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EXPLORING THE ANTIOXIDANT POWER OF *EUCALYPTUS CAMALDULENSIS* (SUFAIDA) AND ITS POTENTIAL ROLE IN AFLATOXINS DEGRADATION

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Abstract
Aflatoxins are secondary metabolite compound produced by fungus *Aspergillus flavus*, *Aspergillus nomius* and *Aspergillus parasiticus.* Aflatoxin degraded by the extract of *Eucalyptus camaldulensis.* The extract leaf of *Eucalyptus Camaldulensis* analyzed for determination of antioxidant and degradation of aflatoxin. The identification and quantification degaradation of aflatoxin analyzed by Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography techniques (HPLC) respectively. In this research, the mean values of water, Acetone, Hexane and Methanol extract of DPPH, FRAP and TPC were (13.41 %, 58.51%, and 27.72%), (68 mg GAE /100g, 466.5 mg GAE /100g, 180 mg GAE /100g, and 450 mg GAE /100g) and (268.78 mg GAE /100g, 269.88 mg GAE /100g, 68.22 mg GAE /100g and 276 mg GAE /100g) respectively. The mean of degaradation AFB_1, AFB_2, AFG_1, AFG_2 and TAF) were 3.05±0.29 µg/kg, 2.15±0.17 µg/kg, 4.21±0.25 µg/kg, 2.88±0.20 µg/kg and 12.29±0.09µg/kg respectively. The removal % of total Aflatoxin by Eucalyptus *camaldulensis* was 95.08 %.

Keywords: *Eucalyptus Camaldulensis* (Sufaida) Aflatoxins, Antioxidant, HPLC, TLC, Degradation of aflatoxins

Introduction

Aflatoxins are the most lethal mycotoxins, produced by *Aspergillus flavus*, *Aspergillus nomius* and *Aspergillus parasiticus*. Aflatoxin biotransformation products have been reported mainly in milk and milk products. Major categories of aflatoxins include aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2). There are > 20 types of aflatoxin molecules. Inside the liver of animals, aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2) are produced by the breakdown of AFB_1 and AFB_2, respectively (Ismail, 2016). Aflatoxins are common all-over sub-Saharan Africa and South Asia areas. Environmental stress, incomplete drying of crops and warm, humid conditions, lack of farmer awareness and improper storage conditions are suitable for the growth of fungus on crops and production of the toxins during storage (Xu, 2018). Aflatoxins exposure may result in acute and chronic toxicity to the human and animals and the condition is generally known as aflatoxicosis. Aflatoxins toxicity is about 68 times greater than that of arsenic. Aflatoxins are reported to have carcinogenic, teratogenic, genotoxic, embryotoxic and immunotoxic health impacts on humans and animals. Aflatoxins natural mixture i.e. AFB_1 + AFB_2 + AFG_1 + AFG_2 and AFB_1 alone both are classified as group 1 category human carcinogen by the International Agency for Research on Cancer (IARC). Aflatoxins degraded by several method e.g. physical, chemical, biological and herbal plants extract method. Among them the extract of herbal medicinal plant are mostly used for the degradation of aflatoxin due high antioxidant activities and safe for human and animal health. The Eucalyptus *camaldulensis* (sufaida) is a native Pakistan herb plant. The several diseases have been treated using the *Eucalyptus camaldulensis* e.g. abdominal pain, eye disease, throat troubles and cardiovascular disorders. (Cela et al, 2012). Eucalyptus *camaldulensis* extracts are obtained from natural sources and are considered as safe for human and animal health. The extracts of Eucalyptus *camaldulensis* show antifugus and antimicrobial activities. Antifugus and antimicrobial activities of medicinal plants are thought to have significant impact on their aflatoxins degradation potential (Bibi, 2011). In this Research, DPPH, FRAF and TPC assay used for the determination antioxidant scavenger of Eucalyptus *camaldulensis.* Thin Layer Chromatography and High Performance Liquid Chromatography used for identification and quantifications of aflatoxin using the extract of Eucalyptus *camaldulensis.* Statistix 8.1 software was used for statistical analysis of results obtained in various experiments.

1. Material and Method

The Laboratories of Institute of Food Science and Nutrition, was used for current research course in Bahauddin Zakariya University, Multan, Pakistan. Eucalyptus *camaldulensis* for the analysis of aflatoxins were collected form district of Punjab including Multan (Bahauddin Zakariya University, Multan, Pakistan). Eucalyptus *camaldulensis* were collected in clean plastic bags and immediately transported to the laboratories of the Institute Food Science and Nutrition, Bahauddin Zakariya University, Multan and stored at 30 ^{0}C until further analysis. Before to analysis, the sample were dried under sun light for preparation of powder. All the chemicals used during the experiment were of analytical grade. Good labs practices were adopted during the all research work experiments. The samples were sent to the Botany department of Bahauddin Zakariya University, Multan, Pakistan for identification purposes. Only leaves portion of the plants were used for degradation of aflatoxins.

1.1. The Extract Preparation

The extracts were prepared according to the method of Ponzilacqua et al., (2019). The leaves of Eucalyptus *camaldulensis* were washed with tap water and dried under sunlight. After drying, the dried leaves of Eucalyptus *camaldulensis* were grounded to fine powder by the grinder machine and extraction of compounds from fine powder of plant leaves was performed by suitable solvents (hexane, acetone, methanol, and water). Briefly, the fine powders were weighed by electrical weight balance and solvents were measured by the graduated cylinder. 20 g fine powders of Eucalyptus *camaldulensis* were mixed with solvents 200 ml each solution (hexane, acetone, methanol, and water) taken in 250 ml flask. The samples were shacked by orbital shaker at room temperature for 8

to 12 h. After it, the samples were placed in a dark room for 48 hours. The samples were filtered by muslin clothe and through Whatman filter paper No. 42. The rotary evaporator was used for the evaporation of the solvent from the samples. Remaining extracts were transferred in a test tube. These test tubes were labeled with respective names of each extract with solvent. These extracts were used for further tests.

1.2. Antioxidant Activity

The plants that showed aflatoxins degradation potential were evaluated for their antioxidant activity according to the method as described by Ismail et al., (2016). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method, ferric reducing antioxidant power (FRAP) and total phenolic content (TPC) methods were performed to evaluate the antioxidant activity of medicinal plants.

1.2.1. DPPH Method

The free radical scavenging property of Eucalyptus *camaldulensis* leaves were determined by DPPH method using ascorbic acid as standard according to the method proposed by Keeler et al., (1983). DPPH solution was prepared by adding 1 mg of DPPH with 25 ml methanol, taken in 250ml beaker and the beaker was covered with aluminum file. The solution was placed in dark room for five minutes. 2 ml of homogenously prepared leaves extracts were mixed with 3 ml DPPH solution taken in 15 ml falcon test tubes. The reaction mixtures were kept for 30 min in dark chamber at 30C and absorbance was measured at 517 nm. Standard curve of ascorbic acid was drawn against DPPH at various concentrations (10 µM to 100 µM) and free radicals scavenging properties of methanol, hexane, acetone and water extracts was calculated as mg ascorbic acid equivalent per gram (mgAAE/g) of dried extracts Ismail et al. (2015) Blank/control preparation. By mixing 2mL methanol with 3mL DPPH were used for the formulation of the control and were also calculated. Three replications were made for each sample. Decrease in the absorbance was a measure of the scavenging activity of DPPH radical by spice extracts which was taken as a percentage of free radical scavenging activity and it was calculated by the following formula.

% Inhibition Activity = $\{(A_0-A_1/A_0)\}$ X 100

A_0 = Absorbance of the blank

A_1 = Absorbance of the extract

1.2.2. Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant potential of the extracts of Eucalyptus *camaldulensis* was determined by the method adopted by Zahin et al., (2010). FRAP reagent was prepared in a ratio of 1:1:10 from ferric chloride (20mM), TPTZ (10mM) and sodium acetate buffer (pH 3.6). The reaction mixture was heated for 10min at a temperature of 37 ^{0}C. 300µl of FRAP reagent was pipetted in a test tube. Powdered extracts weighing 25mg were taken in FRAP reagent tubes and shack it by vortex mixture. The spectrophotometer was used for absorbance of samples and control was measured at 593nm. Ferrous sulfates were used as standard and its multiple concentrations ranging from 100 – 1000µM were prepared. The absorbance of the standard solution was accordingly read at 593nm. The results of the samples and control were expressed as µM of Fe (ferrous ions to ferric ion conversion) (Akhtar et al, 2018).

1.2.3. Total Phenolic Content (TPC)

Total phenolic content of the extracts of Eucalyptus *camaldulensis* were determined according to the method of Katsurayama et al., (2018). Extract of Medicinal plants were dissolved with methanol and water solution and the sample 0.5ml aliquots were poured in test tube by pipet. Folin Ciocalteu Reagent (FCR) 2.5 ml was mixed with sample tubes. The Na_2CO_3 2ml of 7.5% was add in sample tube. The sample tube placed in dark room for reaction at 25^{0}C for 30 minutes. Spectrophotometrically (UV-Vis 3000, ORI, and Germany) was used for the absorbance of sample at 760 nm. Series of gallic acid standard with concentration ranging from 10 to 100mg$^/$L were run as

standard concentration to plot the standard curve. Results obtained were expressed as mg gallic acid equivalent (GAE) per 100g.

1.3. Thin Layer Chromatographic (TLC) Study for detection Aflatoxins via different extract leave of Eucalyptus *camaldulensis*

The qualitative analysis of aflatoxins were determined by (TLC) following to the method described by Ramesh et al., (2013). The standards of total aflatoxins (AFB_1 + AFB_2 + AFG_1 + AFG_2) were purchased from Biopure (Romer Labs, Austria). Stock solutions were diluted with methanol to make the working solutions of at cementation 100, 25, 100 and 25 µg/kg for AFB_1 + AFB_2 + AFG_1 + AFG2, respectively, and stored at 4 °C. The extract of Eucalyptus *camaldulensis* were prepared by grinding leaves of Eucalyptus *camaldulensis* powder (10 g each) with 100 ml of sterile distilled water and filtered through two layers of muslin cloth. The solvent was evaporated by rotary evaporator, remain 10 ml extract after evaporations of water poured in falcon tube. The extract was centrifuged at 14,000 g for 20 min and the supernatant used for further studies. 1mL of plant extract was mixed with 50 µL of standard aflatoxin B_1 and incubated at 37 C for 48 h. After incubation, the sample was completely dried by hot oven method. 1 mL methanol was added in each dried sample. After it, the sample was shocked by vortex mixture. The TLC tank was prepared in 95:5 ratios and total volume of tank was 100v/v. 95 ml chloroform was added with 5 ml Acetone in the beaker covered with aluminum foil. The line was made on the TLC by lead pencil. 5 µl standards of total aflatoxins containing aflatoxins B_1, B_2, G_1 and G_2 in the ratios of 2:0.5:2:0.5 and 5 µl samples were spotted on the TLC plates. The TLC plate was placed in TLC tank until the mobile phase reaches to the top. Spots for aflatoxins were observed under UV light. The samples were compared with standard of aflatoxins.

1.4. High Performance Liquid Chromatography (HPLC) study for Qualification of Degradation of Aflatoxin via different extract leaves of *Eucalyptus camaldulensis*

Eucalyptus camaldulensis samples (25 g each) were extracted for aflatoxins through a 100 ml solution of methanol and water (60: 40 v/v). The mixture was placed in an orbital shaker operating at 150rpm for 3-4 hr. The samples were then placed at room temperature for overnight. Next day, the samples were filtered through Whatman No. 42 filter paper. PBS solution (50 mM) was prepared adding NaCl (8.76 g), KH_2PO_4 (1.24 g) and K_2HPO_4 (7.27 g) in 1 liter flask and added with double distilled water to make the volume up to the mark. The pH of PBS solution was adjusted to 7.4 with the help of 1N HCl and 1N NaOH solutions. 4 ml solution obtained through extraction step was diluted with 16 ml of 50mM PBS (pH=7.4). The resulting 20 ml mixture of PBS and extracted sample was passed through immunoaffinity column (Eurofins). The flow rate of the column was maintained 1 – 3 ml /min. The perform the washing step the 50mM PBS solution was diluted to 10 mM PBS and a mixture of 10mM PBS and methanol in ratio of 90 and 10, respectively, was made (wash solution). 5 ml of wash solution was passed from the immunoaffinity column to remove all the particles other than aflatoxins. In order to elute the aflatoxins from immunoaffinity columns, methanol (2 ml) was passed through the columns. The methanol obtained after elution process contains aflatoxins (if any). The methanolic fractions were evaporated to dryness under a nitrogen stream. Derivatization of aflatoxins was performed to improve the fluorescence properties of aflatoxins. Derivatization was performed according to the AOAC method 2005.08 (AOAC, 2005). Briefly, the vials containing dried methanolic fractions of aflatoxins standards / samples were added with 200 µl hexane. Then, triflouroacetic acid (TFA) solution of 50 µl was added and the vials were placed in darkness for 5 minutes. After 5 min, a mixture of acetonitrile and double distilled water in the ratio of 1 and 9, respectively was mixed and vortexed for 1 minute. The fat containing upper layer was removed and the aqueous layer was collected. The aqueous layer was passed through 0.45 µm syringe filters prior to loading on HPLC system (Sykam, Germany). The isocratic mobile phase used in HPLC analysis consisted of water, methanol and acetonitrile in the ratio of 65, 25, and 15, respectively. The flow rate of mobile phase was set at 1.0 ml / min and the column oven temperature

was 37 °C. The emission and excitation wavelengths of fluorescent detector (RF-20 A) were 440 nm and 365 nm, respectively. The injection volume was set at 20 µl and the single analysis run time was set at 20 min (Wong et al., 2017).

2. Results and Discussion

Aflatoxins are secondary metabolites produced by fungus especially *A. flavus*, *A. nomius* and *A. parasiticus*. Aflatoxins are highly toxic for human health and may cause a number of ailments humans depending on the amount of dose, contact time and contact duration. The four major types of aflatoxins are AFB_1, AFB_2, AFG_1 and AFG_2. Based on the toxicity of aflatoxins and their omnipresence, scientists across the world are trying to explore the suitable and commercially implementable methods for the degradation of aflatoxins. In the current study, we used extract *Eucalyptus camaldulensis* to evaluate antioxidant and their aflatoxins degradation potential.

2.1. Antioxidant Activity

Antioxidants are natural compound present in *Eucalyptus Camaldulensis*. Antioxidant inhibited growth of aflatoxins due to the presence of free radicals because of their action to combat with the free radicals. *Eucalyptus Camaldulensis* appeared as a cheap, safe and novel way of naturally found antioxidants. Extract and oil of *Eucalyptus Camaldulensis* are quantified rich in antioxidants activity. Both, extract and oil of *Eucalyptuss camaldulensis* possess a diversity of valuable quantity of bioactive constituents like flavonoids, terpenes, phenolics, alkaloids, anthocyanins, tannins, steroids, isoflavones, saponins, lignin's, coumarins, isocatechins, ascorbic acid and catechin. These bioactive compounds find the use in reduction of aflatoxins or even prevention of a number of chronic diseases i.e hepatocellular carcinoma, cardiovascular diseases (CVD's) and some other degenerative diseases.

2.1.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) activity

Statistical analysis showed significant ($P < 0.05$) difference in the DPPH activity of different extracts *Eucalyptuss camaldulensis* (Table 1). The highest and lowest % DPPH activity of *Eucalyptuss camaldulensis* in Methanol and water were 69.31% and 13.41 % respectively, while the % DPPH activity acetone extract (47.23%) of *Eucalyptuss camaldulensis* higher than Hexane extract (20.28%). Kiendrebeogo *et al*., (2011) analyzed antioxidant activity in methanolic extract of *Eucalyptuss camaldulensis*. The samples were collected at Gampela (25 km, east of Ouagadougou, Burkina Faso). Spectophotomer was used for measuring absorbance reading of extract of *Eucalyptuss camaldulensis.* The percentage of antioxidant activity (DPPH) in methanolic extract of *Eucalyptuss camaldulensis* was recorded as 50%. France *Eucalyptuss camaldulensis* showed maximum antioxidant activity in acetonic extract with percentage was 41.3%. These researches are in line with our findings.

Table 1:

% DPPH (Mean ±SD) **values of plants**

Plants	**Water**	**Acetone**	**Hexane**	**Methanol**	**Overall Mean**
Eucalyptus camaldulensis	13.41 ± 0.23^{k}	47.23 ± 0.32^{d}	20.28 ± 0.23^{h}	69.31 ± 0.44^{a}	25.31 ± 0.10^{b}

The % DPPH antioxidant scavenger activities in water, acetone, hexane, and methanol extracts leave of *Eucalyptus Camaldulensis* graphically represented in fig 1. The graph bar of methanol has been showed highest % DPPH vales that other extract of Eucalyptus Camaldulensis (water, acetone and hexane).

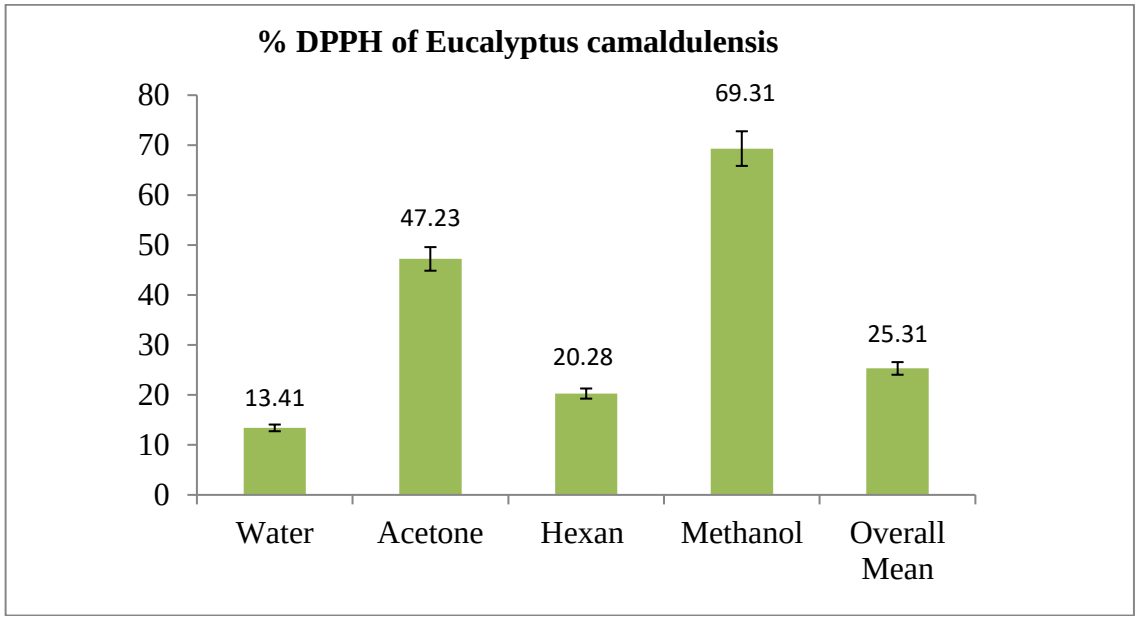

Fig 1: DPPH activity in water, methanol, acetone and hexane of *Eucalyptus camaldulensis*

2.1.2. Ferric reducing antioxidant power (FRAP)

Statistical analysis showed significant ($P < 0.05$) difference in the FRAP activity of different extracts leave of *Eucalyptuss camaldulensis* (Table 2). The mean value of FRAP activity in Water, Acetone, Hexane and Methanol leave extract of *Eucalyptus camaldulensis* were 68±0.71 mg GAE /100g, 466.5±0.7 mg GAE /100g, 180±1.4 mg GAE /100g and 450±14.14 mg GAE /100g respectively. The highest and lowest mean value of FRAP were Acetone (466 ±14.14 mg GAE /100g) and water (68±0.71 mg GAE /100g) respectively. The extract of Hexane has been showed high mean value of FRAP than Acetone. Ashraf et al., (2015) analyzed antioxidant activity in extract hexane and methanolic of *Eucalyptus camaldulensis.* Antioxidant activities determination in extract hexane and methanolic of *Eucalyptus camaldulensis* by FRAP assay. The samples were collated from Lathianwala, District Faisalabad, and Central Punjab, Pakistan. The mean values of FRAP activities was 148.68 and 39.13 mg GAE /100g for methanol and hexane respectively. The review clear show that the mean values of FRAP activities in *Eucalyptus Camaldulensis* high with the data reported from India, Lathianwala, District Faisalabad, and Central Punjab, Pakistan, while higher than the levels reported from Nepal.

Table 2:

Table 2: (mg GAE /100g) FRAP content in *Eucalyptus Camaldulensis* extracts

Plants	Water	Acetone	Hexane	Methanol	Overall Mean
Eucalyptus camaldulensis	68±0.71^{d}	466.5±0.7^{a}	180±1.4^{c}	450±14.14^{a}	291.25±6.61^{a}

The mg GAE /100g FRAP content water, acetone, hexane, and methanol extracts leave of *Eucalyptus Camaldulensis* graphically represented in fig No 2. The graph bar of acetone has been showed highest mg GAE /100g FRAP mean vales that other extract of Eucalyptus Camaldulensis (water, acetone and hexane).

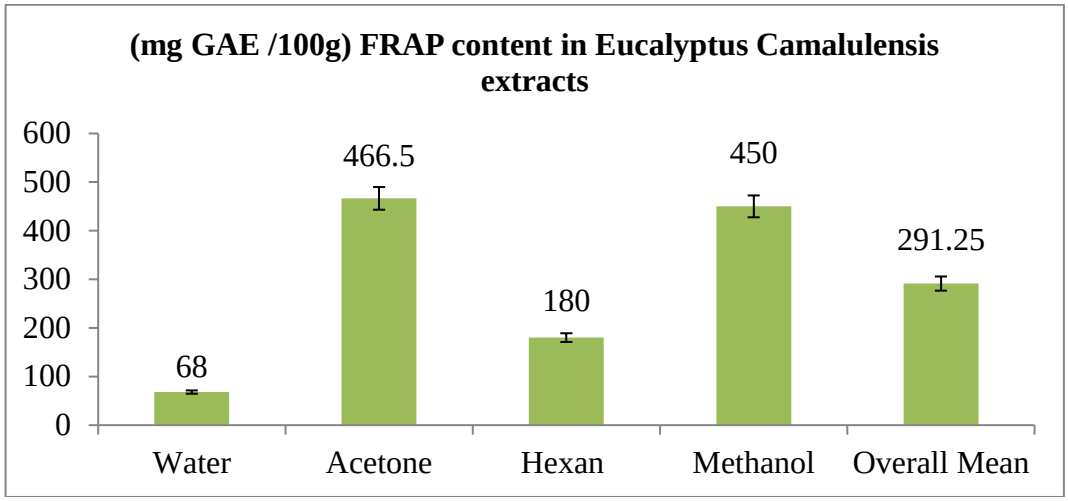

Fig 2: DPPH activity in water, methanol, acetone and hexane of *Eucalyptus camaldulensis*

2.1.3. Total Phenolic Contents (TPC)

Statistical analysis ($P < 0.05$) showed significant difference in the TPC contents of different extracts of *Eucalyptus Camaldulensis* (Table 4.9). The mean TPC contents in Eucalyptus camaldulensis with Water, Acetone, Hexane and methanol was 268.78±0.8 mg GAE /100g, 269.88±3.14 mg GAE /100g, 68.22±0.78 mg GAE /100g and 276±0.78 mg GAE /100g respectively (Table 3). The extract of methanol has been showed highest TPC content in *Eucalyptus Camaldulensis*, while extract of hexane has been lowest mean value of TPC in *Eucalyptus Camaldulensis*. The extract of water and acetone has been showed slight difference of TPC in *Eucalyptus Camaldulensis*. Ashraf et al., (2015) analyzed antioxidant activity in methanol and hexane extract of *Eucalyptuss camaldulensis.* The samples were collected from Lathianwala, District Faisalabad, and Central Punjab, Pakistan. TPC assay was performed for determination of total phenolic compound in extract of eucalyptus camaldulensis. TPC value of methanol and hexane extract of Eucalyptus camaldulensis was 148.68 and 39.13 mg GAE /100g respectively. Pakistani (Multan) extract of hexane and methanol has high TPC values than Pakistani (Faisalabad and Central Punjab) *Eucalyptus camaldulensis* due to the environment of Multan district are very hot than Faisalabad and Central Punjab, Pakistan. Sample preparation, physical properties of sample, polarity of solvents, spermine, solvents types, treatment, temperature, time and method of antioxidant extraction may be influenced the recovery, yield and type of phenolics present in examined extract.

Table 3:

mg GAE /100g TPC content in *Eucalyptus Camaldulensis* extracts

Plants	Water	Acetone	Hexan	Methanol	Overall Mean
Eucalyptus camaldulensis	268.78±0.8^{ab}	269.88±3.14^{ab}	68.22±0.78^{f}	276±0.78^{a}	220.72±1.11^{b}

The mg GAE /100g TPC content water, acetone, hexane, and methanol extracts leave of *Eucalyptus Camaldulensis* graphically represented in fig No 3. The graph bar of methanol has been showed highest mg GAE /100g FRAP mean vales that other extract of Eucalyptus Camaldulensis (water, acetone and hexane).

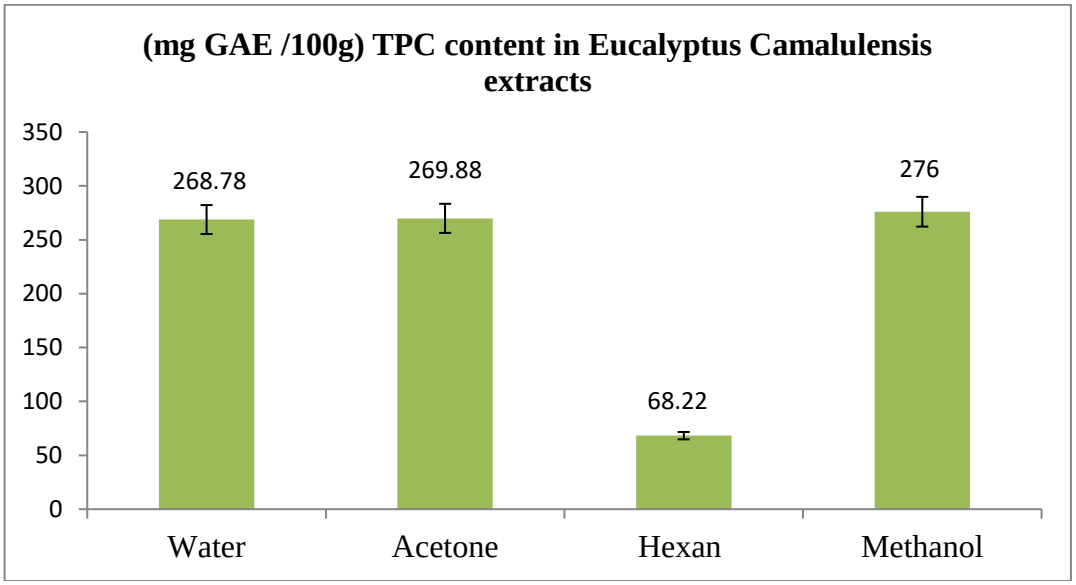

Fig 3: TPC activity in water, methanol, acetone and hexane of *Eucalyptus camaldulensis*

Thin Layer Chromatographic (TLC) Study for detection Aflatoxins via different extract leave of Eucalyptus *camaldulensis*

Degradation of aflatoxins by extracts of *Eucalyptus camaldulensis* was initially detected through TLC method showed in the fig 5. Researchers from different parts of the world have reported the initial screening for aflatoxins through TLC both for aflatoxins degradation and for aflatoxins detection. Uçar et al., (2016) analyzed degradation of aflatoxin by Indian medicinal plants (n=31). TLC method was used for the initial screening of medicinal plants having aflatoxins degradation potenital, while further verification was performed through HPLC method. The extract of *Adhatoda vasica Nees* showed highest potential for the degradation of AFB_1. Velazhahan et al., (2010) evaluated the aflatoxin G_1 degradation via using the extracts of 34 medicinal plants, the initial screening was performed through TLC method while the further verification was performed through ELISA method. *T. ammi* was found to have highest potential among medicinal plants 78 % degradation of aflatoxins. Iram et al., (2016) quantified the degradation of aflatoxins by the extracts of 2 Pakistani medicinal plants i.e. *Ocimum basilicum* and *Cassia fistula*. TLC method was used for detection of aflatoxins degradation while the quantification of percent degradation was performed through HPLC method. Degradation percentages of aflatoxins by *Ocimum basilicum* and *Cassia fistula* were recorded as 82.8–87.7% and 57–68.3%, respectively. The review of above data clearly indicates that the adoption of TLC method to investigate aflatoxins degradation for qualitative purpose only is adopted by the researchers from different parts of the world. Out of the ten medicinal plants that we used in this study only *Cassia fistula* was earlier used by Iram et al. (2016) and in line with her study we also found *Cassia fistula* to have aflatoxins degradation potential.

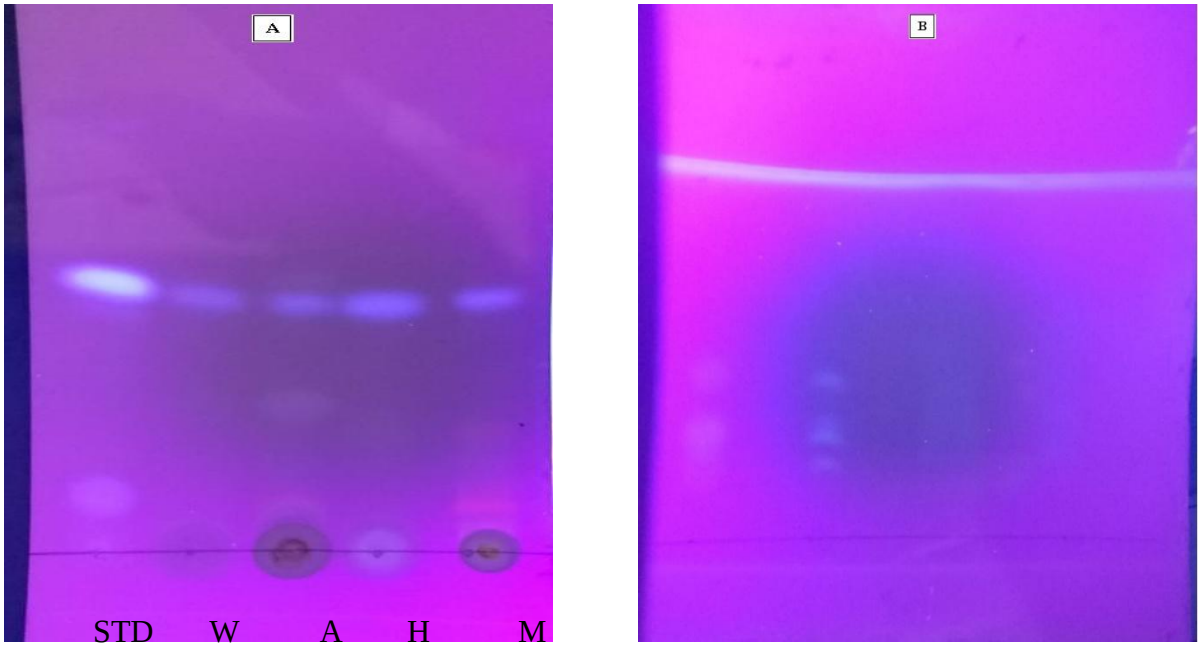

Fig 4: (A and B): Degradation of total aflatoxins by the extracts of *Eucalyptus Camaldulensis* detected via TLC (STD=Standard, W=water, A=Acetone, H= Hexane and M=Methanol)
*A Graph presenting the result of different extract *Eucalyptus Camaldulensis*
*B Graph presenting for as comparing with the result of A graph

2.2. High Performance Liquid Chromatography (HPLC) study for Qualification of Degradation of Aflatoxin via different extract leaves of *Eucalyptus camaldulensis*

Aflatoxin tested for degradation with the aqueous extract leaf of *Eucalyptus Camaldulensis* e.g. AFB_1 + AFB_2 + AFG_1 + AFG_2. HPLC technique is used for determination % removal or degradation of aflatoxins. The % quantified degraded aflatoxin has been shown in the table 4. The mean values % removal of aflatoxin was recorded as range 95.08-100 µg/kg. Scholars from different countries around the world have reported the verification for degraded aflatoxins by HPLC. Ponzilacqua et al., (2019) analyzed degradation of aflatoxins by extract of Brazilian medicinal plants (n=4). The mean percentages for degradation of aflatoxin were 49.0 ± 5.6% and 60.3 ± 2.9% using TLC and HPLC technique respectively. Rao et al., (2017) used the extract of Ethiopian Ageratum *conyzoides* for degradation of aflatoxin using TLC and HPLC method. The Mean value of AFB_1 was recorded as 7.47 ppb. In this review showed that HPLC is most useful for the quantification of aflatoxins. The extract of *Eucalyptus Camaldulensis* has been verified by HPLC method have been shown potential for degradation of aflatoxins. Comparing our results with the above reported studies, it can be stated that the potential in Pakistani medicinal plants (*Eucalyptus camaldulensis)* for degradation of AFB_1 AFB_2, AFG_1, AFG_2 and TAF are higher than Brazilian and Ethiopian medicinal plant

Table 4:

Degradation of different types of aflatoxins by extracts *Eucalyptus Camaldulensis* and verified by HPLC (µg/kg and removal %)

Plant	Level of Spiking (µg/kg)	AFB_1 (µg/kg)	AFB_2 (µg/kg)	AFG_1 (µg/kg)	AFG_2 (µg/kg)	TAF (µg/kg)	Removal %
Eucalyptuss camaldulensis	AFB_1 = 100 AFB_2 = 25 AFG_1 = 100 AFG_2 = 25	**3.05±0.29**	**2.15±0.17**	**4.21±0.25**	**2.88±0.20**	**12.29±0.09**	**95.08**

	TAF = 250						
Eucalyptuss camaldulensis	AFB_1 = 50 AFB_2 = 12.5 AFG_1 = 50 AFG_2 = 12.5 TAF = 125	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**	**100**

The level of spiking 50 µg/kg, 12.5 µg/kg, 50 µg/kg, 12.5 µg/kg and 125 µg/kg for AFB_1, AFB_2, AFG_1, AFG_2 and TAF respectively, then result showed 100 % removal of aflatoxin, on other hand when the level of spiking 100 µg/kg, 25 µg/kg, 100 µg/kg. 25 µg/kg and 250 µg/kg for AFB_1, AFB_2, AFG_1, AFG_2 and TAF respectively, then result showed 95.08 % removal of aflatoxins.

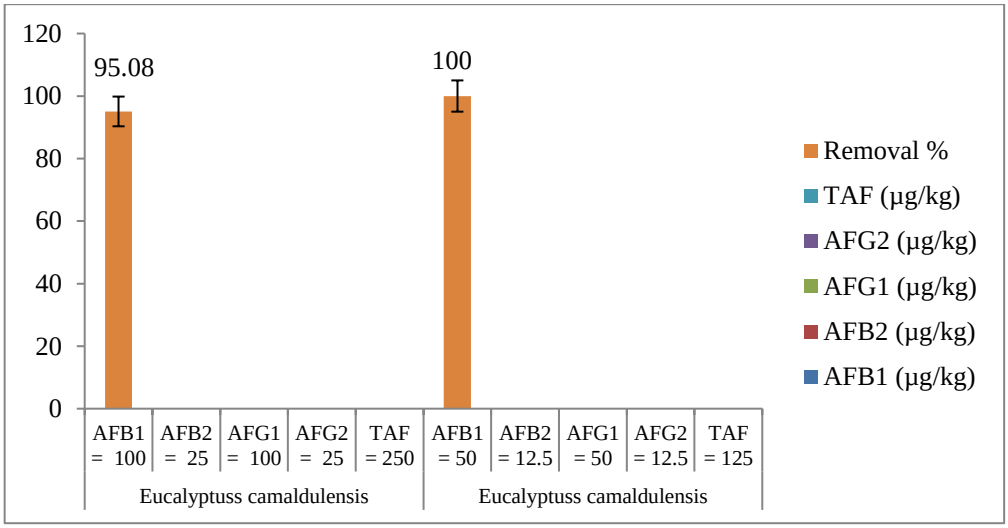

Fig 5: The graphically representing the removal % of aflatoxin (AFB1, AFB2, AFG1, AFG2 and TAF

Conclusion

In the current research the found out us concluded that different solvent such as water, acetone, hexane and methanol used for preparation extract with leaves of eucalyptus Camaldulensis. They showed scavengers antioxidant activities e.g. DPPH, FRAP and TPC, their activities further applied for the degradation of aflatoxin as detection and quantification using the TLC and HPLC respectively. It detected that extract of Eucalyptus Camaldulensis play very important role for the degradation of aflatoxin. However, it was also quantified the degradation of aflatoxin via extract of Eucalyptus Camaldulensis was 95.08-100 %. There is no conflict with other research.

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Author's Contributions

The writers of the original manuscript, Dr. Muhammad Naeem Zubairi, Dr. Muhammad Khurram Afzal and Talha Bin Iqbal were responsible for conceptualization, evaluating, and editing. Hafiz Muhammad Irfan Manzoor and Nayab Rao handled the formal analysis, research, funding procurement, reviewing, and editing. Resources, Naeem Sarwar and Liaqat Ali and data curation and oversight

Declaration of Conflicting Interests

There are no possible conflicts of interest that the authors have disclosed about the research, writing, or publication of this article.

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