International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Antioxidant Activity, Total Phenolic, Flavonoid and Tannin Contents of Callus and Seeds Extracts of Fenugreek (*Trigonella foenum-graecum* L.)

Ammar M. A. Ali[1], Mawahib E. M. ElNour[2]

[1,2]Department of Biology and Biotechnology, Faculty of Science and Technology, AL Neelain University, Sudan

Abstract: ***The research aimed to study antioxidant activity, total phenolic, flavonoid and tannin contents of methanolic extracts of callus and seeds and petroleum ether extract of seeds of fenugreek (Traigonella foenum-graecum). Callus was induced from hypocotyls and cotyledons explants in MS medium supplemented with combination of 2 mg/l of different auxin (2, 4-D or NAA) + 0.5 mg/l Kinetin. Free radical scavenging activity was evaluated by DPPH method. The highest scavenging activity was obtained from cotyledons derived callus (91.5±0.16%) followed by hypocotyls derived callus (85.46±0.29%) and methanolic extract of seeds (80.53±0.01%). Petroleum ether extract of seeds demonstrated weak antioxidant activity with inhibition percentage 24.56±0.12%. Significant differences ($p<0.05$) were detected among different antioxidant potentials of T. foenum-graecum callus and seeds extracts. IC_{50} values of DPPH scavenging capacity of methanolic extracts were evaluated in descending order, plant seeds (1.1185mg/g)>hypocotyls derived callus (0.7159mg/g)>cotyledons derived callus (0.4914mg/g)> ascorbic acid (0.1874mg/g). A lower value of IC_{50} indicates a higher antioxidant activity. Highest phenolic content were observed in callus of cotyledons (412.087 mg/l), compared to callus of hypocotyls (211.1937 mg/l) and methanolic extract of seeds (124.84 mg/l) calculated as mg/l gallic acid equivalent of phenols. Methanolic extract of seeds showed highest level of flavonoid content (424.951 mg/l), compared to callus of cotyledons (217.285 mg/l) and callus of hypocotyls (95.92 mg/l) calculated as mg/l quercetin equivalent of flavonoids. Tannins content were detected only in methanolic extract of seeds (116.259 mg/l calculated as tannic acid equivalent of tannins).***

Keywords: *Trigonella foenum-graecum L., Flavonoids and phenolic compounds, Antioxidant activity, Callus*

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.,) belongs to family *Fabaceae* (*Leguminosae*). It is annual crop mainly grown for use as a spice in many parts of the world [1]. Herbs and spices have been extensively used as food additives for natural antioxidants. Spices and aromatic herbs are considered to be essential in diets or medical therapies for delaying aging and biological tissue deterioration [2]. *T. foenum-graecum* leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses [3]. The medicinal value of plants lies in some chemicals active substances that produce a definite physiological action on the human body [4].

Secondary metabolites produced in plants are low molecular weight natural products [5]. Flavonoids and phenolic compounds are widely distributed in plants that have been reported to exert multiple biological effects including antioxidant, free radical scavenging, anti-inflammatory, and anticarcinogenic [6]. *T. foenum-graecum* categorized as one of Asian vegetables which contain high phenolic contents with very high antioxidant activity [7].

Plant cell cultures are an attractive alternative source to whole plant for the production of high value secondary metabolites [8];[9]. *In vitro* propagated callus cultures can become an alternative to plants grown in their environment due to the fact that under controlled condition, plant tissue can produce significant amounts of metaboliets of interest [10]. However, a considerable progress has been made to stimulate production and accumulation of secondary metabolites using plant cell cultures [11]; [12].

Several strategies have been adopted for the enhancement of bioactive metabolite production in *in vitro* cultures; one of them is using growth regulators which are often a crucial factor in secondary product accumulation [13]. The type and concentration of auxin or cytokinin or the auxin/cytokinin ratio may alter dramatically both the growth and the product formation in cultured plant cells [14]. Therefore, it is of great interest to evaluate the antioxidant activity and poly phenolic compounds of *T.foenum graecum* callus and seeds. It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds [15].

2. Materials and Methods

2.1 Plant Materials

The mature seeds of *T. foenum-graecum* were purchased from local market of Khartoum city, Sudan

2.2 Seeds Surface Sterilization and Germination

Seeds of *T. foenum-graecum* were surface sterilized in 70% ethanol for 30 sec with hand shaking and rinsed 3 times in sterile distilled water to remove trace of alcohol, then seeds were soaked in 20% Clorox (0.5% free chlorine) with two drops of Tween-20 for 15 minute, and rinsed 3-5 times in sterile distilled water. After surface sterilization, the seeds were directly cultured in the germination basal medium MS [16] at 25±2°C and photoperiod of 16 hrs light and 8 hrs dark for 10 days.

2.3 Callus Induction

The hypocotyls and cotyledons were used as explants of fenugreek (*T. foenum-graecum*) in this study for callus induction. To induce callus from explants, MS medium was supplemented with 2mg/l of different auxin (2,4-D or NAA) + 0.5 mg/l Kinetin. Each of the sterilized explants were cut into 2-3 mm pieces using sterile scalpel. Four pieces were inoculated in each vial containing sterile culture MS medium with different combinations of growth regulators. The calli were incubated for 6 weeks in 16 hrs light and 8 hrs dark at 25±2°C, and tissues were subculture at three week intervals.

2.4 Preparation of Plant Crude Extract

20 gm of dry *T. foenum-graecum* seeds were cleaned and ground using a mortar and pestle. The extraction was carried out by soxhlet method. The fine powder was packed tightly in a soxhlet extractor and petroleum ether 200 ml was used as solvent for extraction. The process was carried out for 6 hrs. The fine powder was re-extracted under the same conditions by methanol. The obtained extracts were evaporated by Rot-evaporator under reduced pressure at 60°C to get a dried solid product then stored in dried bottles.

2.5 Preparation of Callus Crude Extracts

This is done in a fashion similar to that of plant extraction except the callus was dried at first by freeze drying using Freeze dryier and then powdered and extracted with two different solvents, petroleum ether and methanol in soxhlet apparatus.

2.6 Antioxidant Activity

2.6.1 Thin Layer Chromatography (TLC) Analysis Antioxidant Constituents:

The antioxidant constituents were analysed using thin layer chromatography (TLC) followed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) technique [17]. The plates were developed in Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26 v/v/v/v) [18], then the surface of developed TLC plates were sprayed with 0.05% of DPPH solution in methanol. The active antioxidant constituents of the extracts were detected as yellow spots produced via reduction of DPPH by resolved bands against purple back ground on the TLC plates.

2.6.2 DPPH Free Radical Scavenging Activity:

The antioxidant activity of the seeds or callus extracts and the standard (Ascorbic acid) were assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity by modified method of Braca *et al.,*[19].

2.6.3 Quantitative Analaysis of Total phenol, Flavonoid and Tannin Contents of Fenugreek Seeds and Callus Extracts:

Determination of Total Phenolic Content

The total phenolic content was estimated according to the method of Wolfe *et al.,*[20]. Total phenolic content was calculated and expressed as mg /l Gallic acid equivalent of phenols.

2.6.4 Determination of Total Flavonoid Content

Total flavonoid content was determined by the aluminum chloride colorimetric method of Singleton and Rossi [21]. Total flavonoid content was expressed as mg/l quercetin equivalents of flavonoid.

2.6.5 Determination of Total Tannin Content

The tannin contents were determined by using $FeCl_3$ and gelatin test of Shivakumar *et al*.,[22]. Total tannin contents were calculated as mg/l tannic acid equivalents of tannin.

2.6.6 Statistical Analysis

Data were analysed by SPSS statistical package software [23].The results are presented as mean ± standard error of three replicates, and analyzed with Duncan LSD.

3. Results and Discussion

3.1 Antioxidant Activity

3.1.1 Screening of Antioxidant Constituents of Fenugreek Seeds and Callus using Thin Layer Chromatography

Thin layer chromatography (TLC) was performed as a preliminary screening process to get an idea about the antioxidant potentiality of different extracts from *T. foenum graecum* seeds and hypocotyls and cotyledons callus. TLC plates were developed in Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26, v/v/v/v) to separate the various constituents of the extracts. The Plates were sprayed with 0.05% 1,1-Diphenyl-2-picrylhydrazyl(DPPH) reagent.

Purple colour of DPPH reagent was bleached by yellow spots which was the indication of positive antioxidant activity. Results provided a clear indication of the presence of free radical scavenging compounds in the methanolic and petroleum ether extracts of *T.foenum graecum* seeds as well as methanolic extracts of hypocotyls and cotyledons derived callus. All extracts displayed antioxidant spots with variable polarities indicative of their different chemical nature. Both callus extracts revealed more antioxidant spots than extracts of plant seeds. The petroleum ether extract of plant seeds exhibited non-polar antioxidant spot whereas, that of methanolic extract of seeds showed less polar antioxidant ones. However, methanolic extracts of callus hypocotyls and cotyledons displayed polar and less polar types of compounds suggesting that callus extracts constitute different types of antioxidant agents. Less polar solvents are particular useful for the extraction of less polar flavonoid aglycones such as flavanone, dihydroflavonles, flavones and flavonols, which are highly methylated while the more polar plant metabolites are generally, isolated from plant materials by extraction with polar solvents like ethyl acetate and alcohol[24].

3.1.2 DPPH Free Radical Scavenging Activity:

The methanolic and petroleum ether extracts *of T. foenum-graecum* seeds and methanolic extracts of callus derived of hypocotyls and cotyledons segments were evaluated for their *in vitro* antioxidant activity using DPPH method and compared with standard ascorbic acid. Percentage inhibition of DPPH free radical scavenging activity of *T. foenum-graecum* callus and plant seeds extracts were presented in Fig.(1). DPPH radical scavenging activity of the tested extracts were concentration dependent. Maximum antioxidant effect was observed in highest concentration (2mg/ml) of all tested samples. Significant differences ($p<0.05$) were detected among different antioxidant potential of *T. foenum-graecum* callus and plant seeds extracts. The highest scavenging activity was obtained from the methanolic extract of cotyledons derived callus (91.5±0.16%) followed by methanolic extract of hypocotyls derived callus (85.46±0.29%) and methanolic extract of plant seeds (80.53±0.01%). These results suggested that the methanolic extracts contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. However, the petroleum ether extract of seeds demonstrated weak antioxidant activity with inhibition percentage 24.56±0.12%.

The IC_{50} values were also calculated for extracts of seeds and callus and compared with that of ascorbic acid. A lower value of IC_{50} indicates a higher antioxidant activity. The IC_{50} values of DPPH scavenging capacity of methanolic extracts were evaluated in descending order, plant seeds (1.1185 mg/g)> hypocotyls derived callus (0.7159mg/g)> cotyledons derived callus (0.4914mg/g)> ascorbic acid (0.1874mg/g) (Fig.2). Thus, calli cultures derived from *T. foenum-graecum* were accompanied by an increasing in free radical scavenging activity suggesting that callus formation enhanced the antioxidative secondary metabolites production in *T. foenum-graecum*. Similar responses were observed for *in vitro* cultures of *Salvia officinalis* and *Rosmarinus officinalis* where callus demonstrated higher antioxidant activity than the mother plant [25].

3.1.3 Total phenol, Flavonoid and Tannin Contents:

Figure (3) represented the total phenolic, flavonoid and tannin contents in methanolic and petroleum ether extracts of seeds and methanolic extracts of callus derived from hypocotyls and cotyledons segments of *T. foenum-graecum*. The highest phenolic content was observed in methanolic extract of cotyledons derived callus (412.087 mg/l) followed by 211.1937 mg/l in methanolic extract of hypocotyls derived callus and 124.84 mg/l in methanolic extract of seeds. The amounts of total phenolic compounds were calculated as mg/l gallic acid equivalent of phenols. These results revealed that there were 2.3 and 0.8 folds increase of phenolic contents in the cotyledons and hypocotyls callus tissues respectively when compared with that of the plant seeds. Previous study carried out by Kaur and Kapoor [7] on the total phenolic content of some Asian vegetables, categorized *T. foenum-graecum* as high phenolic contents vegetables with very high antioxidant activity.

Seasotiya *et al*., [26] reported high value of phenolic contents 186 mg gallic acid/g dry weight in methanolic extracts of *T. foenum-graecum* seeds, whereas Taj al-deen [27] recorded low value of total phenolic content 128.67 mg/g of dry weight in alcoholic extract of callus derived from hypocotyls of *T. foenum-graecum*.

The highest level of flavonoid content were detected in methanolic extract of seeds 424.951 mg/l followed by 217.285 mg/l in methanolic extract of cotyledons derived callus and 95.92 mg/l in methanolic extract of hypocotyls derived callus. The amounts of flavonoid content were calculated as mg/l quercetin equivalent of flavonoids. ALjawfi *et al*., [28] found in their results the total flavonoid contents in *T. foenum-graecum* seeds were 4.99 g/100g dry weight. This variation in the phenolic and flavonoid contents may be due to the variety of *T. foenum-graecum* or the differences in environmental conditions.

Tannin contents were detected only in the plant seeds, where the methanolic extract showed 116.259 mg/l of tannin content calculated as tannic acid equivalent of tannins.

Moreover, the antioxidant effect of this valuable medicinal plant is strongly supported by the presence of phenolic compounds like quercetin[29]. However, the higher antioxidant activity of callus tissues observed in this study might be correlated with the increase in their phenolic composition and it was in accordance with previous studies [30]; [31]; [32] whom demonstrated a significant correlation between phenolic composition and antioxidant activity.

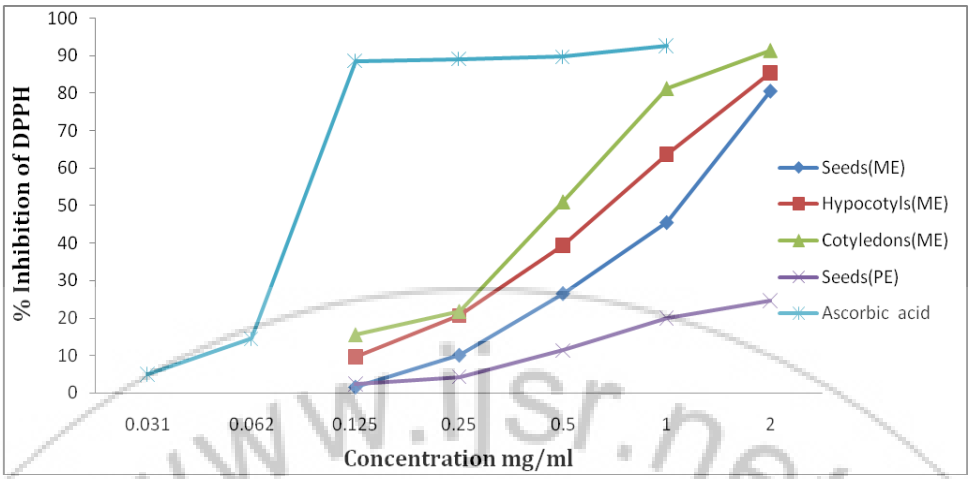

Figure 1: DPPH radical-scavenging activity of seeds and callus (hypocotyls and cotyledons) extracts of *T. foenum-graecum*. PE, petroleum ether extract; ME, methanol extract; values represent the mean ± S.E. M. (n= 3).

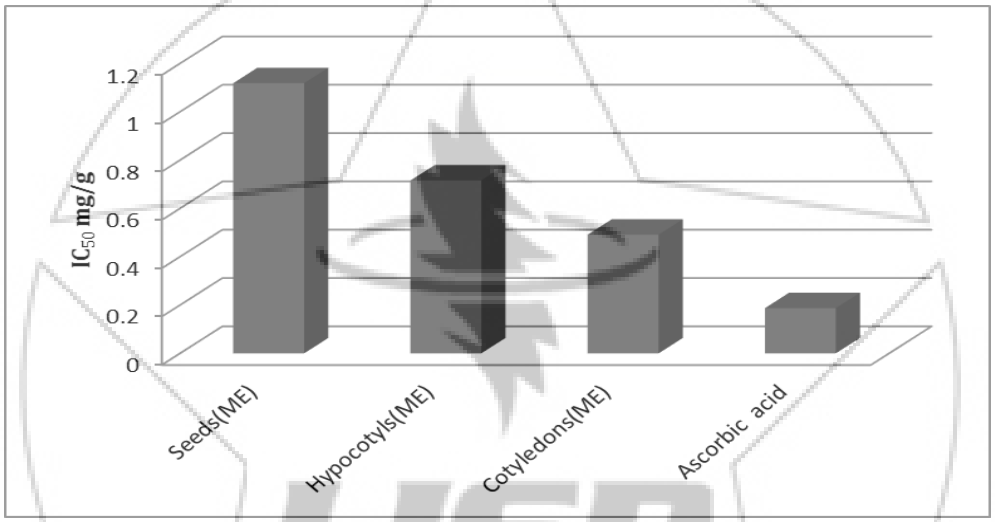

Figure 2: IC_{50} values of DPPH radical-scavenging activity of seeds and callus (hypocotyls and cotyledons) extracts of *T.foenum-graecum.*

PE, petroleum ether extract; ME, methanol extract; IC_{50} values expressed as mg/g.

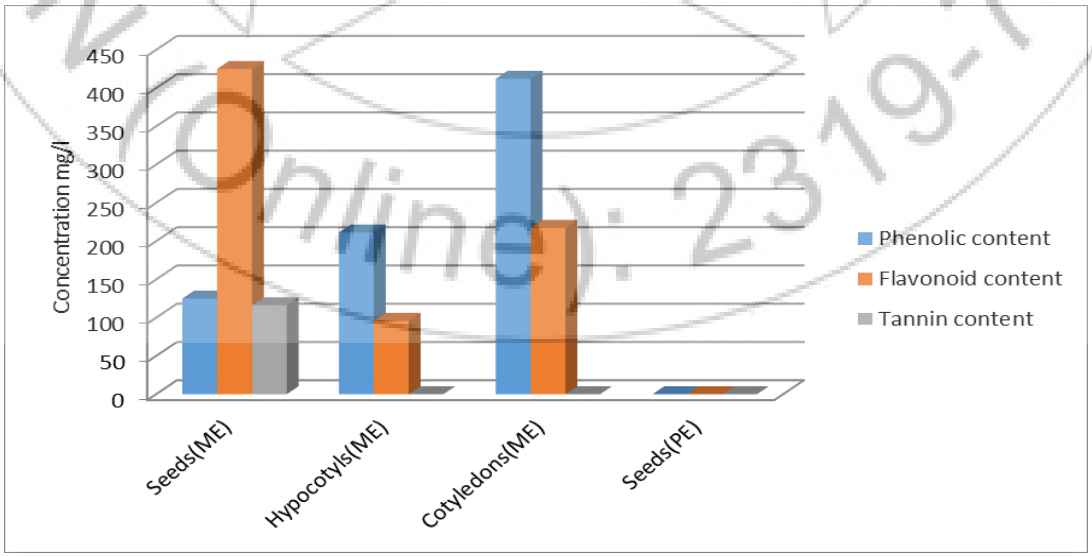

Figure 3: Total phenol, flavonoid and tannin contents in seeds and callus (hypocotyls and cotyledons) extracts of *T.*foenum-*graecum*. PE, petroleum ether extract; ME, methanol extract

4. Conclusion

Methanolic extracts of callus derived from hypocotyls and cotyledons of *T. foenum-graecum* showed higher antioxidant activity than methanolic extracts of seeds. The amount of phenolic contents in methanolic extracts of calli were more, when compared with that of methanolic extracts of *T. foenum-graecum* seeds.

References

[1] Acharya S N, Thomas J E, Basu S K) 2006). Fenugreek: an "old world" crop for the "new world". *Biodiversity* (*Tropical,Conservancy*).**7(3&4)**: 27– 30.
[2] Frankel E N (1996). Antioxidants in lipid foods and their impact on food quality. *Food Chem*. **57**: 51–55.
[3] Basch E, Ulbricht C, Kuo G, Szapary p, Smith M (2003).Therapeutic applications of fenugreek. *Alt. Med. Rev*. **8 (1)**: 20-27.
[4] Edeoga H O, Okwu D E, Mbaebie B O (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J .Biotech* .**4:** 685-688.
[5] Joo S S, Kim Y, Lee D I (2010).Antimicrobial and antioxidant properties of secondary metabolites from white rose flower. *Plant Pathol. J*. **26 (1)**: 57–62.
[6] Miller A L (1996). Antioxidant flavonoids:structure, function and clinical usage. *Alt Med Rev*.**1**: 103-111.
[7] Kaur C, Kapoor H C(2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. *Int. J. of Food Sci & Tech*. **37(2)**: 153-161.
[8] Alfermann AW, Petersen M(1995). Natural products formation by plant cell biotechnology. *Plant Cell Tissue Organ Cult*., **43:**199–205.
[9] Dornenburg H, Knorr D(1997).Challenges and opportunities for metabolite production from plant cell and tissue cultures. *Food Tech*., **51**: 47- 54.
[10] Bolda V V, Botau D, Szôllôsi R, Petô A, Gallé A, Tari I (2011).Studies on elemental composition and antioxidant capacity in callus cultures and native plants of *Vaccinium myrtillus* L. local populations. *Acta Biol Szeged*. **55(2)**:255-259 .
[11] Abouzid S F, El-bassuony A A, Nasib A, Khan S, Qureshi J, Choudhary M I (2010). Withaferin a production by root cultures of *Withania coagulans*. *Int. J. Appl. Res. Nat. Prod*. **3**: 23-27.
[12] Kalidass C, Mohan V R, Daniel A (2010). Effect of auxin and cytokinin on vincristine production by callus cultures of *Catharanthus roseus* L. (apocynaceae). *Trop. Subtrop. Agroecosystems*, **12:** 283-288.
[13] Duangporn P, Siripong P(2009). Effect of auxin and cytokinin on phyllanthusol Aproduction by callus cultures of *Phyllanthus acidus* Skeels. *American-Eurasian. J. Agr. Environ. Sci*. **5**: 258-263.
[14] Mantell S H, Smith H (1984).Cultural factors that influence secondary metabolites accumulations in plant cell and tissue cultures. *In*: Mantell S H, Smith H (Eds.) Plant Biotechnology. Society for Experimental Biology, Seminar Series 18 Cambridge University Press, Cambridge. p_p 75.
[15] Cook N C, Samman S (1996). Flavonoids chemistry,metabolism, cardioprotective effects, and dietary sources. Nutritional Biochemistry. **7:**66-76.
[16] Murashige T, Skoog F(1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Plant. Phys*. **15**:467-497.
[17] Moore J, Yin J, Yu L(2006). Novel fluorometric assay for hydroxyl radical scavenging capacity (HOSC) estimation. *J . Agr Food Chem*. **54(3)**: 617-626.
[18] Males Z, Plazibat M, Vundac V B, Zuntar I (2006). Qualitative and quantitative analysis of flavonoids of the Strawberry tree- *Arbutus unedo* L. (Ericaceae). *Acta Pharm*. **56**: 245-250.
[19] Braca A, Sortino C, Politi M (2002).Anti-oxidant activity of flavonoids from *Licania licaniaeflora*. *J. Ethnopharmacol*. **79**: 379-381.
[20] Wolfe K, Wu X, Liu R H(2003). Antioxidant activity of apple peels. *J . Agr Food Chem*. 51: 609-614.
[21] Seasotiya L, Siwach P, Bai S, Malik A, Bharti P (2014).Free radical scavenging activity and phytochemical analysis of seed s of *Trigonella foenum-graecum. Asian Pac. J. Health Sci*. **1(3)**:219-226.
[22] Shivakumar B S, Ramaiah M, Hema M R, Vijay Kumar M, Vaidya VP(2012). Quantitative Determination of Total Content of Phenol, Flavonoid and Tannin In Leaf Extract of *Barlaria Buxifolia* Linn. *Am. J. Pharm Tech Res*. **2(5)**:418-422.
[23] Griffth A (2007). SPSS for Dummies. Wiley Publishing, Inc.Indiana Pdis, Indiana. p_p.363.
[24] Harborne J B (1973). Phytochemical methods, London. Chapman and Hall, Ltd. P_P. 49-188.
[25] Grzegorczyk I, Matkowski A, Wysokinska H (2007). Antioxidant activity of extracts from *in vitro* cultures of *Salvia officinalis* L. *Food Chem*. **104(2)**: 536-541.
[26] Seasotiya L, Siwach P, Bai S, Malik A, Bharti P (2014).Free radical scavenging activity and phytochemical analysis of seed s of *Trigonella foenum-graecum. Asian Pac. J. Health Sci.* **1(3)**:219-226.
[27] Taj al-deen A M (2010). Application tissue culture technique on some medicinal and aromatic plants used in Yemen.Ph-D thesis faculty of science University of Sanaa.
[28] ALjawfi Y, ALsayadi M, Benmansour A, Chabane S D, Lazoni H A (2013).Chemical and phytochemical analysis of some antidiabetic plants in Yemen. *Int. Res. J. Pharm*.**4(9)**:72-76.
[29] Arfan M, Gul S, Usman R, Khan A, Rauf A, Muhammad N, Ali Shah S U, Khan A, Ali M (2013). The Comparative Free Radical Scavenging Effect of *Trigonella foenum-graecum*, *Solanum nigram* and *Spinacia oleracea. Academic. J. of Plant Sci*. **6 (3)**: 113-116.
[30] Awika J M, Rooney L w, Wu X, Prior R L, Cisneros-Zevallos L(2003). Screening methods to measure antioxidant activity of Sorghum (*Sorghum bicolor*) and Sorghum products. *J . of Agr and Food Chem*. **51**: 6657- 6662.
[31] Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N(2006). Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chem*. **97 (4)**: 654–660.
[32] Jerez M, Selga A, Sineiro J, Torres J L, N´u˜nez M J (2007). A comparison between bark extracts from Pinus pinaster and Pinus radiata: antioxidant activity and procyanidin composition. *Food Chem*. **100(2)**: 439–444.