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Evaluation of Various Solvent Extracts of *Eucalyptus globulus* Leaves on Aflatoxin Production by *Aspergillus flavus* Isolated from Pasta

Monira R. AL-Othman[1]*, Abeer R. M. Abd El- Aziz[1], Saleh A. Al-Sohaibani[1] and Mohamed A. Mahmoud[1,2]

[1]Botany and Microbiology Department, College of Science, King Saud University, Riyadh 1145, Kingdom of Saudi Arabia.
[2]Plant Pathology Research Institute, ARC, Giza, Egypt.

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The aim of this study was to isolate aflatoxigenic *Aspergillus flavus* from ten sample pasta in Riyadh (Saud Arabia) and evaluation of various solvent extracts of *Eucalyptus globulus* leaves on aflatoxin production. Six isolates (No. A2, A3, A6, A7, A9 and A10) of *Aspergillus flavus* expressed as positive for aflatoxin production while four isolates *Aspergillus flavus* expressed as negative by detection of aflatoxigenic isolates under UV radiation, whereas isolates no A6 was gave a negative result when estimating the aflatoxin by HPLC. The antiaflatoxin of Eucalyptus (*Eucalyptus globulus*) were extracts by three various solvents (Acetone – Ethanol – Methanol). Methanol and ethanol extract of *Eucalyptus globules* were more responsible for decrease aflatoxin production followed by acetone extract. In this study, Extraction by methanol was the best in separation many component by GC-MS followed by ethanol while extraction by acetone were recorded very few compounds. Photochemical analysis (Tannins, Glycosides, Reducing sugars, Anthraquinones, Phenolics and Alkaloids) of the leaves of *Eucalyptus globulus* using various solvents (acetone, ethanol and methanol) have been described in previous studies.

Key words: Pasta, *Aspergillus flavus*, antiflatoxin, *Eucalyptus globulus,* GC mass.

Durum wheat (*Triticum durum* desf.) is one of the most important cereal crops in the world, pasta is the most common product made from durum wheat, it is also used in the preparation of bulgur , noodles , Couscous , and various type of bread (Trocoli *et al*.,2000). Pasta has an excellent nutritional profile, it is a good source of complex carbohydrates and a moderate source of protein and some vitamins. For example, a two-ounce portion of dry pasta contains about 210 calories and is about 75 percent carbohydrate, 13 percent protein, and 1.5 percent fat. (Anonymous, 1997). The foods most susceptible to aflatoxin contamination are locally produced or imported cereals such as wheat. its consumption in the form of couscous, pasta, macaroni, spaghetti, bread and frik is a cultural tradition. The mycobiota of wheat and wheat products was found to be dominated by *Aspergillus* section *Nigri* and *Flavi* species (Riba *et al.,* 2008; Pildain *et al*., 2008). Some species of the genus produce secondary metabolites as aflatoxins which are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus.* Common aflatoxins produced in foods are aflatoxins B1, B2, G1 and G2, it's affected by temperature, pH, water activity and nutritional factors (Pitt and Hocking, 1985). Flour as the preliminary material for the pasta production can be contaminated with aflatoxin producing fungi (Halt, 1989, Gallo *et al*., 2008, Othman and Al-Delamiy, 2012).

* To whom all correspondence should be addressed.
E-mail: Monira_alothman@yahoo.com

MATERIALS AND METHODS

Collection of Samples

Ten samples of pasta were collected randomly from different markets in Riyadh, Saudi Arabia, during 2012.

Fungal isolation

The pasta were disinfected using 2% sodium hypochlorite for two minutes, rinsed three times in sterile distilled water and dried between layers of sterile filter paper (Whatman No. 1). Then, three grains were placed randomly onto potato dextrose agar (PDA) and incubated at 25 °C for 7 days. The isolates obtained were purified by the single-spore method and then transferred to PDA slants. The fungal isolates were identified based on their morphological and microscopic characteristics according to the method proposed by Dugan (2006). The identification of isolates were confirmed by Regional Center of the Fungi and their Applications, Al-Azhar University, Cairo, Egypt.

Detection of aflatoxigenic isolates under UV radiation

The culture media used were Cazpek's agar, potato dextrose agar and YES agar, were incubated at 28°C for 4 days in darkness. The presence or absence of fluorescence in the agar surrounding the colonies assayed was determined under UV radiation (365 nm) and expressed as positive or negative according to Franco *et al*., (1998).

Preparation of solvents plant extracts

A total weight of 100g of the dried *Eucalyptus globulus* leaves were soaked separated in each three tested solvents (Acetone – Ethanol - Methanol) for 48h., then the flask was shaken for 30 min. and finally filtered. The solvent was evaporated under temperature not exceeding 50c. yielded extracts were used as crude gum weighted and redissolved again in the same solvent was used (1gm crude extract/20 ml solvent) to give concentration 20%. (Abd-Rabboh, 2000; Abdel-Rahman, 2001).

Yield extract

The percentage extract yield was estimated according to Parekh and Chanda (2007) as: Dry weight / Dry material weight × 100

Aflatoxin inhibition

Ten ml from different concentrations of each solvents *Eucalyptus globulus* leaves (10, 15 and 20%) were prepared separately and added to 90 ml SMKY liquid medium (sucrose, 20 g; magnesium sulfate, 0.5 g; potassium nitrate, 3 g; yeast extract, 7 g and distilled water, 1000 ml) **,**the flasks were inoculated with discs of 6 mm diameter of the toxigenic *A. flavus* and incubated at 25 °C for 7 days. and the control set was kept parallel to the treatment without extract. After incubation, content of each flask was filtered (Whatman, No. 1), then the filtrates of each flask were treated three times with 50 ml of chloroform in a separating funnel. The chloroform extract was separated and dehydrated with anhydrous sodium sulfate and evaporated till dryness on water bath at 50°C under vacuum. The residues were dissolved in 10 ml methanol (Mostafa *et al*., 2011)

High -Performance Liquid Chromatography (HPLC)

The toxins were measured using high-performance liquid chromatography (HPLC) (model PerkinElmer series 200 UV/VIS) with a C18 column with an internal diameter of 3.9 x 300 mm. The HPLC was providing with an UV detector and fluorescence detection with 365nm excitation and 430nm emission wavelengths. The liquid mobile phase yielded results of methanol:acetic acid:water (20:20:60 v/v/v). The total run time for the separation was approximately 30 min at a flow rate of 1 ml/min (Christian, 1990). The aflatoxin inhibition was calculated as follows: percentage of inhibition toxin = [A – a / A] x 100, where" A" is the concentration of aflatoxin in the treated sample and "a" is the concentration of aflatoxin in the control (Mostafa et al., 2011).

GC/GC-MS analysis of *Eucalyptus globulus* leaves which extracted with acetone, ethanol and methanol

Acetone, ethanol and methanol extracts of *Eucalyptus globulus* leaves were analyzed according to the method described by Priyanka *et al.* (2009) through gas chromatography (model PerkinElmer clarus 500) equipped with a flame ionization detector, and quantitation was carried out by the area normalization method neglecting response factors. The analysis was carried out using a VF-5MS capillary column (30 m x 0.25 mm; 0.25 ìm film thickness). The operating conditions were as follow: injection and detector temperature, 250 and 300°C respectively; split ratio, 1: 50; carrier

gas, Helium with flow rate (1.0 ml/min). Oven temperature programme was 50 to 300°C at the rate of 7°C/min. Mass spectrometer conditions were: ionization potential, 70 eV; mass range from, 40 to 400 m/z; electron multiplier energy, 2000 V. The components of plant extracts were identified by comparison of their relative retention times and the mass spectra with those authentic reference compound shown in the literature and by computer matching of their MS spectra with Wiley and Nist mass spectral library.

Phytochemical analysis of *Eucalyptus globulus* leaves using various solvents

Phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, anthraquinones, glycosides, reducing sugars, phenolic compounds and tannins by the following procedure.

Test for Alkaloids

The extract of *Eucalyptus globulus* leaves with three tested solvents were evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent (Siddiq and Ali, 1997). The samples were then observed for the presence of turbidity or yellow precipitation (Evans, 2002).

Test for Glycoside

To the solution of the extract in glacial acetic acid, few drops of Ferric chloride and concentrated sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer (Siddiq and Ali, 1997).

Test for Reducing Sugars

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate. (Sakthi and Geetha, 2011).

Test for Phenolic Compounds

300 mg of extract was diluted in 5 ml of distilled water and filtered. To the filtrate, 5% Ferric chloride was added and observed for dark green colour formation. (Sakthi and Geetha, 2011).

Test for Tannins

To 0.5 ml of extract solution, 1 ml of water and two drops of ferric chloride solution were added. Green black for catecholic tannins and blue colour was observed for gallic tannins (Iyengar, 1995).

Test for Anthraquinones

0.5g of the extract was shaken with 10 ml of benzene, then 5 ml of 10 percent ammonia solution added to the filtrate then, the mixture was shaken. The presence of a pink, red or violet colour indicated the presence of anthraquinones (Sofowora, 1993).

RESULTS AND DISSCUSION

Three culture media included Cazpek's agar, Potato dextrose agar (PDA) and yeast extract sucrose agar (YES agar) were used to screen examined for aflatoxin production. Production of aflatoxins was readily detectable by direct visualization of a beige ring surrounding colonies after four days from incubation time. The presence or absence of fluorescence in the agar surrounding the colonies assayed was determined under UV radiation (365 nm) and expressed as positive or negative . Data in table (1) show that six isolates of *Aspergillus flavus* expressed as positive for

Table 1. Detection of aflatoxigenic isolates under UV radiation

No. of isolates	Culture media		
	Cazpek's agar	Potato dextrose agar	YES agar
1	-	-	-
2	+	+	+
3	+	-	+
4	-	-	-
5	-	-	-
6	-	-	+
7	+	-	+
8	-	-	-
9	+	-	+
10	-	-	+

Table 2. Effect of various solvents on yield extracts of *Eucalyptus globulus* leaves

Solvents	Yield extract (%)
Acetone	8. 2
Ethanol	10.1
Methanol	13.4

Table 3. Effect of different solvents of crude extract of *Eucalyptus globulus* leaves on aflatoxin produced by *A. flavus*

No. of isolates	Control	Conc. %	B1(ppb) A	% inhibition	E	% inhibition	M	% inhibition	B2(ppb) A	% inhibition	E	% inhibition	M	% inhibition
A2	20	10	17	15	12	40	9	55	14	30	10	50	9	55
		15	12	40	9	55	7	65	12	40	7	65	8	60
		20	9	55	7	65	7	65	11	45	6	70	6	70
A3	19	10	18	5.3	16	15.8	14	26.3	17	10.5	15	21.1	13	31.6
		15	17	10.5	13	31.6	13	31.6	14	26.3	14	26.3	12	36.8
		20	17	10.5	12	36.8	10	47.4	14	26.3	12	36.8	9	52.6
A7	17	10	16	5.9	12	29.4	12	29.4	14	76.5	11	35.2	9	41.2
		15	14	17.6	8	52.9	7	58.8	10	41.2	9	47.1	7	58.8
		20	11	35.3	5	70.6	5	70.6	9	47.1	8	52.9	6	64.7
A9	15	10	9	40	8	46.7	7	53.3	11	26.7	8	46.7	9	40
		15	7	50.3	6	60	5	66.7	9	40	7	53.3	6	60
		20	6	40	5	66.7	3	80	7	53.3	4	73.3	4	73.3
A10	9	10	7	22.2	6	33.3	6	33.3	9	0.0	7	22.2	6	33.3
		15	5	44.4	3	66.7	2	77.7	6	33.3	3	66.7	3	66.7
		20	3	66.7	4	55.6	0.0	100	4	55.6	2	77.8	2	77.8

A: Acetone, E:Ethanol, M:Methanol

aflatoxin production while four isolates *Aspergillus flavus* expressed as negative.

Yield extract (%)

The total amount of crude extract obtained with the different solvents showed that methanol was quantitatively the best yield extract was 13.4% followed by acetone 8. 2 and ethanol 10.1%. This result agreement with Abubakar (2010).

Effect of various solvents of crude extract of *Eucalyptus globulus* leaves on aflatoxin produced by *A. flavus* by HPLC

Table 3 reveals that effect of various solvents of crude extract of Eucalyptus globulus leaves on aflatoxin produced by *A. flavus* at three concentrations were isolates from ten kinds of pasta. Isolates no A2, A3, A6, A7, A9 and A10 expressed as positive for aflatoxin production by UV detection, whereas isolates no A6 was gave a negative result when estimating the aflatoxin by HPLC. This is due to the use UV for detection aflatoxin production is inaccurate. Three tested solvents were effective on aflatoxin production by *A. flavus* at three concentrations. Methanol and ethanol extract of *Eucalyptus globulus* were more responsible for decrease aflatoxin production followed by acetone extract. Extraction of *Eucalyptus globulus* with methanol prevented aflatoxin synthesis at 20% concentration for B1 was production by isolate no. A10. Fungitoxicity of plant extracts against the toxigenic *A. flavus* may be due to constituents (Cavaleiro *et al*., 2006). The anti-fungal properties of these extracts may also be due to their phytochemical contents which caused inhibitory effects alkaloids, phenols, glycosides, steroids, essential oils and tannins (Rasooli *et al*., 2009). The antimicrobial activity of *E. globulus* may be due to the presence of a mixture of monoterpenes and oxygenated monoterpenes (Vratnica *et al*., 2011) The antiaflatoxigenic actions of essential oil may be related to inhibition of the ternary steps of aflatoxin biosynthesis involving lipid peroxidation and oxygenation (Bluma *et al*., 2007). Many inhibitors of aflatoxin biosynthesis may be due to: change environmental and physiological factors affecting aflatoxin biosynthesis, inhibit signaling transduction of biosynthetic pathway, or directly inhibit gene expression or enzyme activity in the pathway. (Holmes *et al.,* 2008). Synthesis of aflatoxins is control by specific enzymes which are expressed by DNA through many steps. Each step in gene expression can be inhibited by natural plant products (Trail *et al*., 1995).

Difference of extracting compounds from plants may be dependent on the type of solvent was used (Masoko *et al*., 2007). The percentage of each component differs from solvent to another due to their different solubility in each one (Bakkour *et al*., 2011).

Evaluation of three solvents (acetone, ethanol and methanol) for extraction main component by GC-MS from *Eucalyptus globulus*

Show that evaluation of three solvents (acetone, ethanol and methanol) for extraction main component by GC-MS from *Eucalyptus globulus* according to their retention time (Rt) and Percentage of area. In this study, Extraction by methanol was the best in separation many component followed by ethanol (Masoko *et al*., 2007; Ezekiel *et al*., 2009) while extraction by acetone were recorded very few compounds. 21 compounds characterized methanol extract of *Eucalyptus globulus*, the major constituents was 1.8-Cineole (28.14%) in agreement with other observations (Yu-Chang *et al*, 2006; Batish *et al*., 2008; Song *et al*., 2009; derwich *et al*., 2009; Ouazzou *et al*., 2011; Subramanian *et al*., 2012) other components present in appreciable contents were Globulol (19.25%), Alpha pinene (14.22%), Limonene (6.56%), Valencene (6.33%), β-Eudesmol (5.30%) and Solanone (5.20%) .15 compounds characterized ethanol extract of Eucalyptus globulus, the major constituents was 1.8-Cineole (28.14%) other components present in appreciable contents were Globulol (19.21%), Alpha pinene (15.12%), Geraniol (10.21%) and Limonene (5.34%) while extraction by acetone were recorded 12 compounds1.8-Cineole (22.28%) Globulol (15.20%) and Geraniol (8.50%). The variations may be due to the polarity of solvents which determines solubility of compounds. (Thompson *et al*., 2011). The different components is probably due to plant species, climate, soil composition, part used and age of the plant. (De Paula *et al*., 2004).

Photochemical analysis of the leaves of *Eucalyptus globulus* using various solvents (acetone, ethanol and methanol) have been described in previous studies. Data in table (4) show that tannins, reducing sugars and phenolics were found in Eucalyptus *(Eucalyptus globulus)*

Table 4. Chemical composition of three solvents leaf crude extract of *Eucalyptus globulus* leaves

Compounds	*Eucalyptus globulus*	
	Rt (min)	Relative concentration
acetone		
Limonene	27.311	5.0
Geraniol	26.295	8.30
α-Eudesmol	26.201	4.15
Globulol	23.244	15.20
Aristolene	21.456	1.14
aromadendrene	20.897	3.58
naphthalene	20.458	2.80
Trans-pinocarveol	13.938	2.32
Eucalyptol (1,8cineole)	11.290	22.28
Ethanol	6.570	3.22
Pentanol	3.514	1.63
9,12-Octadecadienoic acid	3.112	1.14
Ethanol		
epiglobulol	29.231	0.09
Limonene	27.311	5.34
naphthalenemethanol	23.919	1.74
caryophallen	23.444	3.20
Globulol	23.242	19.21
Solanone	21.560	6.31
aromadendrene	20.458	2.89
Trans-pinocarveol	13.938	2.54
Eucalyptol (1,8Cineol)	11.290	34.55
2,6,10 dodecatrien-1-ol-3,7,10 trimethyl	22.798	2.14
Geraniol	26.295	10.21
cyclohexanol	15.804	1.54
α terpinol	15.057	2.16
α-Pinocarvone	13.938	4.30
α-Pinene	8.845	15.12
Methanol		
Limonene	27.323	6.56
β-Guaiene	26.854	2.36
β-Eudesmol	26.121	5.30
Epiglobulol	24.740	4.34
Cymene	23.601	0.24
Globulol	23.278	19.25
Valencene	22.952	6.33
β-Phellandrene	22.851	0.19
5 acetoxymethyl-2,6,10 trimethyl-2,9-undecadien-6-ol	22.534	1.26
Solanone	21.412	5.20
Cycloprop azulene (aromadendrene)	20.902	3.68
Sabinene	20.451	1.11
methanoazulene	20.458	2.44
α -Terpineol	15.714	2.87
pinocarveol	14.0	0.45
Trans pinocarveol	13.938	2.12
linalool	12.632	0.13
Eucalyptol(1,8Cineol)	11.290	28.14
Alpha pinene	8.845	14.22
β-pinene	8.152	0.52
Camphene	6.526	0.77

Table 5. Phytochemical analysis of three solvents extract of *Eucalyptus globulus*

Constituent	Ethanol	Methanol	Acetone
Tannins	+	+	+
Glycosides	-	-	-
Reducing sugars	+	+	+
Anthraquinones	-	-	-
Phenolics	+	+	+
Alkaloids	+	+	-

"+"present, "–"absent

while glycosides and anthraquinones were absent when extracted by any three tested solvents such as acetone, ethanol and methanol. This result agree with (Ahmad and Beg 2001; Egwaikhide *et al*., 2007; Selvamohan *et al*., 2010; Yadav *et al*., 2010) on the other side, Alkaloids were presence in methanol extract and ethanol extract, while absent in acetone extract. In many reports, ethanol or methanol is used for alkaloids extraction (Kumaraswamy *et al*., 2008; Ezekiel *et al*., 2009). Antibacterial properties of plant extracts may be attributed to secondary metabolites (Edeoga *et al*., 2005; Okoli and Iroegbu 2005). Antimicrobial action of tannin may be related to their ability to inactivate microbial adhesions, enzymes, cell envelops transport proteins (Ya *et al*., 1988).

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REFERENCES

1. Abd- Rabboh, M.S.Studies on stalk rot disease of maize. M.Sc. Thesis. Fac. Agric., Menofiya Uni, 2000; pp 164.
2. Abdel-Rahman, Saida, S. Further studies on soybean anthracnose disease in Egypt. Ph. D. Thesis, Fac. Agric., Cairo Uni, 2001; pp 147.
3. Abubakar, E. Antibacterial potential of crude leaf extracts of *Eucalyptus camaldulensis* against some pathogenic bacteria, *African Journal of Plant Science*, 2010; **4**(6): 202-209.
4. Ahmad I., Beg, A. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human. *Pathogens Journal of Ethnopharmacology*, 2001; **74**: 113-123.
5. Anonymous . Data compiled by National Pasta Association, *Pasta Journal*, 1997; **79** 12–18.
6. Bakkour, Y., Kassir, S., Kanj, D., El-Omar F., Mouneimne Y. Analysis of the essential oils of *Salvia Libanotica* and *Origanum Syriacum*. *Journal of Natural Products*, 2011; **4**: 51-56.
7. Batish, D.R., Singh, H.P., Kohli, R.K. , Kaur, S. Eucalyptus essential oil as a natural pesticide, *Forest Ecology and Management,* 2008; **256**: 2166-2172.
8. Bluma R., Amaiden, M.R. Daghero J., Etcheverry, M. Control of *Aspergillus* section Flavi growth and aflatoxin accumulation by plant essential oils. *J. Appl. Microbiol*., 2007; **1723**: 1364-5072.
9. Cavaleiro, C., Pinto, E. Goncalves, M.J., Salguerio, L. Antifungal activity of Junipers essential oils against dermatophytes, *Aspergillus* and *Candida* strains. *J. Appl. Microbiol*., 2006; **100**: 1333-1338.
10. Christian, G. HPLC Tips and Tricks. Great Britian at the Iden Press, Oxford. 1990;pp 608.
11. De Paula, J.P., Farago, P.V., M.Checchia, L.E., Hirose, K.M. ,. Ribas, J.L.C. Effect of *Punica granatum* flowers: Effect on hyperlipidemia, pancreatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. *Food Chem. Toxicol*., 2004, **47**: 50-54.
12. Derwich E., Benziane Z., Boukir, A. GC/MS Analysis of Volatile Constituents and Antibacterial Activity of the Essential Oil of the Leaves of *Eucalyptus globulus* in Atlas Median from Morocco. *Advances in Natural and Applied Sciences*, 2009; **3**(3): 305-313.
13. Dugan F.M. The identification of fungi: an illustrated introduction with keys, glossary and guide to literature. American Phytopathological Society, 2006;Pp. 179.
14. Edeoga, H.O., Okwu, D.E. , Mvaebie, B.O. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol*., 2005;4: 685 688.
15. Egeaikhide, P. A., Okeniyi, S.O., Gimba, C.E. Screening for anti microbial and phytochemical constituents of some Nigerian medicinal plants. *Advances in biological research*, 2007; **1**(5-6): 155-158.
16. Evans, W.C. Trease and Evan's Pharmacognosy. 5th edition, Haarcourt Brace and Company: 2002; 336.
17. Ezekiel, C.N., Anokwuru, C.P., Nsofor, Odusanya,E.O.A. , Adebanjo, O. Antimicrobial activity of the methanolic and crude alkaloid extracts of *Acalypha wilkesiana* cv. macafeeana

copper leaf. *Res. J. Microbiol*., 2009; **4**: 269-277.

18. Franco, C.M., Fente, C.A., Va´zquez, Cepeda, B.I., Mahuzier , A.G., Prognon, P. Interaction between cyclodextrins and aflatoxins Q1, M1 and P1: fluorescence and chromatographic studies. *J. Chromatogr*., 1998; **815**: 21-29.
19. Gallo G., Lo Bianco, M., Bognanni, R., Saimbene. G. Mycotoxins in Durum Wheat Grain: Hygienic-Health Quality of Sicilian Production. *Journal of food science*, 2008; **73**(4): 42-47.
20. Othman G.A., Al-Delamiy, K.S. Aflatoxin b1 production by *Aspergillus flavus* in different media and containers and the antifungal activity of garlic and black cumin. *Research Journal in Engineering and Applied Sciences*, 2012; **1**(2): 117-121.
21. Halt, M. Microflora of flour with a special emphasis on aflatoxin-producing fungi. *Znan. Prak. Poljopr. Tehnol*., 1989; **19**: 397-413.
22. Holmes, R.A. , Boston, R.S., Payne, G.A. Diverse inhibitors of aflatoxin biosynthesis. *Appl. Microbiol. Biotechnol*. 2008; **78**: 559-572.
23. Iyengar, M.A. Study of drugs. 8th edition, Manipal Power Press, Manipal, India: 1995; 2.
24. Kumaraswamy M.V., Kavitha H.U. , Satish, S. Antibacterial evaluation and phytochemical analysis of *Betula utilis* D. Don against some human pathogenic bacteria. World *J. Agric. Sci.*, 2008; **4**: 661-664.
25. Masoko, P., Picard, J., Eloff, J.N. The antifungal activity of twenty four Southern African *Combretum* species (*Combretaceae*). *S. Afr. J. Bot.*, 2007; **73**: 173-183.
26. Mostafa, A.A., Al-Rahmah, A.N. , Abdel-Megeed, A. Evaluation of some plant extracts for their antifungal and antiaflatoxigenic activities. *J. of Medicinal Plants Res.*, 2011; **5**(17): 4231-4238.
27. Okoli, S. Iroegbu, C.U. *In vitro* antibacterial activity of *Syncliasa scarbrida* whole root extracts. *Afr. J. Biotechnol.*, 2005; **4**: 946 952.
28. Ouazzou, A., Lorán , S. Bakkali, M., Laglaoui, A., Rota, C., Herrera, A., Pagán, R., Conchello, P. A., Lorán, S., Bakkali, M., Laglaoui, A., Rota, C., Herrera, A., Pagán, R., Conchello, P. Chemical composition and antimicrobial activity of essential oils of Thymus algeriensis, Eucalyptus globulus and Rosmarinus officinalis from Morocco. *J. Sci. Food Agric.,* 2011; **91**(14): 2643-51.
29. Parekh J., Chanda, S. *In vitro* antimicrobial activity of *Trapa natans L. fruit rind* extracted in different solvents. *Afr. J. Biotechnol.*, 2007; 6(16): 1905-1909.
30. Pildain, M.B., Frisvad, J.C. Vaamonde,C., Cabral, G. ,Varga, D.J. , Samson,R.A. Two novel aflatoxin-producing *Aspergillus* species from Argentinean peanuts. *Int. J. Syst. Evol. Microbiol*., 2008; **58**: 725-735.
31. Pitt J. , Hocking A. Fungi and food spoilage. Academic press, Australia, 1985;
32. Priyanka B, Mohd, A. Vidhu, A. Malay, B., Shahnaz, S. Antidiabetic Atividade repelente do o´ leo essencial de Ocimum selloi Benth (variedade eugenol) contra o Anopheles braziliensis Chagas. *Acta Farm. Bonaerense*., 2009; **23**: 376-378.
33. Rasooli, I. , Fakoor, M. K., Allameh, A.A., Rezaee, M.B., Owlia, P. Phytoprevention of aflatoxin production. *J. Med. Plants*, 2009; **8**(supplement 5): 97-104.
34. Riba, A., Mokrane, S., Mathieu, F. Lebrihi ,B. , Sabaou, A. N. Mycoflora and ochratoxin a producing strains of *Aspergillus* in Algerian wheat. *Int. J. Food Microbiol*, 2008; **122**: 85-92.
35. Sakthi S , Geetha, M. Pharmacological screening of *datura metel* and *acalypha indica* for its antifungal activity against pathogenic fungi. *International journal of pharmaceutical science and health care,* 2011; **1**(2): 15-30.
36. Selvamohan, T., MarakathaValli, T. Sujitha. S. Antimicrobial activity and phytochemical studies on some Indian medicinal plants against selected human pathogens. *Der Pharma Chemica*, 2010; **2**(5): 38-45.
37. Siddiq A.A., Ali, M. Practical pharmaceutical chemistry. First edition, CBS Publishers and distributors, New Delhi: 1997; 126-131.
38. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria, 1993; 151-153.
39. Song, A. , Wang. Y. Study on the chemical constituents of the essential oil of the leaves of *Eucalyptus globulus* Labill from China Yanmei Liu. *Asian Journal of Traditional Medicines*, 2009; **4**(4): 134-140.
40. Subramanian, P.A., Gebrekidan A. , Nigussie K. Yield, contents chemical Composition Variations in the essential oils of Different *Eucalyptus globulus* trees from Tigray, Northern Ethiopia. *Journal of Pharmaceutical and Biomedical Sciences*, 2012; **17**(11): 1-6.
41. Thompson, S., Ashok A. ,. Sukesh, K. A study on the antibacterial effect of selected medicinal plants of Western Ghats against dental caries bacteria. *International Journal of Phytomedicine*, 2011; **3**: 416-421.
42. Trail, F., Mahanti N., Linz,. J. Molecular biology of aflatoxin biosynthesis. *Microbiology*, 1995;

141: 755-765.

43. Troccoli , A., Borrelli ,G.M., De – vita , P., Fares, C., C , Di – fonzoet, N . Mini review: Durum wheat quality, a multidis ciplinary concept. *J. Cereal Sci,* 2000; **32**: 99-113 .
44. Vratnica1, B., Dakov, T. Šukoviæ, D., Damjanoviæ, J. Antimicrobial Effect of Essential Oil Isolated from Eucalyptus globulus Labill. from Montenegro. *Czech J. Food Sci*., 2011; **29**(3): 277-284.
45. Ya, C., Gaffuey, S.H., Lilley, T.H., Haslam, E. Carbohydrate polyphenol complexation, Chemistry and significance of condensed tannins, Plenum Press: New York. 1988.
46. Yadav, P., Kumar, A. Mahour, K., Vihan, V.S. Phytochemical analysis of some indiegenous plants potent against endoparasite. *J. Adv. Lab. Res. Biol.,* 2010; **7**(1): 132-140.
47. Yu-Chang, S., Lung-Ho, C., Wang, E.I.C., Chang, S.T. Antifungal Activities and Chemical Compositions of Essential Oils from Leaves of Four Eucalypts, *Taiwan J. Sci*., 2006; **21**(1): 49-61.