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RESEARCH ARTICLE

SAPONINS, GLYCOSIDES AND FLAVONOIDS IN CELLS AND TISSUES OF *BALANITES AEGYPTIACA CULTURED* ON SOLID AND LIQUID CULTURE MEDIA

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Abstract

Objectives: The main objective of the present study is to obtain callus and cell suspension culture from *Balanitesa egyptiaca* sterile plantlets grown *in vitro* and to compare growth and the biosynthetic potential of saponins, flavonoids and glycosides by callus and cell suspension culture *Balanitisa egyptiaca*.
Methods: Callus was induced from the mother plants on MS culture media supplemented with 2.0 mg/l BA + 2.0 mg/l 2,4-D with and without agar gelling. Total saponins, glycosides and flavonoids were estimated in both types of cultures over a period extending from 1 to 5 weeks to compare the productivity of such secondary metabolites in callus and cell suspension cultures.
Results: 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 processes. 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 and of total glycosides reached up 6.11±0.25 dry biomass compared to 5.06±0.05 mg/g in callus culture.
Conclusions: The results obtained in this study may indicate the promising role that plant cell culture will play in the future in phytopharmaceutical industry.
Keywords: Biotechnology, desert palm, phytopharmaceutics, plant tissue culture, secondary metabolites.

INTRODUCTION

Balanitesa egyptiaca, which is known as desert palm or heglig[1], 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]. This 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 *B. egyptiaca* (diosgenin and yamogenin) are used in the partial synthesis of steroidal drugs[11]. Plant cell culture based product is a modern perspective biotechnological application of plant tissue culture. Cell culture systems are preferable over conventional whole plant cultivation in production of phytopharmaceuticals to avoid the adverse effects of external factors on secondary metabolites biosynthesis; 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 costs 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 *B. egyptiaca*.

MATERIALS AND METHODS

Callus and cell suspension cultures

B. egyptiaca growing *in vitro* in Plant Tissue Culture and Biotechnology laboratory, Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt was used as a mother plant from which explants were taken. For induction of primary callus from 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^{o}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^{o}C 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 hours. 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 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 0.5 % p-anisaldehyde reagent were mixed and kept for 10 minute. Later, 2 ml of 50% sulphuric acid was added, and then tubes were vortexed. 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), 2 ml 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% $AlCl_3$, w/v) solution was added and mixed well in a tube. 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 thorough 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 glycosides

Glycosides 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 8 ml 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. Total 10 ml of purified filtrate were transferred into clean flask and treated with 10 ml Baljet reagent. 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].

Data analysis

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 Graph Pad Prism 7 software. In the estimation of contents different character is showing significant different and the same character is showing no significant different.

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 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 1) may show that the amount of total flavonoids estimated on the basis of the dry *B. egyptiaca* biomass depended on both age and type of the culture.

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

Contents	Type of culture	Age of culture in week				
		1	2	3	4	5
Total saponins	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
Total flavonoids	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
Total glycosides	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

After one week of incubation, the amount of flavonoids in the callus culture was 1.08±0.03 mg/g compared to 1.51±0.02 mg/g dry weight in microcalli and cell suspension culture. The gradual increase in the amount of flavoinoids 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.

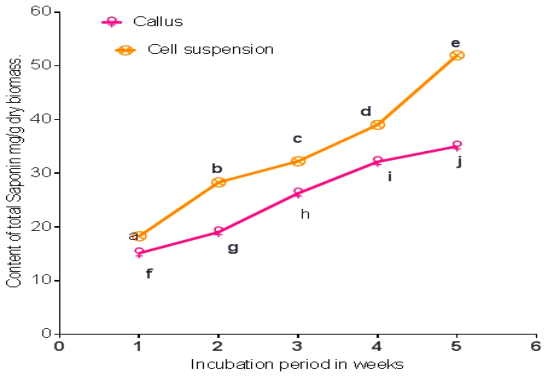

Figure 1: Effect of incubation period of calli and cell suspension culture of *B. aegyticaca* on content of total Saponin (mg/g).

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 1), 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.

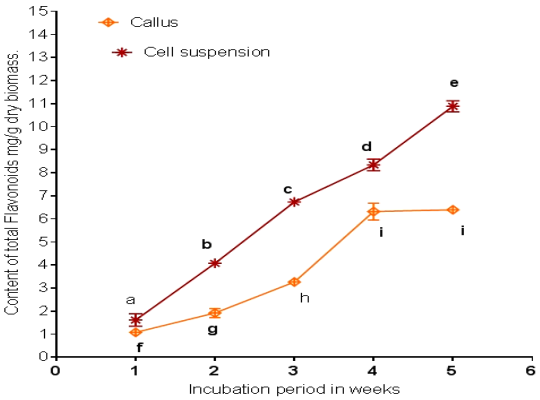

Figure 2: Effect of incubation period of calli and cell suspension culture of *B. aegyticaca* on content of total Flavonoids (mg/g).

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 incybation, the amount recorded in callus culture was 5.06±0.05 compared to 6.11±0.25 mg/g dry weight recorded in the cell and microcalli suspension culture.

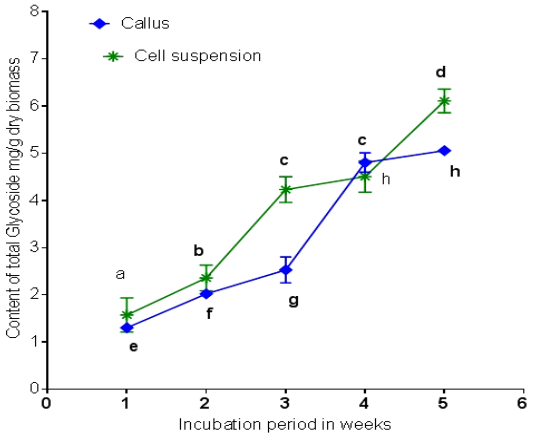

Figure 3: Effect of incubation period of calli and cell suspension culture of *B. aegyticaca* on content of total Glycosides (mg/g).

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 *Spilanthe sacmella* Murr used successfully to produce scopoletin[18]. It has been mentioned that Callus induction and multiplication have been extensively used in Product Development and Manufacturing Center **"**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].

It has been reported that the factors that induce callogenesis are well studied and that callus cultures are used for production of secondary metabolites[20] and stated also that medicinal plant cell suspension cultures (MPCSC) with plant cell totipotency can be regarded as a promising alternative for production of secondary metabolites[21].

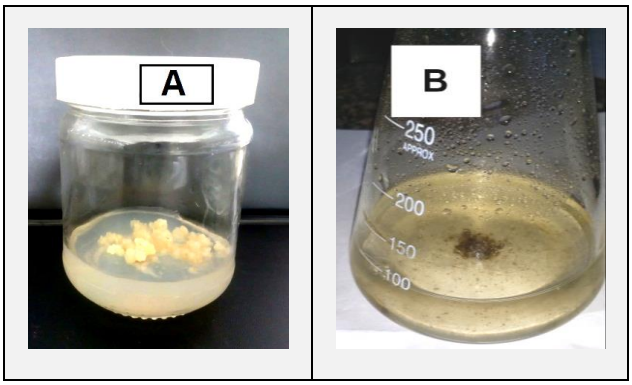

Figure: 4 (A): Callus culture five week old, germination on MS media supplemented with 2.0 mg/l BA+2.0 mg/l 2,4-D. (B): Cell suspension culture five weeks old, germination on MS media supplemented with 2.0 mg/l BA+2.0 mg/l 2,4-D.

CONCLUSIONS

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 (saponins, flavonoids and glycosides). The increase in the age 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 was more active in accumulation of the targeted metabolites. The present study represents additional evidence that plant cell culture will play a major role in the future of industry of phytopharmaceuticals.

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AUTHORS' CONTRIBUTIONS

ALdeen AMT: writing original draft, methodology. **El-Sadek MEA:** investigation, conceptualization. **Abdul-hakim AHS:** supervision, formal analysis, conceptualization. **Hussein EA:** lab censor, research proposal. **Al-kaf AG:** investigation, statistical analysis. All authors revised the article and approved the final version.

DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

CONFLICT OF INTEREST

None to declare.

REFERENCES

1. Orwa C, Mutua A, Kindt R, *et al*. Agroforestree Database: a tree reference and selection guide. World Agroforestry Centre, Kenya 2009; version 4.0.
2. Von Maydell HJ. Arbes and shrubs of the Sahel: their characteristics and uses. GTZ, Eschborn 1984 ;531.
3. Charity UZ, Alexander AJ, Hassan IG, Yarkasuwa IC. Application of Desert Date (*Balanitesa egyptiaca*) seed oil as potential raw material in the formulation of soap and lotion. American J Analyt Chem 2018 ; 9: 423-437.
4. Linda DFB, Marie S, Daniel R, Didier A, Blin J, Salifou KO. Biofuel from *Balanitesa egyptiaca*. Optimization of the Feedstock Supply Chain. Sustainability 2018; 1-15. *https://doi.org/10.3390/su10124501*
5. Naik NS, Balakrishna. A comparative study of B10 biodiesel blends and its performance and combustion characteristics. Int J Amb Energy 2018; 39(3): 257-263. *https://doi.org/10.1080/01430750.2017.1303629*
6. Montasser AOS, Saleh H, Ahmed-Farid OA, *et al*. Protective effects of *Balanitesa egyptiaca* extract, Melatonin and Ursodeoxycholic acid against hepatotoxicity induced by Methotrexate in male rats. Asian Pac J Trop Med 2017; 10: 557-565. *https://doi.org/10.1016/j.apjtm.2017.06.003*
7. Al-Thobaiti SA, Abu Zeid IM. Phytochemistry and pharmaceutical evaluation of *Balanitesa egyptiaca*. J Exp Biol Agri Sci 2018; (3): 53-465. *https://doi.org/10.18006/2018.6(3).453.465*
8. Gnoula C, Mégalizzi V, Ribaucour F, *et al*. Balanitin-6 and -7: Diosgenylsaponins isolated from *Balanitesa egyptiaca* Del. display significant anti-tumor activity *in vitro* and *in vivo*. Int J Oncol 2008; 32: 5-15. *https://doi.org/10.3892/IJO.32.1.5*
9. Patil SV, Salunke BK, Patil CD, *et al*. Potential of extracts of the tropical plant *Balanitesa egyptiaca* (L) Del. (Balanitaceae) to control the mealy bug, *Maconellicoccu shirsutus* (Homoptera: Pseudococcidae). Crop Protection 2010 ; 29: 1293-1296. *https://doi.org/10.1016/J.CROPRO.2010.05.016*
10. AI Ashaal HA, Farghaly AA, Aziz MMA, Ali MA. Phytochemical investigation and medicinal evaluation of fixed oil of *Balanitesa egyptiaca* Ethnopharmacol 2010; 127(2):495-501. *https://doi.org/10.1016/j.jep.2009.10.007*
11. Eltohami MS. Medicinal and Aromatic Plants in Sudan. Research Institute proceedings of the international expert meeting organized by the forest product division. FAO Forestry Department & the FAO Regional Office for the Near East, Cairo Egypt 1999.
12. Efferth T. Biotechnology application of plant callus cultures Engineering 2019; 5 (1):50-59. *https://doi.org/10.1016/j.eng.2018.11.006*

13. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologiaplantarum1962; 15: 473-497. *https://doi.org/10.1111/j.1399-3054.1962.tb08052.x*
14. Ing-Luen S, Tzenge-Lien S, Ya-Nang W, *et al*. Quantification for saponin from a soapberry (*Sapindus mukorossi* Gaerth) in cleaning products by a chromatographic and two colorimetric assays. J Faculty Agriculture Kyushu University 2009; 54: 215-21.
15. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999; 64:555-9. *https://doi.org/10.1016/S0308-8146(98)00102-2*
16. El-Olemy MM, Al-muhtadi FJ, Afifi A. Experimental phytochemistry.A laboratory manual. King Saud University Press, Saudi Arabia1994; 3-19.
17. Chandran H, Meena M, Barupal T, Sharma K. Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. Biotechnol Rep (Amst) 2020; 26:e00450. *https://doi.org/10.1016/j.btre.2020.e00450*
18. Abyari M, Nasr N, Soorni J, Sadhu D. Enhanced accumulation of scopoletin in cell suspension culture of *Spilanthes acmella* Murr, using precursor feeding. Braz Arch Biol Technol 2016;59:1–7. *https://doi.org/10.1590/1678-4324-2016150533*
19. Kapoor S, Raghuvanshi R, Bhardwaj P, *et al*. Influence of light quality on growth, secondary metabolites production and antioxidant activity in callus culture of Rhodiolaimbricata. J Photochem Photobiol B: Biology 2018; 183: 258-265. *https://doi.org/10.1016/j.jphotobiol.2018.04.018*
20. Ahmad N, Rab A, Ahmad N. Light-induced biochemical variations in secondary metabolite production and antioxidant activity in callus culture of *Stevia rebaudiana* (Bert.). J Photochem Photobiol B: Biology 2016; 154: 51-56. *https://doi.org/10.1016/j.jphotobiol.2015.11.015*
21. Yue E, Nibg QL, Kin B, Rahman, K, Cheng Z, Qin L. Medicinal plant cell suspension cultures: Pharmaceutical applications and high-yielding strategies for the desired secondary metabolites. Crit Rev Biotech 2014; 36(2):1-18. *https://doi.org/10.3109/07388551.2014.923986*