Original Research Article

TOTAL SAPONINS, GLYCOSIDES, AND FLAVONOIDS IN CALLUS AND CELL SUSPENSION CULTURE OF *BALANITES AEGYPTIACA*

Abstract

In the present study trials were done to obtain callus and cell suspension culture from *Balanites aegyptiaca* sterile plantlets grown in our lab in vitro. Total saponins, glycosides and flavonoids were estimated over a period extending from 1 to 5 weeks old of the cultures. The results obtained indicated that both calli and cell suspension cultures were able to synthesize the target active ingredients and that cell suspension culture was superior to the callus culture in the biosynthesis and accumulation of the adopted secondary metabolites. By the end of the incubation period the amount of total saponins in cell suspension culture reached up 51.97±0.26 dry biomass compared to 35.02 ±0.06 mg/g in callus culture. The amount of total flavonoids in cell suspension culture reached up 10.88±0.24 dry biomass compared to 6.40 ±0.02 mg/g in callus culture. The amount of total glycosides in cell suspension culture reached up 6.11±0.25 dry biomass compared to 5.06 ±0.05 mg/g in callus culture. Statistical analysis have shown that the increase of saponins and flavonoids in cell suspension culture over the corresponding content in callus culture is significant. The results obtained in this study may indicate the promising role that plant cell culture will play in the future in phytopharmaceutics industry.

Keywords: Biotechnology, Heglig, Phytopharmaceutics, Plant tissue culture, Secondary metabolites.

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Introduction

Balanites aegyptiaca (L.) Del. which is known as desert palm or heglig, belongs to the family *Balanitaceea*[1]. It is a multipurpose evergreen spiny tree species that can be used as a fodder, a source of wood for charcoal industry, a timber, a fuel wood and a raw material for many other purposes[2]**.** More recently, the oil of the seeds of this tree is being used in many industries such as soap, shampoo, cream, herbal medicine and even the production of biodiesel[3,4,5,6]. The plant synthesizes many secondary active metabolites like saponins, flavonoids and glycosides and this may explain the many pharmacological effects of the plant[7], which were reported by many researchers[8,9,10]**.** Saponins of *Balanites aegyptiaca* (diosgenin and yamogenin) are used in the partial synthesis of steroidal drugs[11].

Plant cell cultures based products is a modern perspective biotechnological application of plant tissue culture. Cell culture systems are preferable over conventional whole plant cultivation in production of phytopharmaceutics because the plant compounds of choice can be generated independently of external factors; the cultured cells are not threatened by the attacks of microorganisms or insects; cells of any plant even rare or endangered ones can be cultured *in vitro*; regulation of secondary metabolite production decreases costa and improves productivity[12].

The aim of the present study is to compare growth and the biosynthetic potential of saponins, flavonoids and glycosides by callus and cell suspension culture the adopted plant species.

Materials and method

Callus and cell suspension cultures

For induction of primary callus from *Balanites aegyptiaca,* segments of leaves detached from *in vitro* grown sterile mother plant were used as explants and cultured on Murashig and Skoog's culture media[13], containing 3% sucrose and supplemented with 2.0 mg/l BA + 2.0 mg/l 2,4-D and the pH was adjusted to 5.7 prior to autoclaving. Gelling was achieved with 0.7% (w/v) Anachemia agar (Sigma). Autoclave sterilization (20 minutes, 121 ^{0}C and a pressure equal to 1.5 Atmosphere) was applied. The cultures were allowed to grow for 4 weeks.

Equal weight pieces of the 4 weeks old primary calli obtained were used to develop callus and cell suspension cultures for comparison of growth and secondary

metabolite biosynthesis. The same nutritional and cultural conditions were applied except for that agar was omitted from the culture media used to develop cell suspension culture and shaking was applied 100 in a rate of 100 rounds per minute.
Each treatment was represented by 10 replicates. The cultures incubated at 25 ± 1^0C and illumination intensity of 1500 lux day light at the top of cultures level from white fluorescent lamp (120 cm long 40 watts) the photoperiod was 16 hour. Light and 8-hour dark automatic controlled.

Phytochemical analysis of callus and cell suspension cultures

Extraction:

Calli and cell suspension cultures were harvested washed and excessive water was removed. The harvest of both types was then dried in in shade. After constant weights were attained, calli and cell suspensions were grinded into fine powder. One gram of dry fine calli and cell suspension powders was soaked in pure methanol (150 ml) separately at room temperature for 72 hours with stirring from time to time. After that, filtration was carried out and the residue was washed with three successive rinses (100 ml) of pure methanol. The filtrate and washings were combined and evaporated to 10 ml. the obtained extract was used for determination of total saponins, flavonoids and glycosides.

Determination of total saponins

For total saponins estimation, 0.5 ml of methanolic extract for each of callus and cell suspension cultures extracts and 0.5 ml of o.5 % p-anisaldehyde reagent were mixed and kept for 10 minute. Later, 2ml of 50% sulphuric acid was added, and then tubes were vortexes. Then kept in water bath with constant tempura of 60 ^{O}C for 10 minute then cooled and the absorbance of the developed yellow color was measured at 435 nm. The amount of saponins was calculated as saponin equivalent from the calibration curve of standard saponin 100 – 1000 µ/ml[14].

Determination of total flavonoids

Total flavonoid content of the methanol extracts of both callus and cell suspension cultures were determined by Aluminum chloride method. To 0.5 ml aliquots of the extracts and standard solution (0.01-1.0 mg/ml), 2ml of distilled water and 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) were added and mixed then left to stand 6 minutes. Then 0.15 ml of (10% AlCl3, w/v) solution was added and mixed tube well. Allowed to stand for further 6 min and after that 2 ml of sodium hydroxide (4% NaOH, w/v) solution were added, mix and the final volume of each was completed to 5 ml distilled water. After through mixing the solutions, allowed to stand for another 15 min. The absorbance of each mixture was determined at 510 nm against the same mixture without callus extract as a blank[15].

Determination of total glaycosides

Glaycosides determination was done using Baljet reagent [95 ml of Picric acid (1%) with 5 ml of sodium hydroxide (10%)]. Eight ml of callus extract were transferred to a 100 ml flask; 60 ml of H_2O and 8ml of 12.5% lead acetate were added, mixed and filtered. Fifty ml of H_2O and 8 ml of the filtrate was transferred into another 100 ml flask and 8 ml of 47% Na_2HPO_4 were added, mixed and completed the volume with distilled water and filtered twice. Ten ml of purified filtrate were transferred into clean flask and treated with 10 ml Baljet reagent. This was allowed to stand for one hour at room temperature for complete color development. The color intensity was measured calorimetrically at 495 nm against the blank of 10 ml distilled water and 10 ml Baljet reagent incubated for one hour at the same conditions[16].

Results and discussion

Results of the present study (table 1) show that with respect to callus culture, the amount of saponins increased from 1.51±0,02 mg/g dry callus biomass after one week of growth to 30.02±0.06 mg/g after 5 weeks of growth. With respect to cell suspension and microcalli developed on the liquid media, the amount of saponins increased from18.28±0.42 mg/g dry biomass after one week of growth up to 51.97±0.26 mg/g dry biomass. The results obtained may indicate that both callus and cell suspension cultures of *B. aegyptiaca* were able to synthesize saponins but with

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different potentialities; The accumulation of saponins increased with the increase of age of both cultures from 1 to 5 weeks of incubation and in general, the cell suspension culture was much more active in accumulating saponins. The differences between callus and cell suspension culture in the biosynthesis or accumulation of saponins was statistically significant in all stages of growth. Callus and cell suspension cultures can be seen in figure (1).

Results of the present study (table 2) may show that the amount of total flavonoids estimated on the basis of the dry *B*. aegyptiaca biomass depended on both age and type of the culture. After one week of incubation, the amount of flavonoids in the callus culture was1.08±0.03 mg/g compared to 1.51±0.02mg/g dry weight in microcalli and cell suspension culture. The gradual increase in the amount of flavonoids continued to the fifth week of growth and the accumulation of flavonoids in microcalli and cell suspension was statistically significant over the corresponding callus culture determinations. By the end of the incubation period, the amount of flavonoids in both types of cultures reached up from 5-6 times the amounts recorded after the first week of growth.

With respect to the total glycoside contents (as illustrated in table 3), there were almost no significant differences between callus and cell suspension culture regardless of the general increase in the amount of glycosides determined from the first to the fifth weeks. The amount estimated in the first week was 1.30±0.03 in callus culture compared to 1.57±0.36 mg/g in the cell suspension culture. By the end of the fifth week of incubation, the amount recorded in callus culture was 5.06±0.05 compared to6.11 ±0.25mg/g dry weight recorded in the cell and microcalli suspension culture.

The results obtained in this study may agree with many of the most recent papers on many other plants. Plant tissue culture techniques being independent of climatic and geographical conditions will provide an incessant, sustainable, economical and viable production of secondary metabolites[17]. Cell suspension culture of *Spilanthes acmella* Murr used successfully to produce scopoletin[18].

It has been mentioned that Callus induction and multiplication have been extensively used in PDMC *in vitro* production. It is an efficient approach to produce PDMCs in large scale when compared to other techniques, mainly because the *in vitro* callus induction is a straightforward and rapid system of cell multiplication[19]. Besides, the factors that induce callogenesis are well studied[19], highly consistent, and used commercially already for some decades in tissue culture for other applications[20]. Stated that medicinal plant cell suspension cultures (MPCSC), which are characterized with the feature of fermentation with plant cell Totipotency, could be a promising alternative "chemical factory"[21].

Conclusion

Callus was initiated from *B. aegypiaca* plantlets growing in vitro in our lab. Both types of cultures were able to synthesize and accumulate antioxidative secondary metabolites (saponines, flavonoids and glycoside). The increase in the ago of the culture was accompanied by an increase in the accumulation of such metabolites from the second to the fifth weeks of growth. In general cell suspension culture were more active in accumulation of the targeted metabolites. The present study represents an additional evidence that plant cell culture will play a major role in the future of industry of phytopharmaceutics.

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Conflict of Interests

No conflict of interest associated with this work.

Results and Discussion

Table (1): Effect of different incubation periods (weeks) on contents of total saponins of *B. aegyptiaca* callus and cell suspension cultures (mg/g dry biomass)

Type of culture	Age of culture in week

	1	2	3	4	5
Callus	1.51 ± 0.02	19.05 ± 0.19	26.27 ± 0.23	32.15 ± o.38	35.02 ± 0.06
Cell suspension	18.28 ± 0.42	28.31 ± 0.20	32.24 ± 0.36	39.02 ± o.12	51.97 ± 0.26

Table (2): Effect of different incubation periods (weeks) on contents of total flavonoids of *B. aegyptiaca* callus and cell suspension cultures (mg/g dry biomass)

Type of culture	Age of culture in week				
	1	2	3	4	5
callus	1.08 ± 0.03	1.92 ± 0.20	3.26 ± 0.13	6.32 ± 0.36	6.40 ± 0.02
Cell suspension	1.51 ± 0.02	4.07 ± 0.19	6.74 ± 0.08	8.35 ± 0.26	10.88 ± 0.24

Table (3): Effect of different incubation periods (weeks) on contents of total glycosides of *B. aegyptiaca* callus and cell suspension cultures (mg/g dry biomass)

Type of culture	Age of culture in week				
	1	2	3	4	5
Callus	1.30 ± 0.03	2.02 ± 0.09	2.53 ± 0.28	4.80 ± 0.21	5.06 ± 0.05
Cell suspension	1.57 ± 0.36	2.36 ± 0.27	4.23 ± 0.27	4.50 ± 0.33	6.11 ± 0.25

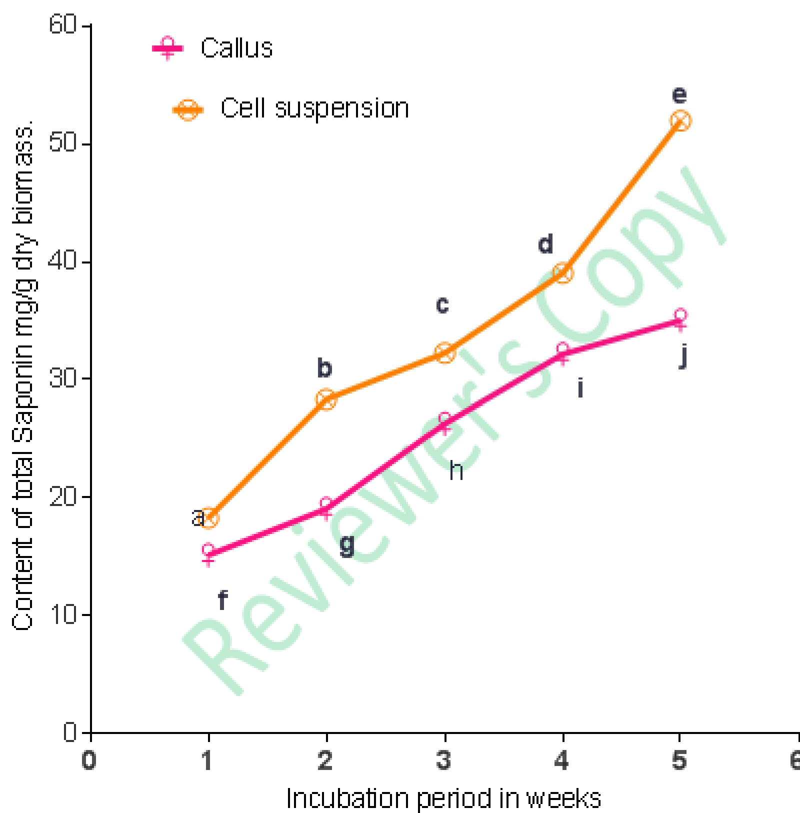

Fig: (1)

Data represent mean ± standard deviation of 3 different values. Statistical significance was determined using Two-way ANOVA: and $p<0.0001$; post hoc test: Tukey using GraphPad Prism 7 software. In the Saponin different character is show significant different and the same character is show no significant different .

Effect of incubation period of calli and cell suspension culture of *B.aegyptiaca* on content of total Flavonoids mg /g.

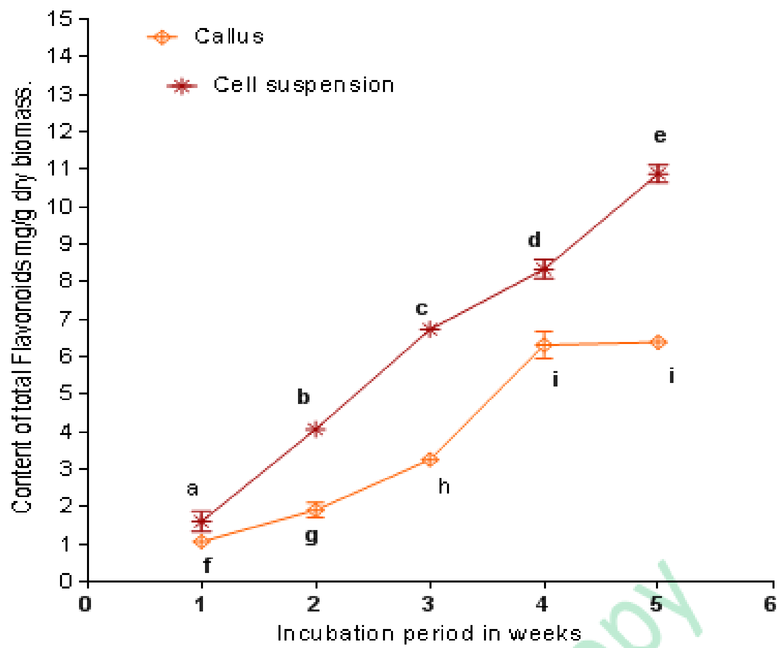

Fig : (2) Data represent mean ± standard deviation of 3 different values. Statistical significance was determined using two-way ANOVA: and $p<0.0001$; post hoc test: Tukey using GraphPad Prism 7 software. In the flavonoids different character is show significant different and the same character is showing no significant different.

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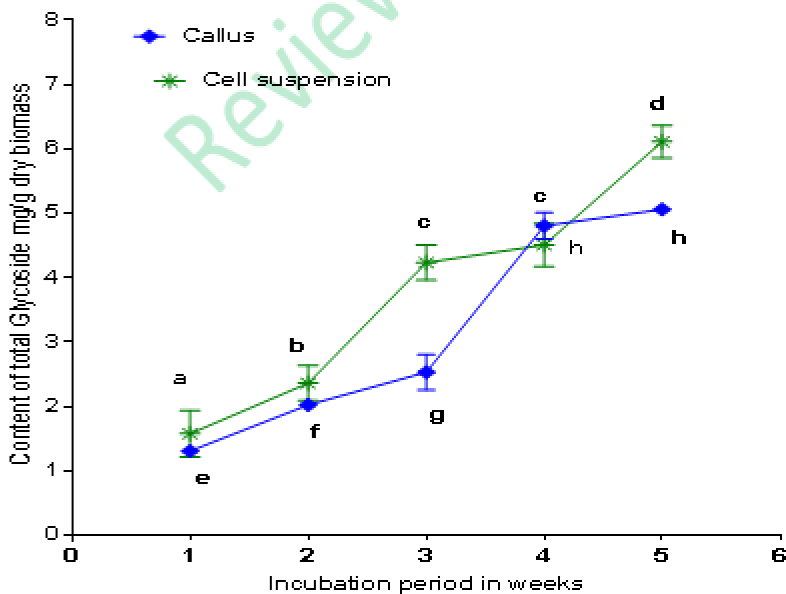
Effect of incubation period of calli and cell suspension culture of *B.aegyptiaca* on content of total Glycoside mg /g.



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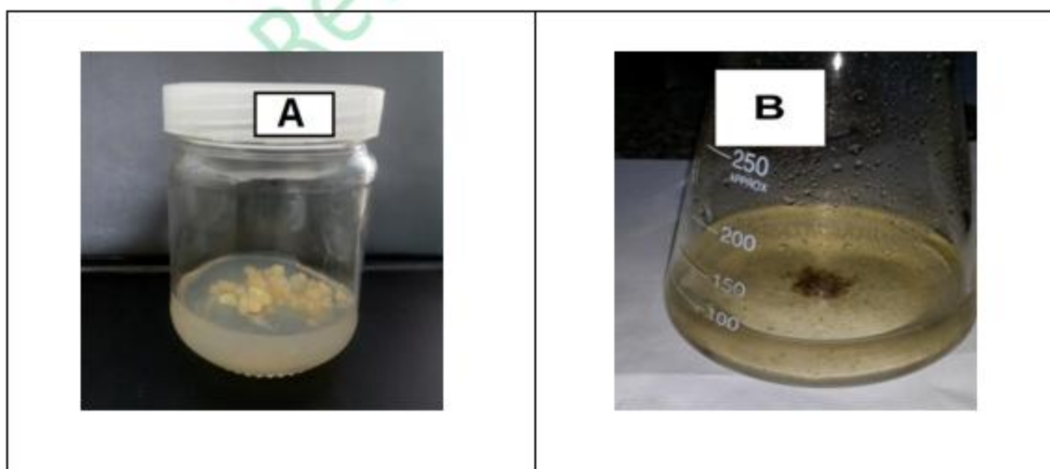

Plate (1):
(A) Callus culture five week old, germination on MS media supplemented with 2.0 mg/l BA + 2.0 mg/l 2,4-D.
(B) Cell suspension culture five weeks old, germination on MS media supplemented with 2.0 mg/l BA + 2.0 mg/l 2,4-D.

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