Chemical Composition and Antibacterial Activity Studies on Callus of *Fagonia arabica* L.

Eman, A. Alam*; Gehan, H. Amin; Yassin, M . ElAyouty** and Mohamed, S. Abdel-Hady***

*,Botany Department, National Research Centre, Dokki,Giza, Egypt.

**, Botany Department, Faculty of Science, Zagazig University, Egypt.

Corresponding author: man200980@hotmail.com

Abstract: *Fagonia spp.* are wild medicinal plants which contain many bioactive constituents used for the treatment of many dangerous diseases, however this fact there were few studies regarding *in vitro* production of these bioactive substances, so we will try to use organ culture technique for this purpose. Production of callus cultures from leaf, hypocotyle and terminal bud explants of *Fagonia spp.* (*Fagonia arabcia*, *Fagonia indica* and *Fagonia bruguieri*) was studied. This study revealed that, leaf of *F. arabica* was the most suitable explant to induce calli especially on MS medium supplemented with 5mg/l kinetin + 1 mg/l NAA, this medium gave the highest percentage of calli induction, while the highest amount of calli was obtained using 5mg/l kinetin + 1 mg/l 2,4-D after six weeks, while MS medium supplemented with 6 mg/l kinetin+ 2 mg/l NAA represented the maintenance medium for giving large amount of yellow healthy calli after four weeks. The best sucrose concentration for obtaining the highest amount of both callus fresh and dry weights is 40 g/l. Maximum growth rates of this callus on both solid and liquid media was recorded after 20 and 10 days respectively. Preliminary phytochemical screening on this callus revealed the presence of carbohydrates and / or glycosides, saponins, sterols and/or triterpenoids, alkaloids, cardiac glycosides, cyanogenic glycosides, flavonoids, coumarins, irodoids, chlorides and sulphates, but this callus devoid of tannins and anthraquinones. Studying the chemical composition of this callus showed that it contains; raffinose, fructose, ribose and sucrose, the most dominant type of sugars is fructose (7.77mg/g fresh weight).Callus contains also amino acids; aspartic acid, glutamic acid, serine, glycine, histidine, argenine, threonine, valine, isoleucine, leucine and phenylalanine, the most dominant type of amino acids is phenylalanine (25 mg/g fresh weight). Total phenols, alkaloids, flavonoids, saponins and oils present in fresh callus were 1.95, 113.40, 0.78, 10 mg/g and 0.68 % respectively. Six fatty acids were isolated and identified; myristic, palmitic, stearic, oleic, lenoleic and lenoleinic acids, the most dominant type of these fatty acids is oleic acid (45.7%). Comparative study through the antibacterial activity was carried out between callus and the intact leaf showed that, the antibacterial effect of this callus superior that of the intact leaf.

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Key words: *Fagonia arabica*, *Fagonia indica*, *Fagonia bruguieri*, callus, chemical composition, antibacterial activity.

Introduction

Family Zygophyllaceae includes many medicinally important plants, in this study we will concentrate on *Fagonia* species. *Fagonia* species were extensively studied by many workers regarding their medicinal uses, since these plants were antitumor, antioxidant, analgesic, astringent, febrifuge and prophylactic against small-pox agents, species of *Fagonia* were also used for the treatments of cancer in the indigenous system, fever, asthma, urinary discharges, toothache, stomach troubles and kidney diseases (Ahsan *et al*., 2007 and Satpute *et al*., 2009). Concerning antimicrobial activity, *Fagonia* species were found to be potent antifungal and antibacterial agents (Zhang *et al.,* 2008 and Gupta *et al.,* 2009). The crude extract of *Fagonia arabica* from Sinai showed broad antimicrobial spectrum against Gram-positive, Gram-negative, spore-forming and acid-fast bacteria (El-Hefnawi, 1999). The previous studies on the medicinal importance of *Fagonia* species ascertain that they contain many biologically active chemical constituents. Species of *Fagonia* have been found to contain saponins (Abdel-Khalik *et al.,* 2001) , alkaloids (Sharawy and Alshammari, 2009), terpenoids (Perrone *et al.*, 2007), sterols (Shoeb *et al.,* 1994), flavonoids (Ibrahim *et al.,* 2008), proteins and amino acids (Sharma *et al*., 2010), coumarins (Zhan *et al.,* 2008), trace elements (Fatima *et al*., 1999). So there is a trend to cultivate different genera of Zygophyllaceae using tissue culture technique in order to isolate more biologically active compounds such as ascorbic acid from callus of *Fagonia cretica* (Kapoor, 2002), diosgenin from callus of *Balanites aegyptiaca* (Gour and Kant, 2006) and beta -carboline and serotonin alkaloids and fatty acids from callus of *Peganum harmal*a (Ibrahim and Khafagi, 2004; Khafagi *et al*., 2004 and Piacetini *et al* ., 2004).

Aims of the work

1 - Determination of calli induction of different explants of different species of *Fagonia* .

2 - Determination of calli induction and calli growth of different explants of *Fagonia arabica* to determine the promised one regarding the amount.

3- Preliminary phytochemical screening to determine the promised one regarding its chemical composition.

4 - Determination of chemical composition of both callus of *Fagonia arabica* leaf explants and the intact leaf.

5 - Determination of antibacterial activity of both callus of *Fagonia arabica* leaf explants and the intact leaf.

Materials and Methods

Plant materials:

Samples of *Fagonia arabica* L. var. *viscidissima* Marie., *Fagonia bruguieri* Dc. were collected from Quatamia- Suez desert road (155 Km away from Suez City). The samples of *Fagonia indica* Burm f. var. *indica* (= *Fagonia parviflora* Boiss.) were collected from Cairo - Alexandria desert road, Km 106 (El-Sadat City, Km 16).

Samples were identified by Prof. Dr. Abdel-Salam Al-Nowahi; Professor of Plant Taxonomy, Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt, where voucher specimens were deposited.

Tissue culture study:

Calli induction:

The time needed for different calli induction from different *F. arabica* explants extended to 6 weeks.

Composition of media:

Media	Sucrose concentration	Hormones	MS medium	Agar
1	30 g/l	5mg/l Kinetin+1 mg/l 2,4-D	(4.43 g/l)	(10g/l)
2	30 g/l	5mg/l Kinetin+1 mg/l NAA	(4.43 g/l)	(10g/l)

Calli growth:

The following six cultural media were used to select the best one for subsequent experiment. The time needed for the growth of different calli of different *F. arabica* explants extended to 4 weeks.

Media	Sucrose concentration	Hormones	MS medium	Agar
1	30 g/l	5 mg/l Kinetin + 2mg/l NAA	(4.43 g/l)	(10g/l)
2	30 g/l	5 mg/l Kinetin +2 mg/l 2,4-D	(4.43 g/l)	(10g/l)
3	30 g/l	5 mg/ l Kinetin +1 mg/l NAA	(4.43 g/l)	(10g/l)
4	30 g/l	5 mg/ l Kinetin+1 mg/l 2,4-D	(4.43 g/l)	(10g/l)
5	30 g/l	6 mg/ l Kinetin+2 mg/l NAA	(4.43 g/l)	(10g/l)
6	30 g/l	6 mg/ l Kinetin+2 mg/l 2,4-D	(4.43 g/l)	(10g/l)

Sucrose concentrations and callus growth:

The callus of *F. arabica* leaf explants growing on medium containing 6mg/l Kinetin+2mg/l NAA and 30 g/l Sucrose (after 4 weeks on growth medium) was transferred to solid media containing different concentrations of sucrose (30,40,50,60 g/l)

Growth rate of calli obtained from leaf of *F. arabica:*

Fresh weight of calli (F.W.) was recorded every 10 days on both solid and liquid media according to Hoda, 1994. Increasing value (I.V.) of fresh and dry weights of callus was determined according to Szoke *et al.*, (1979) using the following equation:

$$\text{I.V.} = \frac{Ge - G_{start}}{G_{start}}$$

Where: - Ge is the mass (mg) of the callus at the end of every 10 days during 50 days of inoculation on both solid and liquid media.

Gstart: Initial mass (mg) of the callus.

Growth Rate (G.R.) of callus was determined according to Dung *et al.,* (1981) using the following equation:

$$\textbf{G.R.} = \frac{Ge - G_{start}}{No.\ of\ \ days}$$

Determination of dry weight:

Samples of callus were dried in an oven supplied with hot air stream at 105ºC for one hour then at 70ºC till a constant weight was obtained (48- 72 hours) according to A.O.A.C., (2000). Dry weight of calli was recorded every 10 days during 50 days of inoculation on both solid and liquid media.

Preliminary phytochemical screening:

Flavonodis (Mabry *et al.,* 1970); Anthraquinones (Farnsworth *et al.,* 1969); Tannins (Trease and Evans, 1978); Alkaloids (Shellard, 1957); Saponins (Hungund and Pathak, 1971); Carbohydrates and / or Glycosides (Stank *et al.,* 1963); Irodoids (Weiffering, 1966); Coumarins (Feigl, 1960); Chlorides and Sulphates (Islam *et al.,* 1993); Sterols and / or Triterpenes (Claus, 1967 and Schmidt, 1964); Cardiac glycosides (Balbaa *et al.,* 1981) and sublimable substances (Afifi, 1972). The previously mentioned substances were investigated for their presence / amount within different plant parts and different obtained calli of *Fagonia arabica* L. var. *viscidissima* Marie., *Fagonia indica* Burm f. var. *indica* (= *Fagonia parviflora* Boiss) and *Fagonia bruguieri* Dc., to select the promised one regarding its chemical composition.

Chemical composition of callus:

20 days old callus of *F. arabica* leaf explants growing on solid medium containing 6 mg/l Kinetin +2 mg/l NAA+40 g/l sucrose was studied for its chemical composition as follows:

1- Determination and Identification of sugars:

The 80% aqueous ethanolic extract (2 ml) containing a known weight of callus (0.758 gram) was analyzed for carbohydrates using HPLC instrument following the method of Farag, 1997.

2-Amino acids analysis:

Amino acids analysis was carried out using HPLC instrument following the method of Millipore Cooperative, (1987).

3 - Determination of total oils and Identification of the isolated fatty acids:

A-Determination of total oils:

Total oils of callus (10 grams dry weight) were extracted by petroleum ether (40- 60°C) in soxhlet apparatus, according to A.O.A.C., (1990) and by Rosese - Gottlieb methods (Farag, 1997). The total oils were calculated as follows:

Percentage of total oils = {(Initial weight of sample - Final weight of sample after extraction) / Initial weight of sample} ×100

B- Determination and Identification of the isolated fatty acids:

Preparation of fatty acid methyl esters using Trans-esterification with cold methanolic solution of potassium hydroxide (EEC, 1991).

C- Chromatographic equipment:

The apparatus used is GC 6890 N. DB 23 column (60 mm × 320 mm × 0.25 mm); maximum temperature of column is 250°C. Temperature of detector is 275 °C.

Operating conditions:

Oven ramp

	°C/min	°C	Hold min
Initial	----------	**150°C**	**0.00**
Ramp 1	**6.50**	**170°C**	**0.00**
Ramp 2	**2.75**	**215°C**	**7.0**
Ramp 3	**10.00**	**230°C**	**1.00**
Total run time			**28- 94**

Temperature of detector	**275°C**
Flow rate	**1.2 ml/ min**

4-Extraction and Determination of total alkaloids:

a-Extraction of alkaloids:

Powdered dried samples (20 grams) were subjected to the following processes described in Ghosal *et al* .,(1984).

b- Determination of total alkaloids:

The final residue was dissolved in 2 ml of chloroform. 25 ml of 0.02 N H_2SO_4 were added. The solution was warmed to driven off the chloroform, cooled and titrated back the excess acid against 0.02 N NaOH solution, using methyl red as an indicator.

Calculations:

Each ml of H_2SO_4 (0.02N) is equivalent to 0.0162 grams of alkaloids.

5- Determination of total saponins:

Total saponins were determined using haemolysis test according to Mochida ,R. and Mochida, H. ,(1961).

Preparation of saponins was carried out following Magnesium oxide method (Rosenthaler,1930)

6- Assay for total phenolics:

Total phenolics were estimated following the method of Gursoy *et al.,* 2009. Involving Folin–Ciocalteu reagent and Gallic acid as standard. Concentration of phenolic compounds was calculated according to the following equation that was obtained form the standard Gallic acid graph.

Absorbance = 0.0167 Gallic acid (ug) + 0.017 (R^2: 0.99)

7- Assay for total flavonoids:

Total flavonoids were determined using the method of Gursoy *et al.,* 2009. Concentration of flavonoid contents was calculated according to the following equation that was obtained from the standard Quercetin graph:

Absorbance = 0.0228 Quercetin (ug) – 0.0045 (R^2: 0.9979)

Antibacterial activity study :

This study was carried out to determine the effect of the crude extract of both callus of *F. arabica* leaf explants "20 days old " (growing on solid medium containing 6mg/l Kinetin +2mg/l NAA+40 g/l sucrose) and the intact leaf.

Tested microorganisms:

Antibacterial activity was investigated against human pathogenic bacterial isolates, obtained from Clinical Pathology Department, Faculty of Medicine (Kasr El- Eini), Cairo University, Egypt.

1- *Escherichia coli* (ATCC 25922).
2- *Providencia alcalifaciens* (ATCC 51902).
3- *Acetobacter aceti* subsp. *liquefaciens* (ATCC 14835).
4- *Klebsiella pneumoniae* (ATCC 13883).
5- *Staphylococcus aureus* (ATCC 25923).
6- *Proteus mirabilis* (ATCC 49565)..
7- *Streptococcus salivarius* (ATCC 25975).
8- *Streptococcus faecalis* (ATCC 29212).
9- *Salmonella typhi* (ATCC 19430).
10- *Serratia marcescens* (ATCC 25419).

The purity and viability of cultures were checked by culturing on nutrient agar slants, incubated at 37ºC for 24 hours. Cultures were subcultured regularly (every week) and stored at 4ºC (Yaecob and Tolba, 2006 and Arya *et al.,* 2010).

Inoculum preparation:

A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 37ºC for 4 hours. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 MC farland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate with 99.5 ml 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately 1-2X10^8 colony-forming units per milliliter (cfu/ml), the suspension was then used for further testing (Arya *et al.*, 2010).

Antibacterial bioassay:

The antibacterial bioassay was carried out following Disc Diffusion Method according to Arya *et al.,* (2010). The concentration of each ethanolic extract in case of callus and intact leaf equals 17 mg/disc. The diameter of inhibition zone (measured in mm) is indicated by clear area in the Petri dish which was devoid of bacterial cells growth was measured. Each Petri dish contains four centered disks, r value of each disk=5 mm, one layer, Whattman number 1 filter paper.

Statistical analysis:

Statistical analysis of all results was done using Fisher analysis of variance methodology. A least significant difference test was applied at 5% and 1% probability level to determine differences among treatment means (Steel and Torrie, 1984). The MSTAT computerized package program was subjected to the regular statistical analysis of variance (Nissen *et al*., 1985). Each reading = mean of three replicates $\pm$ SD.

Results and Discussion

Tissue culture study:

Callus cultures obtained from leaf, hypocotyle and terminal bud explants of *Fagonia spp.* (*Fagonia arabcia*, *Fagonia indica* and *Fagonia bruguieri*) were studied (Tables: 1-3, Photos: 1-2 and Figures: 1-5).

This study revealed that, leaf of *F. arabica* was the most suitable explant to induce calli especially on MS medium supplemented with 5mg/l kinetin + 1 mg/l NAA, this medium gave the highest percentage (93.890%) of calli induction, while the highest amount of calli was obtained using 5mg/l kinetin + 1 mg/l 2,4-D after six weeks.

Regarding *F. indica*, hypocotyle was the most suitable explant to induce calli (60.833%) on MS medium supplemented with 5mg/l kinetin + 1 mg/l NAA, this medium gave both the highest percentage and the highest amount of calli under the same conditions.

Regarding *F. bruguieri*, terminal bud was the most suitable explant to induce calli (39.723%) on MS medium supplemented with 5mg/l kinetin + 1 mg/l 2,4-D, this medium gave the highest percentage of calli only, while the highest amount of calli was obtained using 5mg/l kinetin + 1 mg/l NAA under the same conditions.

MS medium supplemented with 6 mg/l kinetin+ 2 mg/l NAA represented the maintenance medium that gave large amount of yellow healthy calli from *Fagonia arabcia* leaf explants after four weeks. The best sucrose concentration for obtaining the highest amount of both callus fresh and dry weights is 40 g/l. Maximum growth rate of this callus on both solid and liquid media was recorded after 20 and 10 days respectively. These results were parallel to Kapoor, 2002; Zhang and Kang, 2004; Khafagi *et al.*, 2004; Ibrahim and Khafagi, 2004; Mohan *et al*., 2004 and Gour and Kant, 2006 since they found that, *Fagonia cretica, Nitraria tangutorum, Peganum harmala, Tribulus terrestris* and *Balanites aegyptiaca* respectively can induce calli using MS medium supplemented with either NAA or 2,4-D. Kapoor, 2002 found that, glucose concentration affected the production of ascorbic acid. Also Khafagi, 2000 found that, sucrose at 3% was the most suitable carbohydrate source to induce callus from *Peganum harmala* . While Ilahi, 2008 found that Kohat samples of *Fagonia cretica* had not any positive results regarding regeneration trails.

Table (1): Responses of different plant parts explants of different *Fagonia* species to calli induction (Percentage of calli induction) on two different media during six weeks.

Incubation period	Media	Plant species	Percentage of calli induction		
			Leaf	Hypocotyle	Terminal bud
1 st week	1	*F. arabica*	8.890**	21.943**	16.390**
	2		33.610**	26.110	16.390
2 nd week	1		23.333**	41.390**	28.610*
	2		43.890**	39.167**	23.333
3 rd week	1		33.057**	61.943**	72.223**
	2		59.723**	48.057**	26.667*
4 th week	1		65.000**	76.943**	86.110**
	2		73.333**	55.000**	29.443*
5 th week	1		86.390**	83.610**	90.277**
	2		85.557**	58.333**	32.777**
6 th week	1		89.167**	89.723**	93.057**
	2		**93.890****	68.610**	36.110**
1 st week	1	*F. indica*	0.000	0.000	0.000
	2		0.000	10.277	0.000
2 nd week	1		0.000	0.000	0.000
	2		0.000	13.610*	0.000
3 rd week	1		0.000	0.000	0.000
	2		0.000	26.667**	0.000
4 th week	1		0.000	0.000	0.000
	2		0.000	39.723**	0.000
5 th week	1		0.000	0.000	0.000
	2		0.000	43.057**	0.000
6 th week	1		0.000	0.000	0.000
	2		0.000	**60.833****	0.000
1 st week	1	*F. bruguieri*	0.000	0.000	6.110**
	2		3.333	0.000	10.277
2 nd week	1		0.000	0.000	10.277
	2		6.110	0.000	13.610
3 rd week	1		0.000	0.000	16.390
	2		8.890*	0.000	19.723**
4 th week	1		0.000	0.000	23.890**
	2		13.057**	0.000	26.667**
5 th week	1		0.000	0.000	33.610**
	2		15.833**	0.000	32.777**
6 th week	1		0.000	0.000	**39.723****
	2		19.167**	0.000	38.890**
L.S.D.(0.05)			8.157	13.127	10.134
L.S.D.(0.01)			10.833	17.432	13.458

***and**=significant at 0.05 and 0.01 levels respectively.**
Media 1= MS+5mg/l Kinetin+1mg/l 2,4-D, 2= MS+5mg/l Kinetin+1mg/l NAA.

Table (2): Survey for calli induction of different explants of different *Fagonia* species at the end of the incubation period (6 weeks).

Media	*F. arabica*			*F. bruguieri*			*F. indica*		
	L.	H.	T.B.	L.	H.	T.B.	L.	H.	T.B.
MS+5mg/l Kinetin+1mg/l 2,4-D	++++	+++	++	–	–	+	–	–	–
MS+5mg/l Kinetin+1mg/l NAA	+++	+++	++	+	–	++	–	+++	–

Where: L. = Leaf, H.= Hypocotyle, T.B. = Terminal Bud, + ≤ 1g, + + ≤ 2g , +++ ≤ 5g , ++++ ≤ 10 g , +++++ >10 g and – = no callus was observed on this medium .

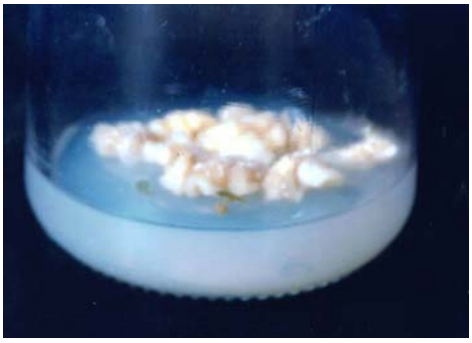


Photo (1): Callus induction of *F. arabica* leaf explants on MS+5mg/l Kinetin+1mg/l 2,4-D.

Table (3): Responses of calli of different explants of *F. arabica* to growth at the end of the incubation period (4 weeks).

Media	Explants		
	Leaf	Hypocotyle	Terminal bud
1	++++	++	+++
2	+++	++	–
3	++++	++	+++
4	++++	+++	++
5	+++++	++++	+++
6	+++	++++	–

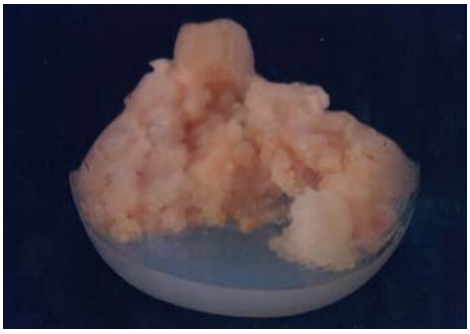


Photo (2): Growth of callus of *F. arabica* leaf explants on MS+6mg/l Kinetin+2mg/l NAA.

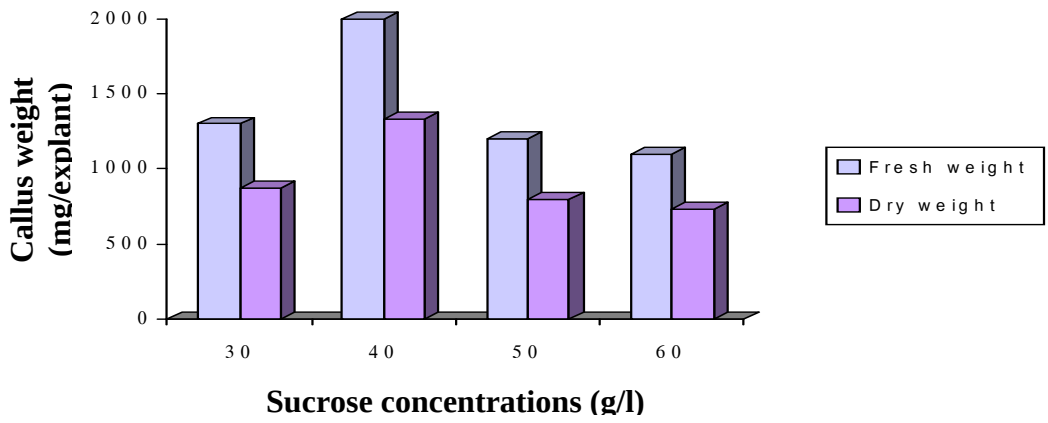


Figure (1): Callus growth with the addition of 40 g/l sucrose.

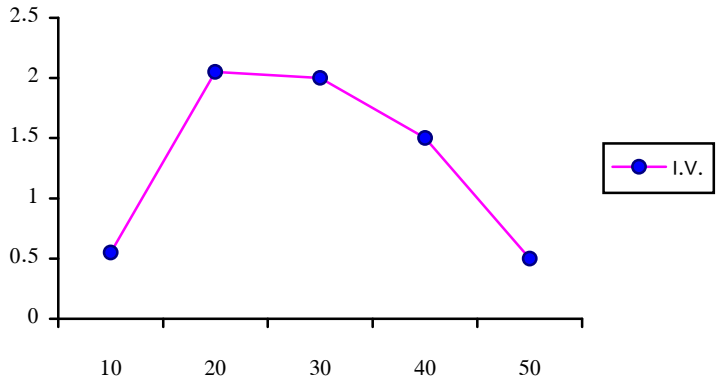


Figure (2): Measurements of callus growth rate (callus fresh weight on solid medium).

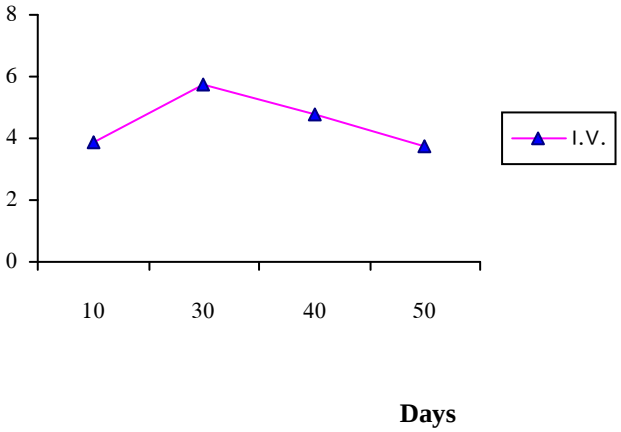


Figure (3): Measurements of callus growth rate (callus fresh weight on liquid medium).

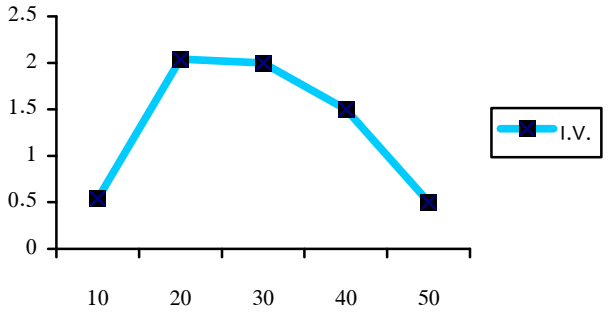


Figure (4): Measurements of callus growth rate (callus dry weight on solid medium).

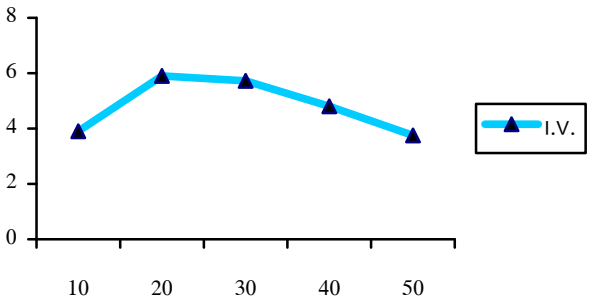


Figure (5): Measurements of callus growth rate (callus dry weight on liquid medium).

Preliminary phytochemical screening on either intact leaf or callus of *F. arabica* leaf explants (20 days old):

Preliminary phytochemical screening (Table: 4) was carried out on calli of different plant parts explants of *Fagonia arabcia*, *Fagonia indica* and *Fagonia bruguieri* and intact plant parts of them revealed that, callus of *Fagonia arabcia* leaf explants that selected quantitatively before (% of calli induction and weight of the obtained callus) was also the best callus regarding active constituents under investigation. Callus was found to contain saponins, alkaloids, coumarins, chlorides more than the intact leaf. These results agreed with others who found that species of *Fagonia* contain saponins (Abdel-Khalik *et al.,* 2001), alkaloids (Sharawy and Alshammari, 2009), terpenoids (Perrone *et al.,* 2007), sterols (Shoeb *et al.,* 1994), flavonoids (Ibrahim *et al.,* 2008), proteins and amino acids (Sharma *et al.,* 2010), coumarins (Zhan *et al.,* 2008), trace elements (Fatima *et al.*, 1999).

Table (4): Preliminary phytochemical screening on either intact leaf or callus of *F. arabica* leaf explants (20 days old) *:*

Experiment	Leaf	Callus
1- Carbohydrates and / or Glycosides	+	+
2- Saponins	+++	++++
3- Tannins	++	-
4- Sterols and / or Triterpenoids	+	+
5- Alkaloids	++	++++
6- Cardiac glycosides	+++	+
7- Cyanogenic glycosides	+	+
8- Flavonoids	+	+
9- Anthraquinones	+	-
10- Coumarins	+	++
11- Irodoids	+	+
12-a-Chlorides	+	++
12-b-Sulphates	+	+

- = The active principle under investigation was not found.

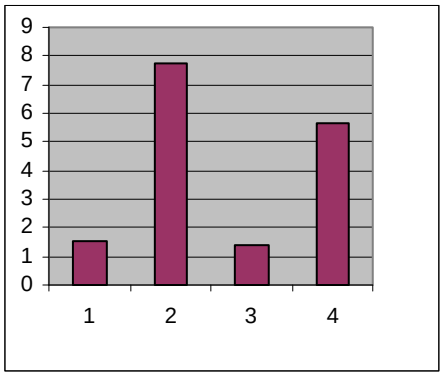
+ = Weak amount of the active principle under investigation was found.

++ = Moderate amount of the active principle under investigation was found.

+++,++++ and +++++ = High amount of the active principle under investigation was found.

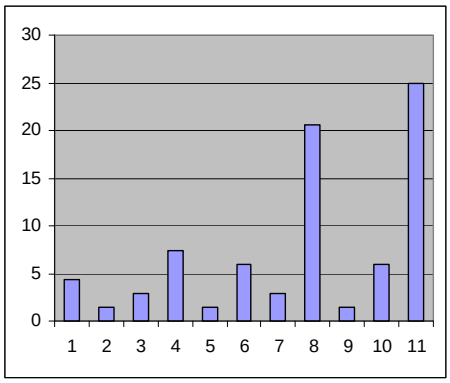
Chemical composition of callus:

Analysis of carbohydrates and amino acids (Figures:6-7) revealed that, callus contains different types of sugars and amino acids which are sucrose, raffinose, ribose and fructose, the last one is the most dominant type of sugars present in the callus (7.77mg/g fresh weight). Meanwhile amino acids have a variable proportion with special reference to phenylalanine (25mg/g fresh weight), meanwhile the least proportion of amino acids was represented by glutamic, histidine and isoleucine. These results agreed with Sharma *et al.*, 2010 who stated that *Fagonia indica* contains amino acids such as alanine, arginine, glycine, isoleucine, leucine, lysine, phenylalanine, proline, tyrosine and valine and sugars such as glucose, arabinose and rhamnose.



(Where : 1=Raffinose,2=Fructose,3 = Ribose and 4 = Sucrose).

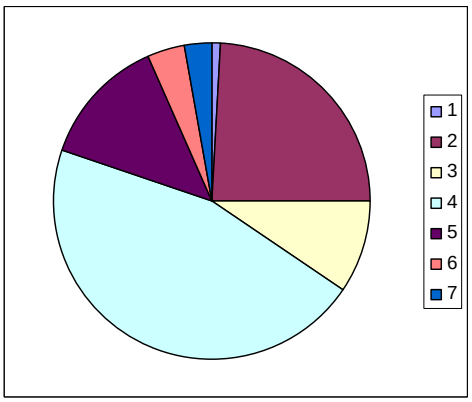
Figure (6): Concentration of different types of sugars in callus (mg/g fresh weight).



(Where:1 =Aspartic, 2 = Glutamic, 3=Serine, 4=Glycine, 5=Histidine, 6=Argenine, 7=Threonine, 8=Valine, 9=Isoleucine, 10=Leucine and 11=Phenylalanine).

Figure (7): Concentration of different types of amino acids in callus (mg/g fresh weight).

Concerning total oils and fatty acids results (Figure:8) revealed that, the percentage of total oils present in callus reached 0.68% and unsaturated fatty acids represent 62.73%, this may be explained on the basis that the unsaturated fatty acid oleic acid had a high proportion (72.85%) from the total unsaturated fatty acids. Such results agreed with Soad, 1994 since she isolated and identified seven fatty acids from *Fagonia cretica*; they were capryroic, caprylic, lauric, myristic, palmitic, stearic and oleic acids. Sharma *et al.,* 2010 found that, *Fagonia indica* contains fatty acids also.



(Where : 1= Myristic, 2= Palmitic, 3= Stearic, 4= Oleic, 5= Lenoleic, 6= Lenoleinic and 7=Unknown).

Figure (8): Percentage of different types of fatty acids in callus.

Quantitative estimation of some secondary metabolites:

Secondary metabolites (Table:5) comprises phenolic compounds, alkaloids, flavonoids and saponins were present in the callus of *F. arabica* leaf explants with a high proportion "especially alkaloids (113.4 mg/g fresh weight) " compared with the intact leaf. Parallely Ahmed *et al*., 1969 found that, the total alkaloids in the plant reached to 0.03%. Moreover, he isolated Harman from *F. arabica, F. bruguieri, F. glutinosa, F. mollis* and *F. parviflora*. Similarly Iyer and Joshi, (1975) isolated and identified harmine from *F. cretica* and it was found that, alkaloidal content in this plant ranged from moderate to high.

Concerning with saponins ratio in callus was 10 mg/g fresh weight. These results agreed with El-Gindi, 1995 who found that, total saponins in *Fagonia arabica* L. was found to be 1.25%.

Concerning with phenolic compounds and flavonoids in callus, their ratios reached 1.95 and 0.78 mg/g fresh weight respectively.

Table (5): Determination of total phenol contents, total alkaloids, total flavonoids and total saponins.

Concentration of different active ingredients (mg/g fresh weight)			
Total phenols	**Total alkaloids**	**Total flavonoids**	**Total saponins**
1.95	**113.40**	**0.78**	**10.00**

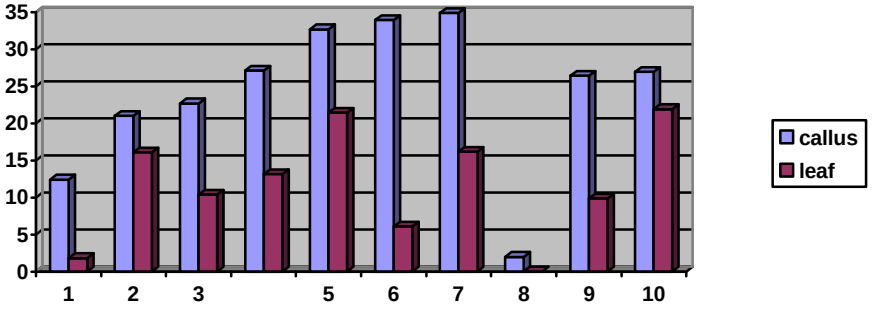
Antibacterial activity study:

Antibacterial activity induced by ethanolic extracts (volume of extract = 17 mg/disc) of either intact leaf or callus of *F. arabica* leaf explants "after 20 days of inoculation on a solid medium containing 6mg/l Kinetin +2mg/l NAA and 40g/l sucrose" (Table: 6 and Figure: 9). Results revealed that, callus extract was more effective against different pathogenic species of bacteria with special reference to *Serratia marcescens, Escherichia coli* and *Acetobacter aceti subsp. liquefaciens* (inhibition zones = 32.67, 33.92 and 34.83 mm respectively) than the crude extract of the intact leaf . The antibacterial effects of callus extract against Gram – ve bacteria were higher than those against Gram + ve bacteria. The most tolerant bacterial isolate was *Staphylococcus aureus*, since the crude extract of the intact leaf has not any effect on it, but the callus has a little effect on it. So there were a positive relationship between chemical composition (with special reference to saponins, alkaloids, coumarins) of the callus and its antibacterial activity. These results agreed with El-Hefnawi, 1999 who found that, the crude extract of *Fagonia arabica* showed a broad antimicrobial spectrum against Gram-negative, spore forming and acid fast bacteria. Also Zhan *et al.,* 2008 and Gupta *et al.,* 2009 found that specie of *Fagonia* were potent antifungal and antibacterial agents.

Table (6): Study of antibacterial activity on ethanolic extracts of intact leaf and callus of *F. arabica* leaf explants.

Bacteria	Clear inhibition zones (mm) (volume of extract = 17 mg/disc)	
	Leaf extract	Callus extract
Gram-ve		
1-Klebsiella pneumoniae	1.833**	12.417**
2-Proteus mirabilis	16.083**	21.000**
3-Salmonella typhi	10.417**	22.667**
4-Providencia alcalifaciens	13.167**	27.083**
5-Serratia marcescens	**21.417****	**32.667****
6- *Escherichia coli*	**6.083***	**33.917****
7-Acetobacter aceti subsp. liquefaciens	**16.167****	**34.833****
Gram + ve		
1-Staphylococcus aureus	**.000**	**2.000**
2-Streptococcus salivarius	9.877**	26.420**
3 -Streptococcus faecalis	21.880**	26.920**
L.S.D. (0.05)	5.434	4.311
L.S.D.(0.01)	7.443	5.906

and*=significant at 0.05 and 0.01 levels respectively.**



*(*Where*:1=Klebsiella pneumoniae, 2=Proteus mirabilis,3=Salmonella typhi , 4=Providencia alcalifaciens,5=Serratia marcescens,6= Escherichia coli, 7=Acetobacter aceti subsp. liquefaciens ,8=Staphylococcus aureus, 9=Streptococcus salivarius* and *10=Streptococcus faecalis).*

Figure (9): Antibacterial effects of ethanolic extracts of intact leaf and callus of *F. arabica* leaf explants.

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Corresponding author:
Eman, A. Alam
Botany Department, National Research Centre, Dokki, El- Behoos Street, Giza, Egypt.

Eman200980@hotmail.com

Mobile: 0179469341-0187180968 (Egypt).

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