	-	-	-	+	-	+
Ficus carica	-	-	-	-	-	-	-	++	+	++
Morus nigra	-	+	-	-	-	-	-	+	-	-
Jasminum sambac	-	-	-	+	-	-	-	-	-	+
Cinnamomum Camphora	++	+	-	++	-	-	-	+	-	+
Ficus benjamina	-	-	-	-	-	-	-	-	-	
Ficus benjamina	-	+	-	-	-	-	-	+	-	-
Agave ferox	++++ +	++	+++	++++	++	+	++	+++	++	+++
Agave americana	+++	++	++	++	++	+	+	++	+	+++
Aloe vera	+	++	+	++	-	-	-	+	-	+

(+): positive antimicrobial activity
(-):negative antimicrobial activity

Table (2): The effect of several factors on antibacterial and antifungal of Agave.

Treatments		*Antifungal*					*Antibacterial*				
		A. flavus	***A. niger***	***P. chrysogenum***	***F. oxysporium***	***C. albicans***	***Staph. aureus***	***E. coli***	***P. aeruginosa***	***B. subtilis***	***K. pneumoniae***
Drying	**Before**	14.2±1.13	6.6±1.15^{a}	5.3±0.57	7.2±1.0	5.8±0.57	3.1±0.41	3.2±0.67	4.5±0.31	4.4±0.48	4.9±0.34
	After	14.0±1.73	4.3±0.57^{b}	4.7±1.15	6.0±1.0	5.5±0.34	2.9±0.71	2.9±0.59	4.5±0.29	4.3±0.57	4.8±0.37
LSD at P=0.05		NS	1.4	NS	NS	NS	NS	NS	NS	NS	NS
Leaf part	**Outer**	11.3±0.57	6.2±0.88^{a}	4.8±1.08	5.7±0.4^{c}	4.0±0.54^{b}	3.3±0.41	3.0±0.17	3.6±0.54	3.4±0.21	3.5±0.27
	Inner	11.7±0.57	7.9±0.67^{b}	4.8±1.13	6.7±0.37^{b}	4.7±0.37^{c}	3.5±0.27	3.0±0.11	3.7±0.31	3.3±0.34	3.5±0.13
	All	14.0±0.67	7.5±0.34^{a}	5.0±1.2	7.0±0.61^{a}	5.3±0.21^{a}	3.3±0.77	3.2±0.22	3.6±0.24	3.4±0.11	3.6±0.41
LSD at P=0.05		NS	1.115	NS	0.907	0.544	NS	NS	NS	NS	NS
Temperature	**Control**	12.4±1.05^{a}	6.8±0.81^{a}	2.7±1.03^{a}	3.7±0.73^{a}	3.4±1.12^{a}	3.0±0.88^{a}	2.2±1.02^{a}	3.4±1.05^{a}	3.2±0.91^{a}	3.0±0.88^{a}
	40°C	13±1.21^{a}	7.1±1.05^{a}	2.9±1.21^{a}	3.7±0.81^{a}	3.6±1.05^{a}	3.2±0.41^{a}	2.4±1.34^{a}	3.5±0.84^{a}	3.4±0.99^{a}	3.2±0.41^{a}
	70°C	14.1±1.34^{b}	7.9±0.57^{b}	3.7±0.97^{a}	5.1±1.08^{b}	4.8±1.23^{b}	4.0±0.57^{b}	3.2±1.16^{a}	4.0±0.66^{a}	4.1±0.81^{a}	3.9±0.6^{a}
	100°C	15.3±1.08^{c}	9.4±0.81^{c}	6.0±1.51^{b}	6.7±1.02^{c}	5.7±1.34^{c}	4.9±0.47^{c}	4.5±1.22^{b}	5.3±0.93^{b}	5.1±0.77^{b}	4.8±0.6^{b}
	121°C	10.7±1.41^{d}	2.0±1.34^{d}	3.0±1.43^{c}	2.0±1.21^{d}	4.0±1.21^{d}	2.0±0.67^{d}	3.0±1.07^{c}	2.7±0.41^{c}	2.4±0.81^{c}	2.5±0.4^{c}
LSD at P=0.05		1.014	0.843	1.712	1.541	1.361	0.821	1.034	1.208	0.997	0.891

- The results expressed as mean ± SD (n=3; P=0.05).
- The least significant differences value (LSD) calculate for each experiment and located in the last raw.

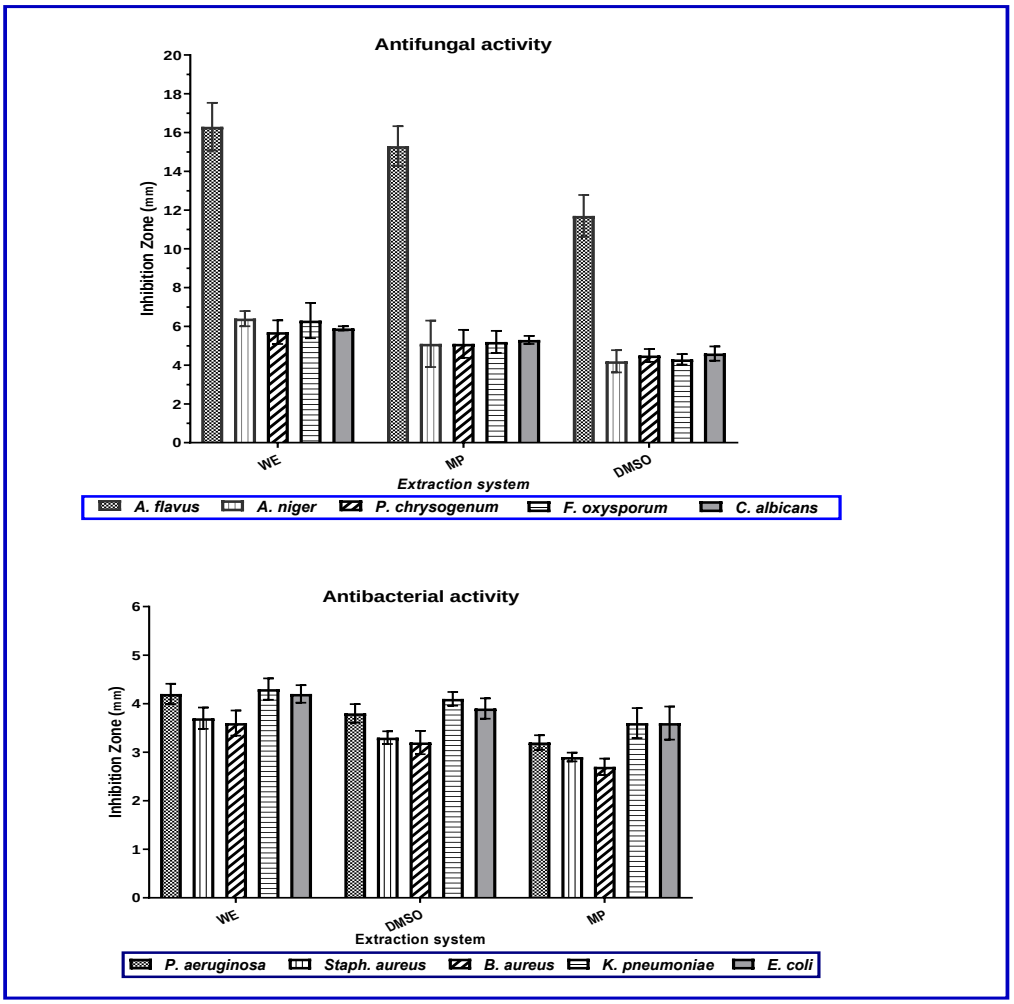


Figure 1. The method impact for the antifungal and antibacterial properties of Agave extract

3.3. The MIC and MFC evaluation of A. ferox

Since solvent extraction gave antimicrobial better than the mechanical press, the MIC and MFC were evaluated just for the WE and DMSO. Data of **Fig. 2** were represented the MFC and MIC for *A. ferox* juice and extract. The MFC values were indicated high sensitivity for *A. flavus* ITEM 698 by Agave solutions (8.7 mg/Kg for DMSO and 12.3 mg/Kg for the WE), followed by an impact on *A. niger* ATCC 1015. Moreover, *P. chrysogenum*, *F. oxysporium*, and *C. albicans* recorded The MFC at 17.4, 5.6; and 27.2 mg/Kg by DMSO extract, these values increased to 21.6, 7.7, and 31.3 mg/Kg using the WE, respectively (**Fig. 2A**).

The MIC impact showed little variations between bacterial strains (**Fig. 2B**), where *P. aeruginosa* NCIB 950 showed a more sensitive response at 125.4 and 189.1 mg/Kg for DMSO and the WE, respectively. The values recorded for *Staph. aureus*, *B. subtilis*, *K. pneumoniae*, and *E. coli* were 141.1, 145.7, 140.5, and 151.2 mg/Kg for the DMSO, respectively. While it was 193.6, 199.4, 191.6, and 199.8 mg/Kg for the WE influence on previous strains.

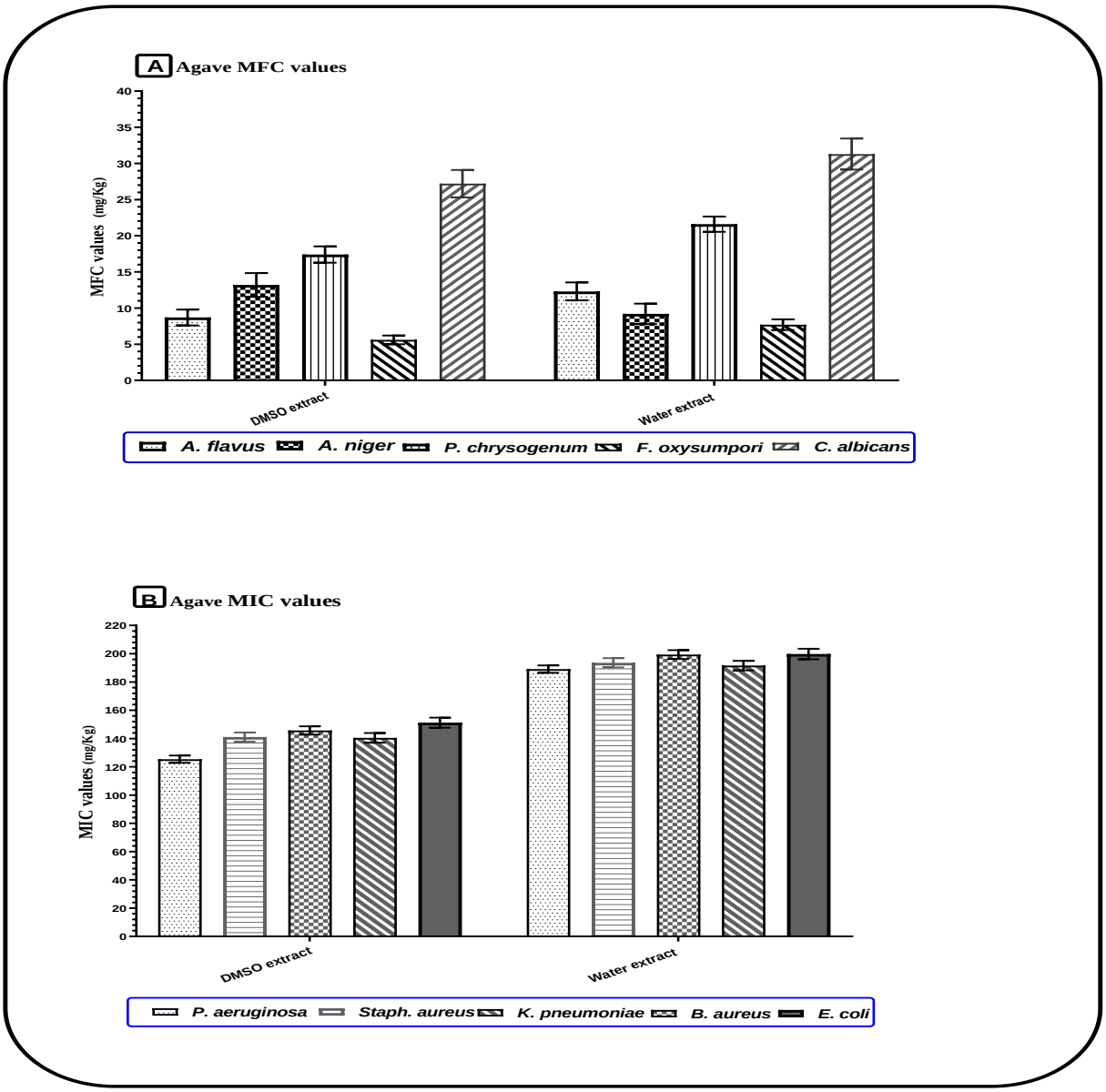


Figure 2. The minimal fungicidal concentrations and minimal inhibitory concentration of Agave extract

3.4. Determination of active ingredient by GC-MS

The active ingredient of Agave extract was analyzed using the GC-MS apparatus. **Table 3** manifested the presence of several components; it is clearly shown components with antibacterial and antifungal properties. For instance, in the extract, n-Undecane, and n-Pentadecane have existed where they noted by antibacterial activity in early studies [19]. While 3, 7-Dimethyl-1, 6-octadien-3-ol (Linalool), and Tetracosanoic as antifungal was also recorded.

Other components with antimicrobial characteristics, such as n-Docosane, Eicosane, and Mono-2-methylhexyl phethalate were also present. The active components that act as an antifungal agent have existed in considerable amounts, where the chemical structure for some of these components were presented in **Fig. 3**. This explains the extract.

This explains the extract suppression behavior on *A. flavus* fungi and its related influence on aflatoxin production. This suppression also affected the secretion amount of aflatoxins that produced form this type of fungi.

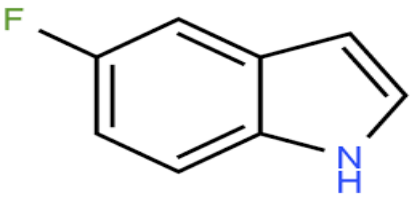
3.5. The FTIR capture of Agave

The changes of FTIR capture of two agave plants (*A. ferox* and *A. americana*) by different treatments were evaluated for discussing the changes of antimicrobial efficacy that recorded before. The data obtained are shown in **Fig. 4 and 5** were explained the difference. Focusing on the specific curves of carbohydrates that ranged between 1280 to 800 cm^{-1}, the various changes of agave observed clearly in **Fig**. **4A** and **4B,** where *A. forex* manifested more content of active components in the specific area from 1000 to 820 cm^{-1}.

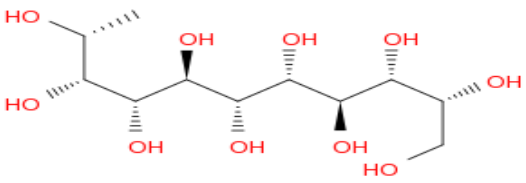
Table (3): The GC-MS main components of *A. forex* that represent antimicrobial and antifungal activities

Component Name	Rt	Percentage (%)	Functionality	Reference
n-Undecane	4.7	4.71	Antifungal anti-tumor	**[20]**
n-Pentadecane	5.1	3.07	Antimicrobial	**[20]**
n- Hexadecane	5.9	2.99	Antifungal	**[21]**
1,2 Benzene dicarboxylic	6.1	6.17	Antimicrobial antifungal	**[22]**
n-Octadecane	7.8	2.54	Antibacterial	**[22]**
Palmitic acid methyl-ester	9.4	4.56	Antifungal antibacterial	**[20]**
9- Octadeconeic acid methyl-ester	10.7	3.59	Antifungal antibacterial	**[22]**
3, 7-Dimethyl-1,6-octadien-3-ol	11.1	2.58	Antifungal	**[23]**
n-Docosane	11.6	6.34	Antibacterial Antifungal	**[23]**
Eicosane	12.4	6.11	Antimicrobial	**[23]**
Tetracosanoic	13.3	2.05	Antifungal	**[23]**
Mono-2-methylhexyl phethalate	14.5	11.39	Antimicrobial antifungal	**[24]**
n-Hexacosane	14.8	4.1	Antifungal	**[25]**
Nonacosane	17.4	3.61	Antifungal	**[23]**

***Rt**: the retention time of the compound according to their appearance on the chromatogram of gas chromatography.
*** (%)**: The percentage of the compound amount compared to the total compound content of the injected extract.

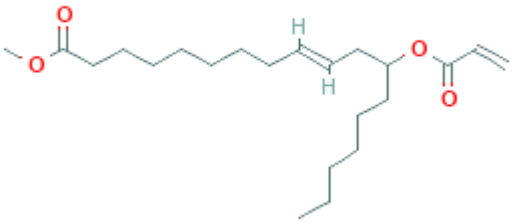


n-Pentadecane

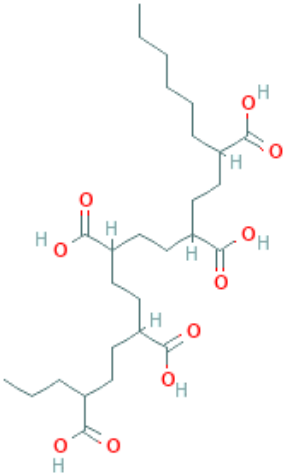


n-Undecane

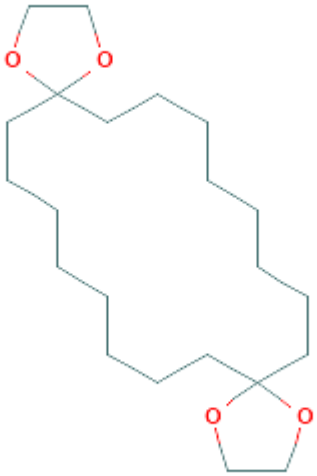
1,2 Benzene di-carboxylic



9- Octadeconeic acid methyl-ester

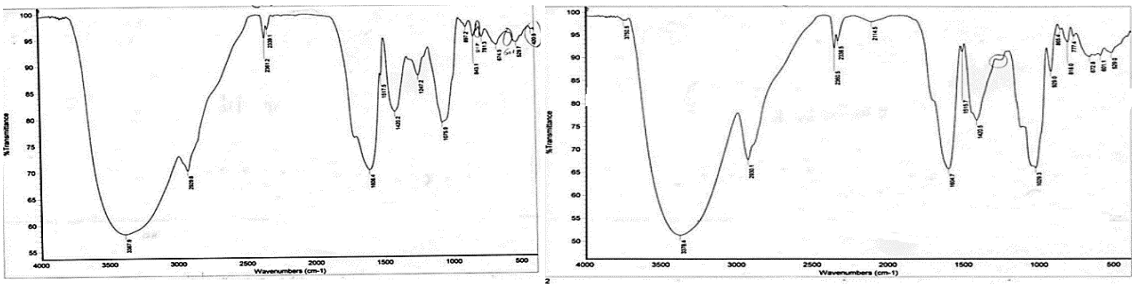


n-Docosane



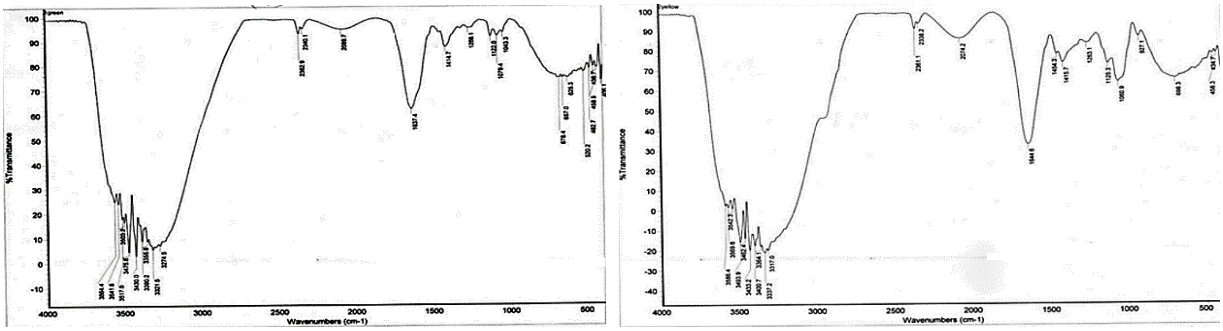
n-Hexacosane

Figure 3: Chemical structure of some isolated antifungal components of Agave extracts



A: FTIR spectrum of *A. forex* **B: FTIR spectrum of *A. Americana***

Figure 4: The FTIR Charts of Agave species extracts



A: FTIR spectrum of boiled A. forex *B: FTIR spectrum of boiled A. Americana*

Figure 5: The FTIR Charts of boiled Agave species extracts

However, the peaks recorded from 800 – 700 cm^{-1} were referred to as aldehydes and methyl alkane's fractions resulted from carbohydrates fragmentation [26, 27]. For *A. ferox*, the peak of 1420 cm^{-1} responses for lignin – CH fraction [28]. The expanded oscillations 1030 -1075 cm^{-1} pointed to the fractions resulted from Fructan [29]. This is more clearly observed in the extract of *A. forex* compared to the extract of *A. americana*. While, expanded oscillations 1245 -1250 cm^{-1} linked by aromatic fractions of hemicellulose and lignin [26], which observed approximate equal in the two extracts. The oscillation of 1600 cm^{-1} in *A. ferox,* and 1604 cm^{-1} in *A. americana* connected to the fiber fractions in the analyzed samples, which were resulted from water-binding by the fiber hydrophilic group [27]. The expanded oscillation of 2930 cm^{-1} is joined to the alkyl group of (C – H) in both types of juice, while The oscillations of 3200 – 3650 cm intensity of the bands were referred to the (-OH groups) which distinguished saccharides and oligosaccharides [27, 30].

3.6. Anti-aflatoxigenic effect of A. forex extract

Aflatoxin concentrations in liquid media of *A. flavus* ITEM 698, which possess the ability to produce aflatoxins, were determined. The media without the extract and contained the fungal strain considered as the control. Aflatoxin concentrations in the control were recorded as 220.1, 58.6, 97.4, and 49.9 ng/100 mL for aflatoxins B_1, B_2, G_1, and G_2, respectively. By application of the WE in the media, just aflatoxin B_1 (AFB_1) was recorded at a concentration of 12.3 ng/100mL. The reduction was almost 99% for AFB_1, where it was 100% for other types of aflatoxins. While the application of DMSO and the boiled extract in fungal media recorded without any detection of aflatoxins concentration.

4. Discussion

The previous studies were pointed out to the plants extracts as rich sources of bioactive components, and it has in many times activity to suppress contamination caused by the microbial infection of food materials [13, 31, 32]. According to the results of the GC-MS and the FTIR apparatus, the antifungal activity of Agave could explain as follows. Firstly, for FTIR results; the area from 1000 to 820 cm^{-1} represents the oligosaccharides, which have a linkage between sugar-molecules [33]. Numerous fractions are represented with significant amounts in this peak area that explains the increment of antifungal efficiency of *A. forex* compared to *A. Americana*. In comparison to the mix of the extract of the two-agave species changes of *A. forex* of oligosaccharides were not shown with a high impact. The results have reflected a distinction between the two types of juice, as *A. americana* contains more alkenes groups than *A. ferox* (**Fig. 4A** and **4B**). This is represented by the peak extended at 2700 to 2800 cm^{-1}. Moreover, the (-OH) expanded oscillation was seemed divided into small peaks in the same area. In light of these mentioned results, the boiled juice shown with fractionation of peaks-area related to oligosaccharides. This meant that new oligo-fractions have existed after boiling treatment of agave extract. In contact with the FTIR results for the boiled solution of Agave, the amelioration, which recorded in the boiled solution, may connect with the oligosaccharides fractions that were resulted. Fructose, Fructan, and its fractions are the main sugar in the Agave plant. In this way, the fructan and fracto-oligosaccharides, which resulted from the boiling treatment (**Fig 5A** and **5B**), could act as an antimicrobial agent, particularly against pathogens [34].

Secondly, the GC-MS results referred to the presence of antifungal components such as n-Undecane, n- Hexadecane, Tetracosanoic, and linalool. These results explained the antifungal activity reported on the diffusion assay. The content of minor components also play an *invivo* function to play a protective action; and minimize the bad impact on the tissue level [35].Variations that were recorded between the WE, DMSO, and MP juice for its zone-inhibition of fungi and bacteria linked to the existing amount of these components. The differentiations are depending on the type of extraction, which changed according to the applied system [36]. The increment of the efficiency may be connected to phenolic contents that are increased by polar extraction. This also harmonizes with results obtained by Stefanović and Comic [37].

As the results indicated the maturation impact on antibacterial and antifungal activities conjugated with maturation degrees, it could be explained by the fact that the leaves are growing as an interactive circle. In this regard, not all leaves have the same dose of lightning, chlorophyll content, and other growth factors. This differentiation affects the chemical composition. In addition, the changes could join to the accumulation of active components in maturing leaves by the growth time increases.

The enhancement of the antifungal activity of boiled extract link to the more solubility of the active components included fibres and oligosaccharides, while the less activity of autoclaved extract could be explained due to the breakdown linkages found between fractions of the molecules, which mainly represented by oligosaccharides and phenolic, converted it to a less or none active fraction. To explain this efficient reduction of aflatoxin production by the fungal strain, it was connected to the minor components represented in the GC results (**Table 3)**, side to the phenolic content, which affected the ability of the strain to produce aflatoxin [38]. The more efficiency of DMSO extracts to suppress aflatoxins production referred to its capability to solve both polar and non-polar active molecules that presented in leaves [39, 40]. While the increase of aflatoxin reduction by the boiled extract could connect to the new fractions that resulted from heat treatment, these fractions were noticed clearly in the FTIR spectra of the boiled extract.

In the same way; the flower extraction of other Agave species was reported by an anti aflatoxigenic fungi impact. The functional properties of *A. sisal* saponins were also reported as an antimicrobial against food pathogens [39, 41]. While methanol extract has an inhibition impact for *Shigella* [42]. Agave reveals significant affluence of phenolic compounds, which have broad biological activities linked to its behaviors to act as antioxidants, antibacterial, and antifungal activity [43].

5. Conclusion

In the antimicrobial comparison of eleven plant leaves, Agave recorded the best results. The extraction was applied using several methods (MP – WE – DMSO); and were tested for antifungal activities. The most effective MFC of the WE was recorded against *A. flavus* ITEM 698. The FTIR analysis of extracts reflects variations in chemical composition by boiling treatment; this explains the increment of activities against fungi. These results pointed to the boiled WE as a better antifungal, particularly against an aflatoxigenic strain of *A. flavus*. The combined influence of phytochemicals and oligosaccharides fractions resulted in the amelioration of antifungal potency. Both the DMSO extract and boiled WE were suppressed aflatoxin production in liquid media, which is recommending their applications in pharmaceutical and food safety application.

6. Conflicts of interest

There are no conflicts of interest to declare for this manuscript.

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