



## Research Article

# Antioxidant Activities and Potential Impacts to Reduce Aflatoxins Utilizing Jojoba and Jatropha Oils and Extracts

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## Abstract

**Background and Objective:** Jojoba as well as jatropha contains many active ingredients that have important roles to give them antioxidant efficiency, furthermore; fatty acids composition give a substantial function as antimicrobial properties. Thus, it may enhance the resistance to pathogenic microbes and toxins. This study aimed to evaluate antioxidant, antimicrobial and antifungal activities of jojoba and jatropha materials. **Materials and Methods:** Jojoba and jatropha oils fatty acid composition were analyzed, total phenolic and flavonoid was determined and the antioxidant activities were evaluated. The antimicrobial activity of extracts was investigated against some pathogens by agar well and disk diffusion method. The oil and pomace extract of jojoba and jatropha were tested for antifungal activities against four toxigenic fungi. Inhibitory activity of materials against aflatoxins production was evaluated using High-performance liquid chromatography (HPLC). Statically analysis using one way ANOVA was done using SPSS v.16. **Results:** The major fatty acids were mono-unsaturated fatty acids, 97.4% for jojoba and 42.2% for jatropha, while palmitic acid and stearic acid being the major saturated fatty acids in jatropha oil. Total phenolic and flavonoid contents were ranged from 12.5-996.5 mg of GAE g<sup>-1</sup> and 5.92-180.83 mg of catechol g<sup>-1</sup>, respectively. Reducing ratio of aflatoxins were vary between 43.8-26.6% for aflatoxin B<sub>1</sub>, 38.61-14.5% for aflatoxin B<sub>2</sub>, 33.2-14.6% for aflatoxin G<sub>1</sub> and 34.3-9.4% for aflatoxin G<sub>2</sub>, while for total aflatoxins were between 38.7-18.75%. **Conclusion:** Jojoba oil was more effective and more antimicrobial than jatropha oil, the pomace extract shows more efficacy for jatropha than jojoba and they have variable effect against pathogens and aflatoxins.

**Key words:** Jojoba oil, jatropha oil, jatropha pomace extract, mycotoxigenic fungi, aflatoxins degradation

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**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Concurrently, geographical and climatic differences between cultivation and storage areas must beware of it; it is considered as a master agent in feed as well foods commodities contamination. Differentiation between regions in climatic factors, which are concurrently the factors that affecting fungal growth and mycotoxin excrete, may resulting as variation in fungal types that will infect the food material along with its impact on toxin type and amount on the stored materials<sup>1-4</sup>. Upon the recent alteration in climate data, from time to time there is a persistent need to render updated researches on the actual contaminants situation of food and feed materials by mycotoxins compounds. A novel way for that as searching for strategies are able to degrade mycotoxin in final food products depending on natural and safe materials<sup>5</sup>.

*Euphorbiaceae* family members, especially jojoba and jatropha are the substantial birthplace of oil, pharmacological offspring, pigments, furniture and agricultural tools. Jatropha as well as jojoba, considered as a manufacturer to produce many phytochemicals, those bioactive components vary between terpenes, cyclic peptides, lignin, alkaloids and essential amino acids along with its utility to be a source of oil. According to the suitability of these plants to grown even if the climate conditions are difficult, the amount of its contains from the bioactive components may increase, the refrating of that can appeared as increasing the antimicrobial effect. Many of other uses may apply for those plants such as for animal feed, pesticides, medicinal supplies and in cosmetics components, anticancer medicines and in soap<sup>6</sup>.

Many strategies were used to inhibit fungal growth and/or reducing toxin production, chemical, physical and biological applications were used to reduce aflatoxin contamination<sup>7-9</sup>, according to the unwholesome impact of mycotoxin, generality research endeavor has focused on the wherewithal for prevention of mycotoxin excretion.

To decrease the hazard of toxigenic fungi which may grow on the food and agriculture product, the past conventional methods were utilized a chemical compound which had antifungal characteristics; the riskiness here was its conjugated to its deleterious for environment as well for human and animal health<sup>10,11</sup>. Recently there is a need to find a safe and natural component that may help to reduce toxigenic fungal hazard concurrently with no bad impact on human, animal and environment. One of the best methods is that use plants extracts as a source for antifungal components.

In the field of food and feed products, recently the consumer demands and industrial applications, interest in the development of environmentally and eco-friendly extracts from agricultural renewable resources as feedstock. This research was aim to utilize, new, safe and eco-friendly extracts from agricultural renewable resources as feedstock, with the ultrasonic-assisted method as way to inhibit the toxigenic fungi growth and/or reduce mycotoxin to increase the safety in food commodities. Those materials basically depending on the extracts of oil manufacturing and oil processing byproducts (jojoba and jatropha oils as well their pomace extracts).

## MATERIALS AND METHODS

**Collection of plant materials:** The oil and its byproducts extract of two oil seed plants were collected to estimate its antioxidant activity, antimicrobial activity, anti-mycotic and finally its anti-mycotoxigenic power. The material under the study was Jojoba oil (JOO), Jojoba pomace extract (JOPE), jatropha oil (JAO) and Jatropha pomace extract (JAPE), those material were collected just after the oil manufacturing steps in the production year of 2016 and it was gained from the farms located in the Suez Canal area, Egypt. Each plant material was labeled, numbered, a noted with the date of collection, locality and their antifungal or antibacterial characteristics were tested.

**Preparation of plant extract:** As in Theodora method with some modifications, 100 g of the air dried (at oven, 40°C) of pomace samples either for jojoba or for jatropha were extracted using ultrasonic assisted techniques (at room temperature for 45 min) with isopropanol: water 1:1 v/v<sup>12</sup>. The aqueous isopropyl solution was added at ratio of 5:1 (v/w), pH was adjusted to 4-5. The last step was done twice, after that; it was collected and centrifuged at 4000 rpm for 20 min. The supernatant containing the extract was then transferred to a pre-weighed flask and the extract was concentrated by rotary evaporator at 50°C. The crude extract was weighed and dissolved in a known volume of dimethyl sulphoxide (DMSO) to obtain a final concentration of 5 mg/1 µL.

**Fatty acid composition:** Methyl esters of fatty acids (FAME) were prepared according to AOCS Official Method Ce 1 k-0<sup>13</sup>. Detector and injector temperatures were set at 240°C. Fatty acids were identified by comparison of the retention times with authentic standards and the results were reported as

weight percentages after integration and calculation using Chem. Station (Agilent Technologies).

**Total phenolic compounds:** The total phenolic compounds assay was carried out using the Folin-Ciocalteu reagent, following the method<sup>14</sup> and based on the reduction of a phosphor-wolframate phosphor-molebdate complex by phenolic to blue reaction products. One milligram extract was dissolved in 1 mL methanol and 500  $\mu$ L of the dissolved sample was taken and added to 0.5 mL of the distilled water and 0.125 mL of Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min before adding about 1.25 mL of 7%  $\text{Na}_2\text{CO}_3$ . The solution was adjusted with distilled water to a final volume of 3 mL and mixed thoroughly. After incubation in the dark for 30 min, the absorbance at 650 nm was read versus the prepared blank. A standard curve was plotted using different concentrations of gallic acid (standard, 0-1000  $\mu\text{g mL}^{-1}$ ). Total phenolic content was estimated as  $\mu\text{g}$  Gallic acid equivalents (GAE)  $\text{mg}^{-1}$  of dry weight.

**Total flavonoid content:** The total flavonoid contents of the extracts were determined by a modified colorimetric method described by Sakanaka *et al.*<sup>15</sup> with some modifications, using catechol as a standard. Extracts or standard solutions (250  $\mu$ L) were mixed with distilled water (1.25 mL) and 75  $\mu$ L of 5% sodium nitrite ( $\text{NaNO}_2$ ) solution followed by the addition of 150  $\mu$ L of 10% aluminum chloride ( $\text{AlCl}_3$ ) solution 5 min later. After 6 min, 0.5 mL of 1 M sodium hydroxide ( $\text{NaOH}$ ) and 0.6 mL distilled water were added. The solutions were then mixed and absorbance was measured at 510 nm. The results were expressed as mg catechol  $\text{g}^{-1}$  of sample. All determinations were performed in triplicate.

### Antioxidant activity assays

**Scavenging activity on DPPH radicals:** The antioxidant activity of extract was determined using DPPH free radicals scavenging assay according the method of Shimada *et al.*<sup>16</sup>, with some modifications. Briefly, 1 mL of solution containing different concentrations of extract was mixed with 1 mL of 0.078 mM DPPH in methanol. The mixture was shaken and allowed to stand at room temperature in dark for 30 min. The absorbance of the solution at 517 nm was measured using a spectrophotometer. All of the tests were carried out in triplicates. The inhibition of DPPH radical was calculated as follows:

$$\text{Inhibition of DPPH radical (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where:

$\text{Abs}_{\text{control}}$  = Absorbance of the control solution

$\text{Abs}_{\text{sample}}$  = Absorbance of the test extract

The  $\text{IC}_{50}$  value ( $\text{mg extract mL}^{-1}$ ) is the inhibitory concentration of the test content at which the DPPH radicals were scavenged by 50% and was calculated interpolation from linear regression analysis.

**ABTS radical scavenging assay:** To determine ABTS radical scavenging assay, the method of Re *et al.*<sup>17</sup> was adopted. The fresh ABTS solution was prepared for each assay. Plant extracts (1 mL) were allowed to react with 1 mL of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of BHT and percentage inhibition calculated as:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where:

$\text{Abs}_{\text{control}}$  = Absorbance of ABTS radical+methanol

$\text{Abs}_{\text{sample}}$  = Absorbance of ABTS radical+sample extract/standard

**Selection of bacterial and fungal strains:** In order to suggest methodologies for screening the natural extracts of antimicrobial activity, two different qualitative methods were evaluated as follows: Agar diffusion test, employing two different types of reservoirs (filter paper disc impregnated with extracts-test and wells in dishes). Bacteria and fungi strains were prepared and reactivate from a lyophilized media of each strain, bacterial strains were divided to Gram positive strain (*Enterococcus faecium* and *Bacillus cereus*) and Gram negative (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*); whereas the toxigenic fungal strain under investigation were *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Fusarium solani* and *Penicillium sp.*, those microorganisms were cultured on PDA medium.

**Antibacterial assay:** Plant extracts were made by isopropanol: water (1:1 v/v), the natural components were dissolved and diluted with solvents as mentioned previously. Same number of subsequent dilutions were performed. The effect of various plant extracts on the several bacterial strains were assayed by the agar well diffusion method and further confirmed by the disc diffusion method. The minimum concentrations of the plants extract to inhibit the microorganisms were also determined by micro-dilution method using plant fractions serially diluted in sterile nutrient broth.

### Detection of antimicrobial activity

**Agar diffusion test:** The bacterial inoculum was uniformly spread using a sterile glass rod on a sterile petri dish PDA agar. Four serial dilutions yielded concentrations of 100, 50, 20 and 10 mg mL<sup>-1</sup> for extracts. Fifty microliter of natural products were added to each of the 5 wells (7 mm diameter holes cut in the agar gel, 20 mm apart from one another). The systems were incubated for 24 h at 37 ± 1 °C, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in millimeter (mm). Control discs were used (chloramphenicol 30 mg, 50 mL). Tests were performed in duplicate.

**Disk diffusion test:** Agar plates were prepared and the test microorganisms were inoculated by the spread plate method. Filter paper discs approximately 6 mm in diameter were soaked with 15 µL of the plant extract and placed in the previously prepared agar plates. Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly so that they are no closer than 24 mm from each other, center to center. The agar plates were then incubated at 37 °C. After 16-18 h of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including the diameter of the disc where the chloramphenicol was used as a control<sup>18</sup>. Whatman filter paper No. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a petri dish and sterilized in a hot air oven. The loop used for delivering the extract is made of 20 gauge wire and has a diameter of 2 mm. This delivers 0.01 mL of extract to each disc.

**Determination of Minimal inhibition concentration of extracts:** The Minimum Inhibitory Concentration (MIC) was determined by a micro dilution method using serially diluted plant extracts according to the NCCLS protocol<sup>19</sup>. The aqueous, methanol and chloroform extracts were diluted to get a series of concentrations from 6.25-100 mg mL<sup>-1</sup> in sterile nutrient broth. The microorganism suspension of 50 µL was added to the broth dilutions. These were incubated for 18 h at 37 °C. The MIC of each extract was taken as the lowest concentration that did not give any visible bacterial growth.

**Determination of minimal fungicidal concentration of extracts:** The fungicidal effect of the plant extracts can be assessed by the inhibition of mycelial growth of the fungus and is observed as a zone of inhibition near the disc or the wells. The activity of the plant extracts on various fungal

strains were assayed by the agar plug method and spore germination inhibition assay.

The commercial potato dextrose agar medium (39 g) was suspended in 1000 mL of distilled water. The medium was dissolved completely by boiling and was then autoclaved at 15 lbs. pressure (121 °C) for 15 min. Agar medium was prepared and poured onto the petri plates. A fungal plug was placed in the center of the plate. Sterile discs immersed in the four plant extracts were also placed on the plates. Nystatin was used as the antifungal control. The antifungal effect was seen as crescent-shaped zones of inhibition<sup>20</sup>.

**Statistical analysis:** Data were statistically analyzed with SPSS software (version 16)<sup>13</sup>. One-way analysis of variance (ANOVA) was used to study significant difference among means, with a significance level at p = 0.05, all data inside tables and figures were represented as values ± SD.

## RESULTS AND DISCUSSION

**Fatty acid composition of jojoba and jatropha oil:** The chemical composition of the jojoba oil described that, many fatty acids were presented in the oil by majority for gadoleic acid which reached about 71.3% of total fatty acid percentage (Table 1). Gadoleic is a monounsaturated, by 20-carbon backbone fatty acid, this long-chain fatty acid had many benefits especially as antimicrobial agent, it is also classified as omega 11 fatty acid<sup>21</sup>. The second majority for erucic fatty acid, oleic fatty acid and arachidic were 14.9, 9.8, 1%, respectively, while palmitic, nervonic and palmitoleic were 1.3, 1.3 and 0.1% of total fatty acid, respectively, otherwise, linoleic, linolenic and behenic fatty acids were not detected. Other side, the chemical composition of the jatropha oil showed that, many fatty acids were absent in the oil by majority for oleic and linoleic acid which reached about 41 and 35.7% of total fatty acid percentage, respectively. The second

Table 1: Fatty acid composition of jojoba and jatropha oils

Fatty acid	Jojoba	Jatropha
C16:0 Palmitic acid	1.3 ± 0.05	15.40 ± 2.44
C16:1 Palmitoleic acid	0.1 ± 0.01	1.20 ± 0.27
C18:0 Stearic acid	0.3 ± 0.01	6.70 ± 0.37
C18:1 Oleic acid	9.8 ± 0.13	41.00 ± 1.08
C18:2 Linoleic acid	Nd	35.70 ± 2.05
C18:3 Linolenic acid	Nd	Nd
C20:0 Arachidic acid	1.0 ± 0.04	Nd
C20:1 Gadoleic acid	71.3 ± 1.23	Nd
C22:0 Behenic acid	Nd	Nd
C22:1 Erucic acid	14.9 ± 1.09	Nd
C24:1 Nervonic acid	1.3 ± 0.31	Nd

values are represented as ± SD, Nd: Not detected

majority was for palmitic fatty acid, that was present by ratio 15.4%; the other fatty acids were stearic fatty acid 6.7%, palmitoleic 1.2%, while, linolenic, arachidic, gadoleic, behenic, erucic and nervonic, were not detected in the jatropha oil use in this experiment.

**Total phenolic and total flavonoid of JOO and JOPE:** Total phenolic and flavonoids are exceedingly known as radical scavengers, mineral chelating agent, reducing factor, hydrogen granter and singlet oxygen quenchers<sup>22</sup>. Among the different phytochemicals, phenolic compounds have to attract the concern of several scopes of implementations like in pharmaceuticals, foods, healthcare as well the industrial cosmetics in the nearby past years. The Folin-Ciocalteu procedure has been suggested to be a rapid evaluation method to estimate the total phenolic in foods and food supplements<sup>23</sup>. The total phenolic substances and amount of the different extracts evaluated varied between  $191.23 \pm 1.53$  and  $996.5 \pm 0.5$  mg GAE  $g^{-1}$  of sample (Fig. 1).

As a recognized information that plants as well their extracts, protect itself against pathogens by different vindication reactions whose contain the antimicrobial outputs. The total phenolic compounds beside the total flavonoid contents either for JOO or for JOPE were determined (Fig. 1).

Total phenolic compounds were determined as mg gallic acid  $g^{-1}$  of material, the values were recorded as  $191.23 \pm 1.53$  mg GAE  $g^{-1}$  of material for JOPE and it was  $12.5 \pm 1.77$  mg GAE  $g^{-1}$  of material for JOO. Along with it; the values of total flavonoids contents recorded  $53.13 \pm 0.8$  mg Cat.  $g^{-1}$  and  $5.92 \pm 2.75$  mg Cat.  $g^{-1}$  for JOPE and JOO, respectively. The results showed more amounts of phenolic compound and flavonoid content in JOPE than the amount in the oil, this may give a reason how the extract appeared to have more antimicrobial characters than the oil extracted from jojoba.

However, the values of total phenolic compound and total flavonoids content of jatropha were estimated, the results showed variation between the values in JAPE and the values in JAO (Fig. 1).

Total phenolic compounds were determined as mg gallic acid  $g^{-1}$  of material, the values were recorded as  $996.5 \pm 0.5$  mg GAE  $g^{-1}$  of material for JAPE and it was  $102.75 \pm 2.38$  mg GAE  $g^{-1}$  of material for JAO. On the other hand; the assessment amount of flavonoids contents was  $180.83 \pm 1.04$  mg Cat.  $g^{-1}$  for JAPE and it was recorded at  $9.99 \pm 3.32$  mg Cat.  $g^{-1}$  for JAO. The results showed an increase of phenolic compound and flavonoid content in JAPE more than the values in the oil, this may give a reason how the extract appeared to have more antimicrobial characters than the oil extracted from jatropha. As in comparable to jojoba

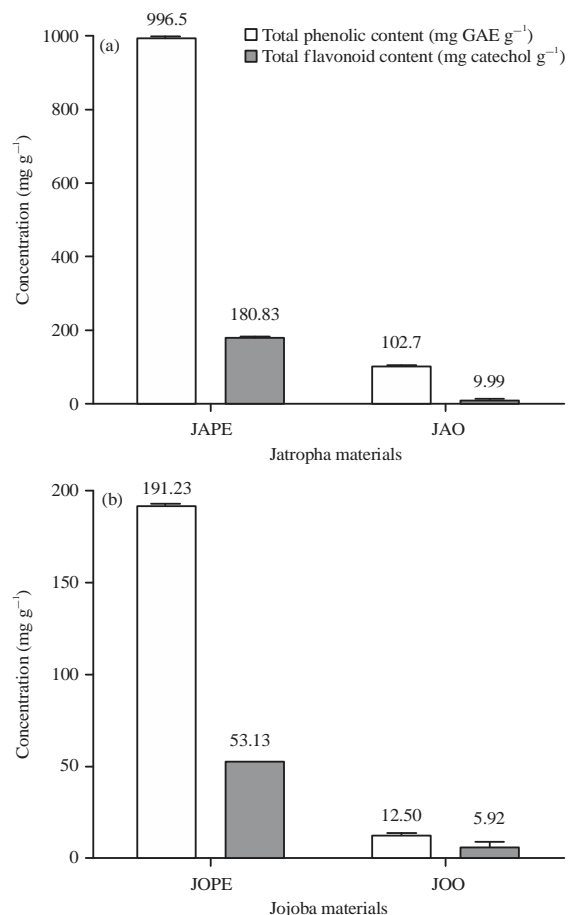


Fig. 1: Total phenolic and flavonoid content of pomace aqueous extract and oil for jojoba and jatropha, (a) Significant difference between JAPE and JAO for phenolic and flavonoid content, (b) Significant difference between JOPE and JOO for phenolic and flavonoid content

Values are obtained  $\pm$ SD

(JOPE and JOO), jatropha appeared as highly content of total phenolic compound and total flavonoid contents either in JAPE or in JAO, the total phenolic of JAPE recorded about five duplicates of the total phenolic of JOPE, while the total flavonoids of JAPE appeared as a duplicates amount of the total flavonoids of JOPE, thus may reflecting as an enhancement of JAPE properties.

**Antioxidant activity:** DPPH or ABTS radical cation assay was utilized to evaluate the free radical scavenging attributes of several extracts<sup>24</sup>. It was found that; jojoba pomace and jatropha pomace showed a good antioxidant capacities compared with ascorbic acid. The results from Table 2 indicate that the radical scavenging activity (percentage of

Table 2: DPPH radical scavenging ability, for aqueous extracts of Jojoba pomace, Jatropha pomace, Jojoba oil and Jatropha oil

Concentration of samples ( $\mu\text{g mL}^{-1}$ )	Inhibition of DPPH (%)				
	Jojoba pomace extract	Jatropha pomace extract	Jojoba oil	Jatropha oil	Ascorbic acid
25	6.71 $\pm$ 4.29 <sup>c</sup>	23.77 $\pm$ 5.99 <sup>c</sup>	15.66 $\pm$ 1.60 <sup>b</sup>	23.66 $\pm$ 1.65 <sup>c</sup>	88.79 $\pm$ 2.08 <sup>a</sup>
50	36.86 $\pm$ 1.33 <sup>c</sup>	45.33 $\pm$ 7.83 <sup>c</sup>	43.36 $\pm$ 2.08 <sup>b</sup>	36.46 $\pm$ 0.92 <sup>c</sup>	89.31 $\pm$ 3.23 <sup>a</sup>
75	57.90 $\pm$ 9.39 <sup>b</sup>	57.62 $\pm$ 6.65 <sup>c</sup>	48.50 $\pm$ 1.05 <sup>c</sup>	44.83 $\pm$ 1.20 <sup>c</sup>	89.53 $\pm$ 3.48 <sup>a</sup>
100	64.56 $\pm$ 3.30 <sup>b</sup>	73.43 $\pm$ 5.38 <sup>c</sup>	56.20 $\pm$ 0.95 <sup>c</sup>	48.10 $\pm$ 1.24 <sup>c</sup>	90.40 $\pm$ 1.77 <sup>a</sup>

Values with different superscripts are significantly different at  $p = 0.05$ , values are represented as  $\pm$ SD

inhibition) of the extract from jojoba pomace extract was measured as 6.71, 36.86, 57.9 and 64.56% with different concentrations of the extract 25, 50, 75, 100,  $\mu\text{g mL}^{-1}$ , respectively, whereas treated with the same concentrations of Jojoba oil reached the percentages of DPPH Inhibition of 15.66, 43.36, 48.5 and 56.2%, respectively. Ascorbic acid was used as a reference standard to compare the antioxidant power activity.

As  $IC_{50}$  values compared between the standard compound, ascorbic acid, with JOO and JOPE, the results showed that the  $IC_{50}$  of ascorbic acid was 14  $\mu\text{g mL}^{-1}$ , JOO showed  $IC_{50}$  value at 77.34  $\mu\text{g mL}^{-1}$  but for JOPE the  $IC_{50}$  value was 66.03  $\mu\text{g mL}^{-1}$ , this finding illustrate how JOPE will be more effective as antimicrobial than JOO.

To make a comparability of DPPH which is the most common radical scavenging assays, the ABTS radical scavenger was used to assure the antioxidant values of jojoba oil and jojoba pomace extract. ABTS is a well-recognized nitrogen-centered, artificial radical and is vastly used to evaluate antioxidant liveliness. The ABTS radical is produced through an oxidation process of ABTS with potassium peroxy-disulfate ( $K_2S_2O_8$ ) as antioxidants added, it is switched to a non-radical form. Different concentrations of extracts extended from 25-100  $\mu\text{g mL}^{-1}$ , were examined for scavenging activity.

According to the data values in Table 3, the radical scavenging activity of the JOPE as ABTS<sup>+</sup> test was measured at 31.51, 47.27, 61.81 and 79.63%, for concentration values of the extract 25, 50, 75, 100  $\mu\text{g mL}^{-1}$ , respectively. At the same concentrations, the values were recorded as 23.66, 36.46, 44.83 and 48.1% inhibition of ABTS<sup>+</sup>, respectively. The  $IC_{50}$  of JOO was 103.99  $\mu\text{g mL}^{-1}$  while the  $IC_{50}$  of JOPE was 53.2  $\mu\text{g mL}^{-1}$ .

Jojoba oil side with the byproducts of oil processing as well as jatropha oil and its processing by-products has great potency as a naturalist antioxidant and polyphenols, it is considered as one of its main phenolic components<sup>25</sup>. Subsequently, the evaluation process of the total antioxidant capacity for any plant part could not be performed rightly just using only one method due to the complex nature of phytochemicals<sup>26</sup>. Also, the antioxidant capacity of herbal and

medicinal plants is precisely correlated with the content of phenolic compounds. Oxidative stress is presently renowned to be correlating for more than two hundred illness cases, along with the normal aging in life cycle system. Usually, the performance of DPPH radical scavenging activity is done as a mensuration antioxidant capacity of electron denoting<sup>27</sup>. The antioxidant substances are matters that balanced the free radicals and its passive effects. It acts as important roles at several levels (prevention, delay and reform), it is chelated acting and trapping for free radicals, using various techniques as reducing agents by donating hydrogen, quenching singlet oxygen<sup>28</sup>.

Also, the radical scavenging of DPPH activity of the JAPE was measured as 23.77, 45.33, 57.62 and 73.43% for concentrations of values of the extract 25, 50, 75, 100  $\mu\text{g mL}^{-1}$ , respectively (Table 2). Moreover, the radical scavenging of DPPH for JAO was measured as 45.16, 58.9, 68.66 and 80.28% at the same concentrations respectively. On the other hand the DPPH radical scavenging activity of ascorbic acid (Standard) was determined as 88.79, 89.31, 89.53 and 90.40% with the same previous concentrations. It was a consideration that the scavenging efficiency of the extracts was raised by the growing of the extract concentrations. The data obviously that the concentration of (100  $\mu\text{g mL}^{-1}$ ) of the JAPE resulted in a percentage inhibition of DPPH (73.43%) almost of the same concentration of ascorbic acid that was 90.40%. Along with a value of radical scavenging activity of the JAO recorded as 80.26% at the same concentration.

The scavenging activity ranged from 23.77-73.43 % for the extract and from 45.16-80.26% for the oil extracted from jatropha seed but it was ranged from 88.79-90.4% for ascorbic acid (Table 2). Along with that, the  $IC_{50}$  values were 65.67, 42.45 and 14  $\mu\text{g mL}^{-1}$  for JAPE, JAO and ascorbic acid, respectively. Besides, the antioxidant activity in the ABTS or DPPH assay were also strongly correlated with the contents of phenolic and flavonoid compounds.

In Table 3, the Antioxidant capacity of the extracts of JAPE and JAO as per ABTS<sup>+</sup>-radical assay expressed as percentage activity was measured, at samples concentrations of 25, 50, 75 and 100  $\mu\text{g mL}^{-1}$ , the percentage inhibition of ABTS<sup>+</sup> for

JAPE was recorded closely to the standard (Ascorbic) at  $100 \mu\text{g mL}^{-1}$ . While, the inhibition of ABTS<sup>•+</sup> for JAO was recorded at 61.04% for  $100 \mu\text{g mL}^{-1}$  concentration as the maximum ratio. For the standard compound, the results for the inhibition ratio were 92.66% for the concentrations of  $100 \mu\text{g mL}^{-1}$ .

The  $\text{IC}_{50}$  which indicates how the power of the material could inhibit the fifty percent of the ABTS was also recorded for JAPE, JAO and ascorbic acid. The  $\text{IC}_{50}$  value of JAPE appeared as so closely to the value in case of use ascorbic acid as standard reference material, it was  $16.07 \mu\text{g mL}^{-1}$  in case of use JAPE and  $14.43 \mu\text{g mL}^{-1}$  in case of use ascorbic acid. Finally, the  $\text{IC}_{50}$  value was recorded as  $68.88 \mu\text{g mL}^{-1}$  of JAO.

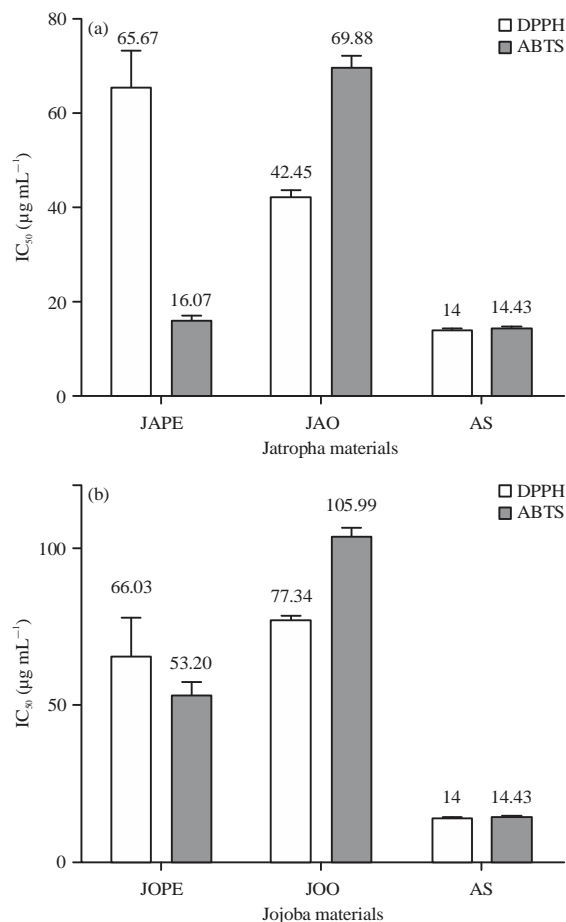


Fig. 2:  $\text{IC}_{50}$  values for jojoba and jatropha materials, (a) Significant difference between JAPE and JAO for  $\text{IC}_{50}$  value, (b) Significant difference between JOPE and JOO for  $\text{IC}_{50}$  value  
Values are obtained  $\pm$ SD, AS: Ascorbic

**Variation of  $\text{IC}_{50}$  values:** In comparison of  $\text{IC}_{50}$  for jojoba and jatropha materials (JOPE, JAPE, JOO and JAO) by two anti-oxidative evaluation methods, the results showed an ascending graduated impact for  $\text{JAO} < \text{JOO} < \text{JOPE}$ , then the high effective one was the JAPE, this results were done against ascorbic acid as a standard material. The results in Fig. 2 showed that; both of JAPE and JOPE which done using ultrasonic assisted in the presence of eco-friendly solvents had an efficiency as antimicrobial material more than the oils either of jojoba or of jatropha seeds.

**Antimicrobial activity of oils and extracts:** More than one methods were used to check the ability of oils and extracts to inhibit fungal growth of toxigenic strains, disk diffusion beside well diffusion assay were used to examine how those material could decrease the fungal contamination and increase the protection effect. JOPE, JOO, JAPE and JAO were inoculate to disk as well to well on media plate, the results showed that; the JOPE was able to decrease the fungal growth of *Aspergillus*, *Penicillium* and *Fusarium* either for disk or for well diffusion assay (Fig. 3). For the same strains; the inhibition ratio by using JAPE was more effective than using JOPE but in case of using JAO, the growth rate of *Penicillium* fungi was increased. By using JOO in well diffusion and disk diffusion assay, it showed a better ability for reducing the fungal growth especially for *Aspergillus ochraceus*.

As described in the first row of the Fig. 3, the data in Fig. 3a-c were showed the effect of JAPE on fungal growth of some mycotoxigenic fungi. The figure showed an effective inhibition for JAPE on the fungal strains under investigation, especially on *Aspergillus*, *Penicillium* and *Fusarium*.

Table 3: Antioxidant capacity of the extracts of jojoba pomace, jatropha pomace, jojoba oil and jatropha oil as per ABTS<sup>•+</sup> radical assay expressed as percentage activity (n = 3) as a function of concentration of extracts

Concentration of samples ( $\mu\text{g mL}^{-1}$ )	Inhibition of ABTS <sup>•+</sup> (%)				
	Jojoba pomace extract	Jatropha pomace extract	Jojoba oil	Jatropha oil	Ascorbic acid
25	31.51 $\pm$ 2.25 <sup>b</sup>	77.93 $\pm$ 5.09 <sup>b</sup>	23.66 $\pm$ 1.65 <sup>c</sup>	31.40 $\pm$ 1.60 <sup>c</sup>	86.60 $\pm$ 2.37 <sup>a</sup>
50	47.27 $\pm$ 3.63 <sup>b</sup>	83.33 $\pm$ 4.03 <sup>b</sup>	36.46 $\pm$ 0.92 <sup>c</sup>	47.86 $\pm$ 1.04 <sup>c</sup>	89.39 $\pm$ 0.21 <sup>a</sup>
75	61.81 $\pm$ 3.63 <sup>b</sup>	86.54 $\pm$ 1.09 <sup>b</sup>	44.83 $\pm$ 1.20 <sup>c</sup>	53.70 $\pm$ 1.86 <sup>c</sup>	92.13 $\pm$ 0.29 <sup>a</sup>
100	79.63 $\pm$ 3.10 <sup>b</sup>	87.75 $\pm$ 1.16 <sup>b</sup>	48.10 $\pm$ 1.24 <sup>c</sup>	61.04 $\pm$ 2.16 <sup>c</sup>	92.66 $\pm$ 0.27 <sup>a</sup>

Values with different superscripts are significantly different at  $p = 0.05$ , values are represented as  $\pm$ SD

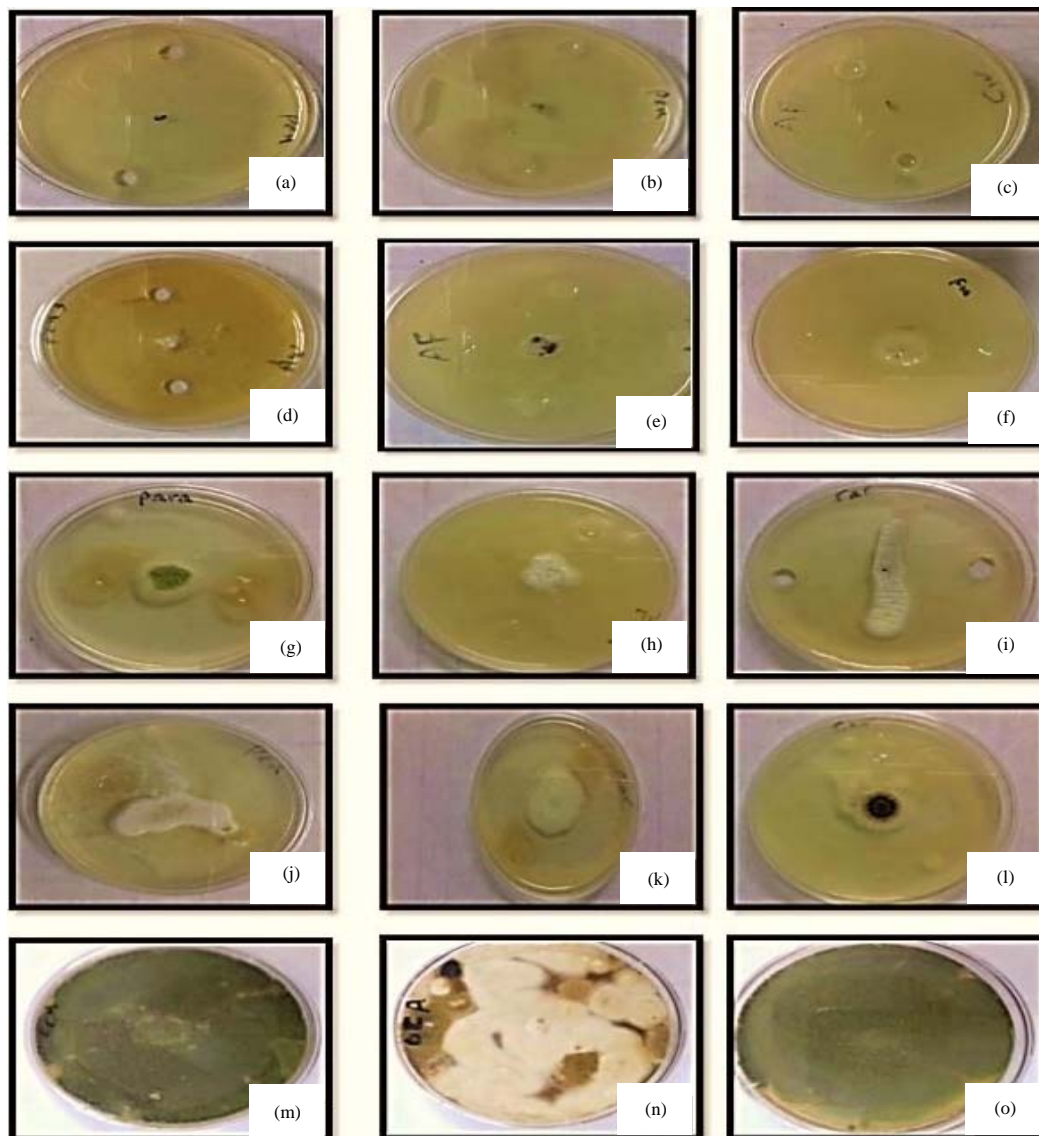


Fig. 3(a-o): Inhibition effect of jojoba and jatropha oils and extracts on some toxigenic fungi (a-c) Jatropha pomace extract, (d-f) Jojoba pomace extract, (g-i) Jojoba oil, (j-l) Jatropha oil and (m-o) Control

Otherwise, in the second row, it was shown a good inhibition also by using JOPE in agar well diffusion assay, the results were showed in (Fig. 3d-f). By using JOO in the well diffusion assay, the results appeared with efficiency as decreasing material for fungal growth. It was a very little growth of fungi on the media (Fig. 3g-i) but in case of using JAO (Fig. 3j-l), the result represented an inhibition effect but not more than using JOO.

#### Determination of the Minimum Inhibitory Concentration (MIC):

The minimum inhibitory concentration is the lowest concentration that used to stop the growth of pathogenic bacteria, many phytochemical components and plant extracts were recorded to have sterilized properties with highly MIC

values. The minimal inhibitory concentration values of the four types of materials were determined against four bacterial strains (Gram positive and Gram negative strains), in the presence of the control. In Table 4 each material had a value of MIC, while the extract of Jatropha pomace (JAPE) appeared as the best extract in MIC value with high ability to inhibit pathogenic bacterial growth at  $60 \mu\text{g mL}^{-1}$ , followed by a Jojoba pomace extract (JOPE) which have MIC value at  $90 \mu\text{g mL}^{-1}$ . The weak efficiency extract of the four materials was the JOO with a value of MIC at  $160 \mu\text{g mL}^{-1}$ .

As mention in the study of Carraro *et al.*<sup>29</sup>, that discuss around better understanding for mode of action of phenolic compound as antimicrobial agent against *E. coli* K12 strain as



Table 4: Minimal inhibition concentration (MIC) values of by-product extracts

Bacterial strains	Ampicillin	Jatropha pomace extract	Jjoba pomace extract	Jatropha oil	Jjoba oil
	----- $\mu\text{g mL}^{-1}$ -----				
<i>Enterococcus faecium</i>	8	60	90	110	160
<i>Bacillus cereus</i>	2	50	90	100	160
<i>Pseudomonas aeruginosa</i>	2	50	80	90	160
<i>Klebsiella pneumoniae</i>	2	50	90	100	160

Table 5: Minimal fungicidal concentration (MFC) of by-product extracts

Fungal strains	<i>Aspergillus parasiticus</i>	<i>Aspergillus ochraceus</i>	<i>Penicillium</i> sp.	<i>Fusarium solani</i>
Jjoba oil ( $\mu\text{g mL}^{-1}$ )	170	170	170	160
Jatropha oil ( $\mu\text{g mL}^{-1}$ )	250	250	300	250
Jjoba pomace extract ( $\mu\text{g mL}^{-1}$ )	150	150	150	150
Jatropha pomace extract ( $\mu\text{g mL}^{-1}$ )	120	120	120	120
Natamycin ( $\mu\text{g mL}^{-1}$ )	70	70	70	50

a microbial model, the experimental study was focused on the extract impact of phenolic components from olive vegetation water at the molecular scale of the bacteria, the result recorded that; there was inhibitory effect of using phenolic water of olive on the gene level of the *E. coli* K 12 strain which reflecting on the decreasing of bacterial biofilm on the surface of liquid food material, this result was very benefit according to its importance in food processing sectors.

Comparing to Silva *et al.*<sup>30</sup>, whose represented that in a previous work on olive extract which done in 1993, it was illustrated how did phenolic compound and oleuropein extracted from olive able to inhibit the bacterial growth of *staphylococcus aureus*, this strain had ability to produce enterotoxin B. Three levels of phenolic compound and oleuropein concentration were used, at low concentration the bacterial growth rate were delayed as well as the ability of toxin production was blocked, while at high concentration the growth of the pathogenic bacteria completely stopped.

#### Determination of the Minimum Fungicidal Concentration

**(MFC):** The minimum fungicidal concentration (MFC) is considered as the less amount the components that had a property to stop grow the fungi strain, thus may happened by using a new material that had a fungicidal effect. In order to determine the minimal fungicidal concentration of the four extracts serial dilutions were done to use against four strains of toxigenic producing fungi, the strains were *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Penicillium* sp. and *Fusarium solani* (Table 5).

The minimal fungicidal concentration value for JAPE and JOPA are 120 and 150  $\mu\text{g mL}^{-1}$ , respectively. The two kinds of extracts were appearing as the best efficiency extracts of the four materials that were used; otherwise, the lowest effective one was the JAO with a MFC value of 250  $\mu\text{g mL}^{-1}$ .

**Eco-friendly plant extracts impact on toxigenic fungi:** The impact of the four types of eco-friendly oils and the extracts by-products of oil manufacturing on four types of fungi and their growth were recorded, toxigenic fungi that used in this study are: *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Penicillium* sp. and *Fusarium solani*. This experiment was targeted to illustrate if there is a positive effect of these extracts on mycotoxigenic fungi growth, so one hundred part per million of each extract inserted into the plate well in each group (group contains one type of fungi), fungi growth were represented as millimeter of fungal circle diameter for 2, 4, 6, 8 and 10 days of fungal growth.

In Table 6 showed a differentiation between jjoba and jatropha extract using eco-friendly solvent which consist of isopropanol: water (1:1), otherwise; there was also a variation between jjoba and jatropha oil about their inhibition effect on mycotoxigenic fungi. Firstly, for *A. Parasiticus* strain which able to produce the four types of Aflatoxins ( $B_1$ ,  $B_2$ ,  $G_1$  and  $G_2$ ). JAPE was the highly efficient material for fungal inhibition on plate media, for more than 6 days, the growth of *A. parasiticus* on media contain the JAPE had not appeared and after 12 days of growth the inhibition ratio reached 88.6%. The less effective were shown for JAO, the maximum inhibition ratio after using JAO on fungal plate of *A. parasiticus*, was reached 56%. In case of *A. ochraceus*, the inhibition ratio was still more effective in the presence of JAPE and less effective in the presence of JAO, inhibition ratio were ordered as JAPE>JOPE>JOO>JAO and the inhibition ratio reached 100% by using JAPE on plate media. The same results also, repeated in case of *F. solani*. The inhibition ratio was equal to one hundred percent by using JAPE against the fungi, while in presence of JAO, inhibition ratio was 49.24%. Finally *Penicillium* Fungi again showed an ascending effect for growth inhibition initiated by JAO then

Table 6: Jojoba and jatropha (oil and oil waste extract's) inhibition impact on toxigenic fungi growth

Treatments	Days			
	3	6	9	12
<b><i>Aspergillus parasiticus</i> (mm of growth diameter)</b>				
Control	47	82	131	150
Jojoba oil	7	19	32	37
Jatropha oil	11	39	53	66
Jojoba pomace	12	33	45	54
Jatropha pomace	0	0	7	17
<b><i>Aspergillus ochraceus</i> (mm of growth diameter)</b>				
Control	33	56	115	144
Jojoba oil	0	7	29	43
Jatropha oil	6	19	38	59
Jojoba pomace	16	30	48	66
Jatropha pomace	0	0	0	0
<b><i>Penicillium sp.</i> (mm of growth diameter)</b>				
Control	42	71	124	150
Jojoba oil	13	17	31	51
Jatropha oil	27	45	58	83
Jojoba pomace	22	41	56	82
Jatropha pomace	0	8	19	26
<b><i>Fusarium solani</i> (mm of growth diameter)</b>				
Control	27	54	88	132
Jojoba oil	0	11	26	39
Jatropha oil	10	24	45	67
Jojoba pomace	8	21	36	48
Jatropha pomace	0	0	0	0

Oils and oil waste extracts were applied on four types of toxigenic fungi

JOO, followed by JOPE and the JAPE was shown the best effective material to decrease *Penicillium* growth.

As statistically, in case of study the effect of jojoba and jatropha materials against *A. parasiticus*. The results showed that, there was no significant JAO and JOPE in 3 days of fungal growth. While, in case of *A. ochraceus* fungi, 3 days of fungal growth but between *A. parasiticus* and *A. ochraceus*, the use of JOO for 6 days growth had no significantly differences. The same was for using JAO for 6 days of growth for *Penicillium* sp., the results showed a non-significant between JAO and JOPE on 9 days of growth, also on 12 days growth rate. For *Fusarium solani*, JAPE was able to stop the fungal growth for about 12 days. The effect was ordered as JAPE>JOPE>JOO>JAO and JAO showed the less inhibition effect.

Thymus recorded as the highest growth inhibition for *Fusarium oxysporum* by a ratio of 77.85% while, it was 67.31% in case of jatropha extract. With regard to using four types of plant extract concentrations (5, 10, 15 and 20%), it was found that it could be able to make 100% inhibition for *Fusarium oxysporum* mycelia growth recorded by using 20% aqueous extracts of all plants under study including jatropha, while inhibition ratios was progressively decreased with extracts concentration lessening.

Anti-inflammatory efficacy of jatropha powder in paste form was confirmed through the investigation conducted on mice<sup>31</sup>. The deoxy-preussomerins and palmarumycins had isolated from the jatropha stems and found to have

antibacterial in nature<sup>32</sup>. Jatropha stem latex included alkaloids such as jatrophine, jatropham and curcin by anti-cancer properties<sup>33</sup>. The study also refers to the plants' extract contains of phenols, flavonoids and alkaloids, those components may fight the pathogens by several modes of action. Furthermore, a powerful antifungal effectiveness discovered in 22 of several plants extracts contra two soil pathogens, *Fusarium solani* and *Rhizoctonia solani* and it had an effect on the fungal mycelium stress.

**Aflatoxins reducing by jojoba and jatropha materials(oils and extracts):** By using jojoba and jatropha materials to decrease the aflatoxins from liquid media. JAPE clearance appeared as the best material for toxins reducing (Fig. 4). The ratio was recorded as 43.81, 38.61, 33.2, 34.3 and 38.7% for AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub> and total AFs, respectively. The second material was shown the better effect for aflatoxins decreases was JOO by ratio of 32.8%, for AFB<sub>1</sub>. In the third order JOPE appeared as the next material which had ability to reduce aflatoxins. The best inhibition ratio was recorded as 28.7%, for AFB<sub>1</sub>. Finally the best inhibition ratio of JAO for aflatoxins reducing was 26.6%, for AFB<sub>1</sub>. Over all the results of aflatoxin reducing, the materials may ordered for aflatoxins decreasing as JAPE>JOO>JOPE>JAO.

As described in Fig. 4, highest decreases ratio was recorded for JAPE on AFB<sub>1</sub> (43.8%) but the low decreases ratio was shown for JAO against AFG<sub>2</sub> (9.4%). The otherwise, the

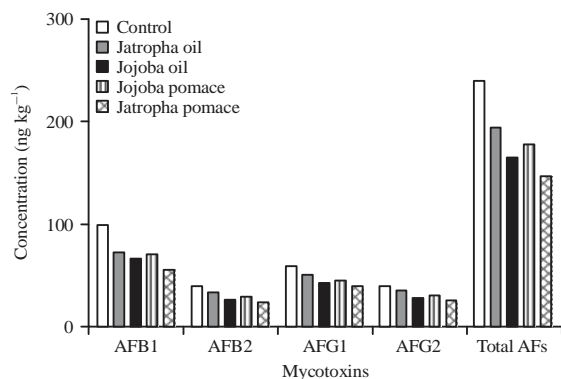


Fig. 4: Aflatoxins inhibitions by jojoba and jatropha materials  
 AFB1: Aflatoxin B1, AFB2: Aflatoxin B2, AFG1: Aflatoxin G1,  
 AFG2: Aflatoxin G2 and AFs: Aflatoxins

highest decrease for total AFs was recorded in case of using JAPE (38.7%) however in case of using JAO and total AFs decrease appeared as the lowest value (18.75%).

Abdel-Wahhab *et al.*<sup>34</sup> study the effect of the ethanolic extract (95% ethanol) of jojoba extract as a hepatoprotective agent on the tissues of rats fed a contaminated diet which contents fumonisin B<sub>1</sub>, the results showed a good results especially in case of the low doses of jojoba extract. The data of Abdel Wahab specified an enrichment of jojoba ethanolic extract in phenolic compounds and protein, side to a significant amount of simmondsin. rats that fed a contaminated diet by Fumonisin offer a serious biochemical and histopathological changes. Treating the contaminated feed only by jojoba extract at two examined doses did not induce a significant modification of parameters under investigation, however, the joint between jojoba extract in presence of FB<sub>1</sub> discarded the hepatotoxicity induced by Fumonisin B<sub>1</sub>, particularly at a low dose of jojoba seed extract.

## CONCLUSION

Although the changes in climate cause difficult weather condition for food plants along with increasing hazard of contamination by toxigenic fungi and mycotoxins, some plants members of Euphorbiaceae family may be considered as a source for bioactive components. Jojoba and jatropha materials (oils and extracts) had antioxidant properites beside its content of phenolics and flavonoids, it has ability to reduce pathogenic and toxigenic hazard. Also, it has a good impact to reduce food risks, JAPE showed the best antimicrobial and antifungal characteristics of all four materials used (JAPE, JOPE, JOO and JAO). Otherwise, those materials were able to reduce aflatoxins especially AFB<sub>1</sub> by ratio upto 43.8%.

## SIGNIFICANCE STATEMENTS

This study explores the possible reducing effect of some non-traditional oils and its by-products on the toxicity of mycotoxins especially aflatoxins. By-products considered as economic low values materials, while it may rich source for many bioactive components. This study will help the researchers to uncover the critical area of losses in agricultural by-products. This article is an attempt to focus on the importance of by-product to food industries and how to raise the value-add of the food industrial wastes. Thus, a new theory on improvement and maximize utilization of by-products as a source of bioactive components or nutraceutical, possibly other combinations may arrive at the followed studies which may decrease an amount of plants manufacturing wastes and turned to a value added materials.

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