The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 20(3): 870-878,2025 www.thebioscan.com

A COMPARITIVE STUDY ON THE CELLULAR VIABILITY AND APOPTOSIS POTENTIAL OF PALMYRA SAP SAMPLE COLLECTED FROM KANYAKUMARI AND TIRUNELVELI DISTRICTS

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DOI: 10.63001/tbs.2025.v20.i04.pp870-878

KEYWORDS

Borassus flabellifer, Invitro Cytotoxicity Analysis, Cell Viability, MTT Assay.

Received on: 17-07-2025

Accepted on: 08-08-2025

Published on: 20-08-2025

ABSTRACT

Borassus flabellifer Linn., of the Arecaceae family is locally called as Tal, English Name: Palmyra palm.Traditional cuisines have been employed the fruit pulp of *Borassus flabellifer* L. and the sap that was collected from the flower section as a sweetener for diabetes patients. Proteins, lipids, carotenoid, vitamin B complex, ascorbic acid and other minerals are found in the Palmyra apart from sugars. Besides all Palmyra fruits are also rich in immunosuppressive effects. Palmyra sap itself is generally not described as cytotoxic in research, but rather is known for its nutritional and various health-promoting properties, including antioxidant, anti-inflammatory, and hepatoprotective effects. Extracts from this part contain compounds that exhibit potent cytotoxic effects against certain cancer cells. In the present research, the cytotoxic effect of Palmyra sap collected from Kanyakumari and Tirunelveli Districts against normal cell lines were studied and the results reported significant increase in the cytotoxic effect in the increasing concentration of the sample. The sap exhibited minimal cytotoxic effects on Normal L929 cells across various concentrations, with a dose-dependent effect noted. The overall results indicate that even if both samples have less toxic effect on normal cell lines, the Palmyra palm sap samples collected from Tirunelveli district showed higher toxic effect with less viability than the samples collected from Kanyakumari district. The cytotoxic effects were more pronounced at higher concentrations, indicating potential for selective use in anticancer applications.

In the present research, the cytotoxic effect of Palmyra sap collected from Kanyakumari and Tirunelveli Districts against normal cell lines were studied and the results reported significant increase in the cytotoxic effect in the increasing concentration of the sample. The sap exhibited minimal cytotoxic effects on Normal L929 cells across various concentrations, with a dose-dependent effect noted. The overall results indicate that even if both samples have less toxic effect on normal cell lines, the Palmyra palm sap samples collected from Tirunelveli district

showed higher toxic effect with less viability than the samples collected from Kanyakumari district. The cytotoxic effects were more pronounced at higher concentrations, indicating potential for selective use in anticancer applications.

Keywords: Borassus flabellifer , Invitro Cytotoxicity Analysis, Cell Viability, MTT Assay.

Introduction

Borassus flabellifer is commonly called Palmyra palm or double palm or toddy palm. The young roots of the tree are taken as diuretic and anti-parasitic drug (Jerry, 2018). Palmyra palm is widely cultivated throughout India in different soil types and climate zones, including the coastal strip, agricultural margins, and wastelands of Karnataka, Kerala, Maharashtra, Madhya Pradesh, Andhra Pradesh, Chhattisgarh, West Bengal, Bihar, and Odisha. Among all Tamilnadu is the largest producer of palmyra fruit in India (Aman *et al.,* 2018, Asmussen *et al.,* 2006). *Borassus flabellifer* contains albuminoids, fats and the fresh pulp is reportedly rich in vitamins A and C (Pullaiah, 1997). *B. flabellifer* is used in folk medicine for multiple purposes, such as a stimulant, anti-laprotic, diuretic and antiphlogistic. The fruits are stomachic, sedative, laxative and aphrodisiac in nature useful in hyperdipsia, dyspepsia, flatulence, skin diseases, haemorrhages, fever and general debility. The roots and juice of the plant are useful in inflammatory reactions. The ash obtained by burning the inflorescence is a good antacid antiperiodic, and is useful in heart burn, splenomegaly and in bilious fever (Kapoor, 2000 and Nadkarni, 1954). Palm sap is a non-alcoholic, refreshing natural beverage that has a plethora of health benefits of various natures. The collection of the sap from the tree itself is a major challenge to the industry. As the sap is rich in microflora, it is vulnerable to fermentation instantaneously after the harvest. Toddy palm nectar (TPN) is a naturally fermented sap from young and matured inflorescences of *Borassus flabellifer* Linn. (Palmyra palm), belongs to

the family Arecaceae, and is commonly referred to as "toddy" (Zeid and Faraj Alla, 2019). Palm sap is tapped from the matured unopened inflorescence of the palm. The palm sap is collected by cutting the head of the inflorescence (Nathanael, 1966). Dietary recommendations for the prevention of cancer, atherosclerosis and other chronic diseases have been established by various health agencies. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centred free radicals and other reactive oxygen species (ROS), which are continuously, produced in vivo, result in cell death and tissue damage. The role of oxygen radicals have been implicated in several diseases, including cancer, diabetes, cardiovascular disease and aging (Halliwell B, Gutteridge, 2000). At an early stage the tender endosperm part is edible, while after ripening, the yellow coloured fibrous mesocarp squeezed to collect the pulp. The ripe fruit pulp contains beta-carotene and has anti-inflammatory effects. Traditionally the matured pulp is used for making pitha and fermented drink preparations (toddy) (Nadkarni, 1954; Vaidyaratnam, 1994). In the present study, the In vitro Cytotoxicity effect of the Palmyra sap collected from Kanyakumari and Tirunelveli Districts were analysed in L929 cell line by using MTT Assay and the sap exhibited minimal cytotoxic effects on L929 cells across various concentrations, with a dose-dependent effect noted. The cytotoxic effects were more pronounced at higher concentrations, indicating potential for selective use in anticancer applications.

Materials and Methods

1. **Collection of Palmyra palm sap samples**

Fresh Palmyra palm sap samples were collected from two geographically distributed areas, namely Kanyakumari and Tirunelveli districts of Tamil Nadu. *Borassus flabellilfer* is extensively found in the Kanyakumari region, particularly in Karungal, where the extraction of sap reaches its peak from August to November. In the Tirunelveli region, it is widely distributed in the area of

Aral, and the extraction of sap is high from April to June. Palm sap is tapped from the mature, unopened inflorescence of the palm. The palm sap was collected by cutting the head of the inflorescence. In rural areas, palm sap was traditionally collected from Palmyra trees by organized practice for its local consumers.

Figure 1. Collection of Palmyra Sap sample

The fresh samples were collected in the early morning by professional tappers. As soon as the collected fresh Palmyra sap samples were brought down from the Palmyra tree, they were transferred to sterilized bottle, packed in a sterile ice box to avoid fermentation during transportation, and transported to the laboratory for analysis. On reaching the laboratory, the Palmyra sap samples were stored in the refrigerator at about 4ºC for further analysis.

2. Invitro Animal Cell culture studies

MTT assay (Joseph *et al.*, 2012)

- **Cell lines and maintenance**

L929 cell line (mouse fibroblast cell line) was procured from the National Centre for Cell Sciences (NCCS), Pune, India.

- **Cell culture media and Maintenance**

The cells were cultured in Dulbecco's Modified Eagles Medium (DMEM-Himedia), supplemented with 10% heat inactivated foetal bovine serum (FBS) and a 1% antibiotic cocktail containing penicillin (100 U/ml), streptomycin (100 µg/ml), and amphotericin B (2.5 µg/ml). The cells containing TC flasks (25cm^2) were

incubated at 37°C at 5% CO_2 environment with humidity in a cell culture incubator (Galaxy® 170 Eppendorf, Germany).

- **Procedure**

L929 cells (2500 cells/well) were seeded on 96 well plates and allowed to acclimatize to the culture conditions, such as 37 °C and a 5% CO_2 environment, in the incubator for 24h. The Palmyra sap samples were prepared in DMEM media (100 mg/ml) and filtered and sterilized using a 0.2 µm Millipore syringe filter. The Palmyra sap samples were further diluted in DMEM media and added to the wells containing cultured cells at final concentrations of 6.25, 12.5, 25, 50, and 100 µg/ml respectively. Untreated wells were kept as control. All the experiments were done in triplicate and average values were taken in order to minimize errors. After treatment, the plates with the test samples were further incubated for 24 hours. After the incubation period, the media from the wells were aspirated and discarded.100 µl of 0.5 mg/ml MTT solution in PBS was added to the wells. The plates were further incubated for 2 hours for the development of formazan crystals. The supernatant was removed and 100 µl of DMSO (100%) were added per well. The absorbance at 570 nm was measured with a microplate reader. Two wells per plate without cells served as blanks. All the experiments were performed in triplicate.

The cell viability was expressed as follows:

$$\text{Percentage of cell viability} = \frac{\text{Average absorbance of treated}}{\text{Average absorbance of control}} \times 100$$

Result and Discussion

Cytotoxicity detection by the MTT assay

Cell viability refers to the number of viable cells in a particular cell population and analysing the living cell number is accepted as a major indicator of the impact of test material on cell survival or death. Usually, cytotoxicity assays are used for screening the retort of the cells alongside a drug or any chemical agent. The MTT assay depends on the conversion of MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) to unsolvable formazan crystals by NAD (P) H-dependent oxidoreductase enzymes released from the mitochondria of living cells. The MTT assay generally helps to detect the number of

viable cells by evaluating mitochondrial activity, which is linked with the concentration of formazan crystals (Sumantran *et al.* 2011; Van Meerloo *et al.*2011; Adan *et al.* 2016). Mosmann *et al.* (1983) reported that the seed coat extracts of *B. flabellifer* were tested for inhibitory cytotoxic effects on the HeLa cell line by MTT assay. *B. flabellifer* was administered in different concentrations and found that the growth of the HeLa cancer cells was significantly inhibited, showing better cytotoxicity to cancer cell lines. Dung Huynh Thi Le *et al.* (2020) investigated the cytoprotective ability of palm granulated sugar in NIH3T3 fibroblast cells. Palm-granulated sugar-treated NIH3T3 cells showed a higher cell viability of 18.10% to 23.68%, and this value was 45.13% for the sample without sugar. The cell viability of sugar-treated cells in this study was higher than that of white sugar and brown sugar and lower than that of jaggery sugar. In the present research, the cytotoxicity of normal L929 cell lines was observed using Palmyra sap samples from both samples by MTT assay. An increase in the cell viability less is the cytotoxic activity of the sample. The results of the two-way ANOVA for the cytotoxicity detection by the MTT assay showed that the variation due to concentration and the variation due to the sampling regions are statistically significant ($P < 0.05$) (Table. 1 and 2). The results of the MTT assay were represented based on the cell viability and cytotoxicity of normal L929 cell lines. Here, the percentage of cell viability was higher in both samples, especially the Palmyra sample of Kanyakumari, which exhibited 97.22% better viability and 96.51% in Tirunelveli, indicating that the samples are less toxic to human normal cell lines. This is an indication that the samples can be applied to normal cells without any toxic impact (Figure 2 and 3). The overall positive impact on the cellular system is reflected in the maintenance of normal cellular homeostasis, as visualized from cell morphology analysis along with the MTT assay.

Table 1.Cytotoxicity detection by MTT assay of *B. flabellifer* sap collected from two different regions

Concentration (µg/ml)	Percentage of viability	
	Kanyakumari	Tirunelveli
6.25	98.74	98.23
12.5	98.32	97.85
25	98.15	97.47
50	97.64	96.97
100	97.22	96.51

Table 2.Two-way analysis of variance on MTT assay as a function of variation between concentration and variation between sampling regions

Source of Variation	SS	df	MS	F	P-value
Variation due to concentration	3.2586	4	0.81465	135.5491	P < 0.0001
Variation due to sampling region	0.92416	1	0.92416	153.7704	P < 0.0001
Error Variance	0.02404	4	0.00601		
Total Variance	4.2068	9			

Fig. 2 L929 Cell images after treatment with Sample Kanyakumari a) control b) 6.25 µg/ ml treatment c) 12.5 µg/ ml treatment d) 25 µg/ ml treatment e) 50 µg/ ml treatment f) 100 µg/ ml treatment

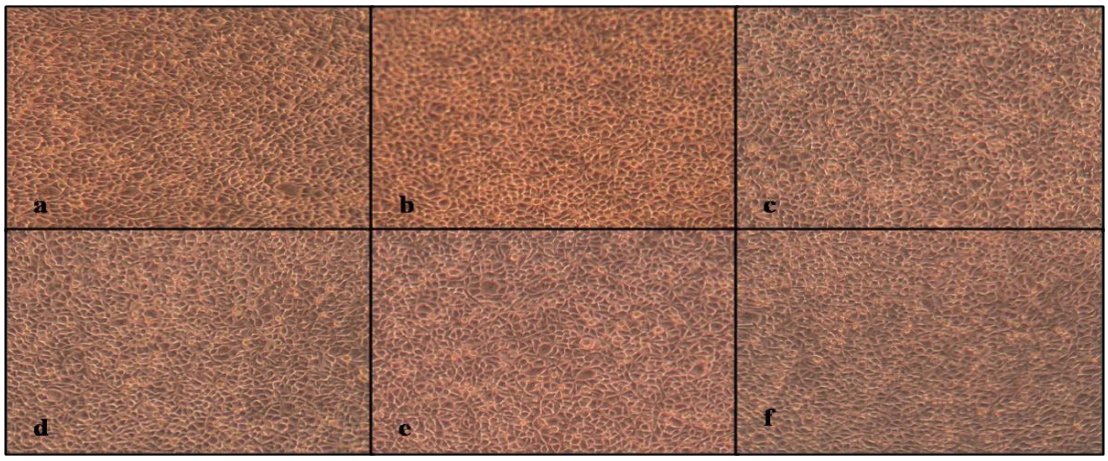
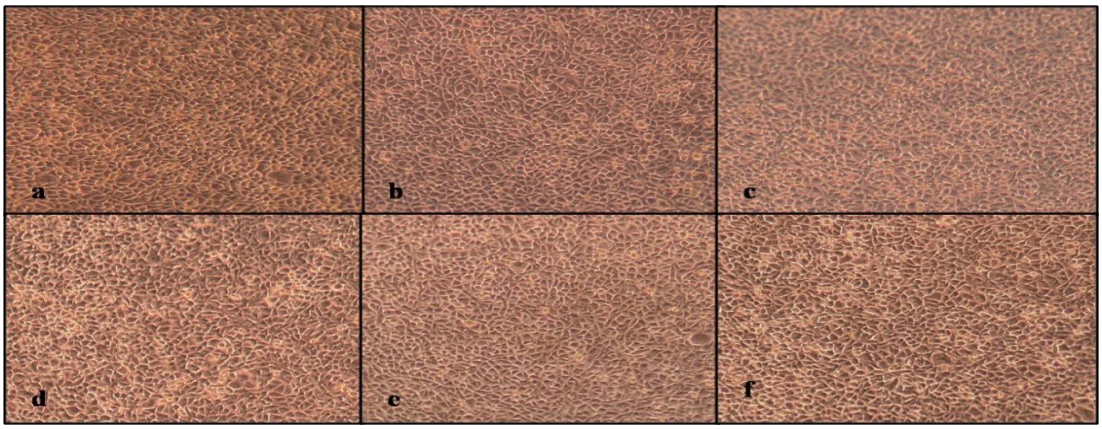


Fig. 3 L929 Cell images after treatment with Sample Tirunelveli a) control b) 6.25 µg/ ml treatment c) 12.5 µg/ ml treatment d) 25 µg/ ml treatment e) 50 µg/ ml treatment f) 100 µg/ ml treatment



Conclusion

Palmyra is one of those trees with every part has its own unique potential health benefits. In conclusion, it was observed from the present study that both Palmyra sap collected from Kanyakumari and Tirunelveli have less cytotoxic property on normal cell lines. While the concentration of *B. flabellifer* sap of Kanyakumari has the impact on the cytotoxicity of cell lines, where the percentage mortality increased with an increase in concentration. The overall positive impact on the cellular system is reflected in the maintenance of normal cellular homeostasis, as visualized with the MTT assay. This is an indication that the Palmyra sap samples can be applied to normal cells without any toxic impact.

Conflict of interest:

The authors declare no conflict of interest in this work.

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