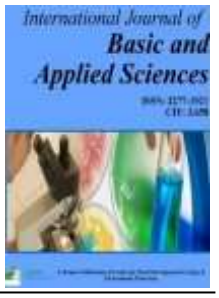
Vol. 11. No.3. 2022

DOI: 10.13140/RG.2.2.14279.16808

Contents available at:

http://www.crdeepjournal.org

International Journal of Basic and Applied Sciences (ISSN: 2277-1921) (CIF:3.658 ; SJIF: 6.823)
(A Peer Reviewed Quarterly Journal)



Full Length Research Paper

Pharmacognostical Studies, Phytochemical Screening and *In Vitro* Cytotoxic Activities of *Prosopis juliflora*, Fabaceae

Husna Kadagothy, Prakash Yoganandam G* , Mahalakshmi S, Balaji N, Sai Monohar, Gopal V

Department of Pharmacognosy, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, A Government of Puducherry Institution, Puducherry, India -605 006

ARTICLE INFORMATION

Corresponding Author:
Prakash Yoganandam G

Article history:
Received: 30-08-2022
Revised: 05-09-2022
Accepted: 11-09-2022
Published: 14-09-2022

Key words:
Anticancer, Prosopis juliflora, Fabaceae, Trypan blue dye exclusion method.

ABSTRACT

Cancer has been a constant battle globally with a lot of development in cures and prevention therapies. Literature reviews suggest that Prosopis juliflora has anti-cancer activity and still under-explored. In vitro cytotoxicity test is performed by using Trypan Blue Dye Exclusion Method. This in-vitro study shows that the plant Prosopis juliflora contains anticancer properties. The results obtained from this research through the in-vitro cytotoxic activity of hydro-alcoholic extracts of leaves of Prosopis juliflora (Sw.) using Dalton's Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) cell lines by Trypan blue dye exclusion method. The study indicated that the hydro-alcoholic extracts significantly inhibit the proliferation of DLA and EAC cell lines in a dose-dependent manner. From these findings, the research proved that the leaves of Prosopis juliflora (Sw.) is an alternative choice for the management of tumors.

Introduction

Cancer has been recognized as a popular disease in recent years. The disease is identified by the loss of programmed cell death due to genetic mutation, environmental factors, radiation, etc. If a tumor is localized to an area, it is benign and if travels to other sites it is malignant. The latter is said to be metastasis. In trend radiotherapy, chemotherapy, and chemically derived drugs are used in the treatment of cancer (Zhou, Yu and Huang, 2017). Chemotherapy may affect the patient's health and may have serious adverse effects. Therefore, alternative treatments and therapies should be focused to overcome the side effects. For ages, herbal sources of medicines have been used and are still used as the primary source of treatment. These herbal medicines may have lesser toxicity than conventional medicine. The plant *Prosopis juliflora* is mostly used as feed for animals and used for firewood. The 'pods' of this plant contain sugar and it is consumed as a pod syrup, fermented beverage and in baked products (Ukande *et al.*, 2019). *Prosopis juliflora* shows many pharmacological activities such as antioxidant, anti-bacterial, anticancer, antifungal etc., (Utage *et al.*, 2018).

The legume is straw-yellow or brown in color (8-29 cm long, 9-17 mm wide, 4-8 mm thick) and the plant has spines that are 0.5-5 cm long (Shiferaw *et al.*, 2004). Conventionally *Prosopis juliflora* is used as indigenous medicine for diseases like inflammation, sore throat, dysentery, cold, diarrhea, swelling, measles, flu, and in the healing of wounds (Badri *et al.*, 2017). Different aspects like anticancer, antioxidant, antagonistic effect and anti-bacterial activities of *Prosopis juliflora* have been described (Preeti, Ram Avatar and Mala, 2015). The Prosopis genus has approximately 44 species; *Prosopis tamarugo*, *Prosopis nigra*, *Prosopis argentina*, *Prosopis glandulosa*, *Prosopis juliflora*, *Prosopis alba* etc., (FA *et al.*, 2016). In our study, we have taken the *Prosopis juliflora* species. Ethyl acetate extracts of *Prosopis juliflora* have shown cytotoxic effect on hepatocellular carcinoma, colorectal carcinoma and breast adenocarcinoma (Elbehairi *et al.*, 2020). The alkaloid-rich pods of *Prosopis juliflora* can be used as a promising feed to reduce the production of gas amid ruminal digestion (dos Santos *et al.*, 2013). *Prosopis juliflora* is a good adsorbent (Gautam *et al.*, 2020). *Prosopis juliflora* pods are an important source of anticancer and chemoprotective compounds like terpenoids (Malik, Ahmed and Khan, 2018).

Materials and methods

Materials, Instruments and Chemicals

Plant material, Grinding mixer, Soxhlet apparatus, Glycerin, Alcoholic ferric chloride, Phloroglucinol, Iodine solution, Ruthenium red, Ethanol, Lead acetate, Sudan III red.

Prosopis juliflora sample collection

Prosopis juliflora parts were collected from matured plants from Gorimedu, Puducherry. Plant parts like leaves, pods and flowers were collected with relevant guidelines and regulations. Leaves, fruits and pods were then washed properly, dried and made herbarium (Voucher specimen no. MTPG/COP/PCG/2020/012). The dried leaves were powdered using a wearing blender in the Pharmacognosy Laboratory, MTPG&RIHS, Puducherry and kept in a moisture-free environment.

Soxhlet extraction

Extraction is carried out by using a Soxhlet apparatus. For the collection of ethanolic extract the continuous hot percolation method (Soxhlation) was used. To extract continuously with fresh solvent this technique uses the principle of reflux and siphoning. 500ml of ethanol is taken in a round bottom flask. The round bottom flask is connected to the Soxhlet extractor and condenser on a thermostatic mantle. Dried and powdered leaves are filled in the thimble and the thimble is kept inside the Soxhlet extractor. The Siphon tube is well plugged with cotton wool. Under reflux, the solvent is heated. Condensation and extraction take place with fresh solvent. This process is repeated. This process was carried out for a total of 24 hours in the Pharmacognosy Laboratory. Using a rotary vacuum evaporator the ethanol is evaporated when the process is finished (Redfern *et al.*, 2014; Tzanova *et al.*, 2020).

Macroscopical studies

The macroscopical study of the collected sample of *Prosopis juliflora* is carried out by naked eyes and in luminescent light for their color, size, and shape. The odour and taste of the sample were noted. All these noted observations are given in the result section.

Phytochemical tests

The detection of alkaloids is carried out by using Mayer's reagent and Dragendroff's reagent. Detection of cardiac glycosides by Keller-kiliani test anthraquinone detection by Borntrager's test. Identification of flavonoids is done by using the Shinoda test and alkaline reagent test. Test for saponins carried out by froth test. Detection of tannins by lead acetate test and ferric chloride test and the ethanolic extract is detected for the presence of coumarins (Sathiya and Muthuchelian, 2008; Mani, 2017).

Trypan Blue Dye Exclusion Method

The test sample is studied for the interim term *in vitro* cytotoxicity by using Dalton's Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) cells. The tumorous cells are collected from the peritoneal cavity of the mice suffering from a tumor. The cells are washed thrice with normal saline or phosphate buffer saline. The potentiality of the cell is determined by the trypan blue dye exclusion method. Potential cell suspension (1×10^6 cells in 0.1 ml) is added to microtitre well tubes which contain different concentrations of the test sample ranging from 10 μg/ml to 200 μg/ml. The volume is made up to 1ml using phosphate buffer saline. The Control tube contains only cell suspension. The assay mixtures are incubated for 3 hours at 37 ℃. The further cell suspension is mixed with 0.1 ml of 1 % trypan blue dye and kept for 2 to 3 minutes and loaded on a hemocytometer.

$$\% \text{ Cytotoxicity} = \text{No. of dead cells} \div (\text{No. of live cells} + \text{No. of dead cells}) \times 100$$

Results and discussion

In this study, the anti-cancer effect of *Prosopis juliflora* leaf extract using the Trypan Blue Dye Exclusion method against DLA and EAC cell lines are reported. Cancer is frequently affecting humans with high mortality rates. It is believed to be the most deadly disease affecting the human population. About 7.6 million people lost their lives due to cancer in 2008 (Priya *et al.*, 2015).

The color and odour of the leaves were noted visually. Thorny shrub with light green leaves with up to 20 leaflets. Flowers were 6-10 cm long greenish yellow colored. The leaves were odorless. The leaves are pinnately compound and the leaflets are oblong in shape with a blunt apex, petiolate, reticulate venation, entire margin, and the petiole is 2-3 cm long. Phytochemical tests done in the hydroalcoholic extracts of leaves of *Prosopis juliflora* gave positive results for flavonoids, tannins, alkaloids, coumarins and anthraquinone glycoside.

Table 1 Percentage cytotoxicity of the hydro-alcoholic extract of leaves of *Prosopis juliflora* on the DLA cell line.

Drug Concentration $(\mu g/ml)$	**%Cytotoxicity**
10	9.9 ± 1.70
20	18 ± 2.84
50	32 ± 2.33

100	57 ± 1.98
200	77 ± 1.82

Results were expressed Mean ± SEM. n = 3

Research on *Prosopis juliflora* by Elbehairi and colleagues investigated the potential anti-cancer effect and identified its chemical composition. They investigated on MCF-7 (breast), HepG2 (liver), and LS-174T (colorectal) cancer cell lines. Anticancer activity of *Prosopis juliflora* by using MCF-7, HePG2 and LS-174T cell lines was determined and the calculated IC_{50} was noted as 18.17, 33.1 and 41.9 µg/ml for MCF-7, HePG2 and LS-174T respectively. Their study revealed that the cytotoxic action of *Prosopis juliflora* extracts was mainly via necrosis not by apoptosis. The major constituents identified in the ethyl extracts of *Prosopis juliflora* leaves were hydroxymethyl-pyridine, nicotinamide, adenine, and poly-(methyl methacrylate) (PMMA) by employing liquid chromatography-mass spectrometry. Their analysis revealed that the ethyl acetate extracts of *Prosopis juliflora* have a potential anti-cancer effect against breast adenocarcinoma, hepatocellular carcinoma, and colorectal adenocarcinoma (Elbehairi *et al.*, 2020). Utage and his co-workers prepared methanol extract of the *Prosopis juliflora* and exposed it to human breast cancer cell lines-MDA-MB-23, and MCF-7 and human keratinocytes HaCaT as a representative of normal cells and 4T1 cel1s, BALB/c xenograft mouse model. The study revealed that the methanol extract of *Prosopis juliflora* leaves holds impressive anti-breast cancer activity more precisely against triple-negative breast cancer cells, while the in vivo studies demonstrated that *Prosopis juliflora* leaves extract significantly repressed the 4T1 induced tumor growth (Utage *et al.*, 2018). The promising results from the Trypan Blue Dye Exclusion method was obtained. The blue color of trypan blue dye was taken up by the dead cells while live cells do not take up the trypan blue dye. Ehrlich ascetic tumor grows rapidly and the local inflammatory reaction results in ascetic fluid formation which provides nutrition to the tumor cells (Fecchio *et al.*, 1990; Segura, Barbero and Márquez, 2000). When the concentration of the sample was increased percentage of cytotoxicity also increased. The cytotoxicity of the sample observed in cancer cells at the highest concentration (200 µg/ml medium) was found to be 77 ± 1.82 % for DLA and 80 ± 1.28 % for EAC cell lines. This research work proved that the plant *Prosopis juliflora* has potential anticancer activity. This method confirms the anticancer activity of the *Prosopis juliflora*. Stained and unstained cells were counted separately using a hemocytometer. Reports obtained are given in table 1 and table 2.

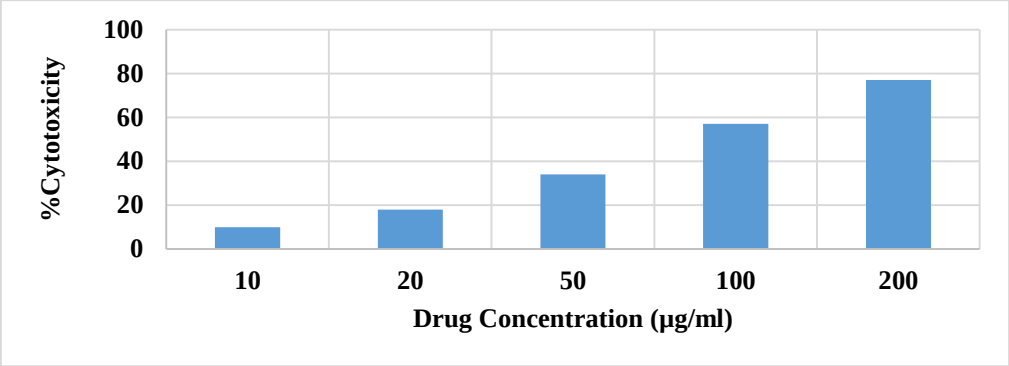


Fig 1 Histogram showing cytotoxic activity of the ethanolic extract of leaves of *Prosopis juliflora* on DLA cell line.

Table 2 Cytotoxicity Of The Hydro-Alcoholic Extract Of Leaves Of Prosopis Juliflora On The EAC Cell Line

Drug Concentration $\mu g/ml$	**%Cytotoxicity**
10	9.2 ± 1.72
20	20 ± 2.18
50	34 ± 2.23
100	59 ± 1.99
200	80 ± 1.28

Results were expressed Mean ± SEM. n = 3

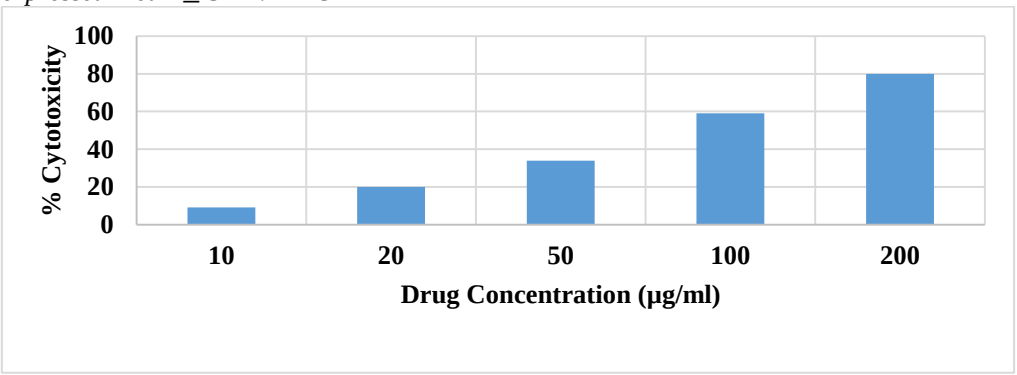


Fig 2 Histogram showing cytotoxic activity of the ethanolic extract of leaves of *Prosopis juliflora* on EAC cell line.

Conclusion

The present study involves the Pharmacognostic aspects such as collection, authentication, solvent extraction, macroscopical studies, analysis, preliminary phytochemical screening and the *in vitro* cytotoxic study of fresh leaves of *Prosopis juliflora* (Sw.) DC, (Fabaceae). Based on preliminary studies and literature surveys revealed that the plant has been extensively used as a herbal anti-cancer drug. The results obtained from our research through *in vitro* cytotoxicity of hydro-alcoholic extracts of *Prosopis juliflora* (Sw.) DC on DLA and EAC cell line by using Trypan blue exclusion method indicated that the hydro-alcoholic extracts significantly inhibits the proliferation of DLA and EAC cell lines in a dose-dependent manner. From the findings, this research proved that the plant *Prosopis juliflora* has promising cytotoxic action and can act as a promising molecule in near future. This plant should be further investigated and formulated for treating cancer. Further preclinical studies may provide a promising way to utilize this pant.

Acknowledgement

Authors are thankful to Dean and Principal, College of Pharmacy, Mother Theresa Post Graduate & Research Institute of Health Sciences, Puducherry for their encouragement and support and we are thankful to the Amala Cancer Research Centre Society, Thrissur for providing *in vitro* study services.

References

Badri, A.M. *et al.* (2017) 'Antioxidant activity and phytochemical screening of Prosopis juliflora leaves extract', *Advancement in Medicinal Plant Research*, 5(3), pp. 37–40. Available at: https://doi.org/10.30918/AMPR.53.17.020.

Elbehairi, S.E.I. *et al.* (2020) 'Prosopis juliflora leave extracts induce cell death of mcf-7, hepg2, and ls-174t cancer cell lines', *EXCLI Journal*, 19, pp. 1282–1294. Available at: https://doi.org/10.17179/excli2020-2830.

FA, P. *et al.* (2016) 'Overview of Genus Prosopis Toxicity Reports and its Beneficial Biomedical Properties', *Journal of Clinical Toxicology*, 06(05). Available at: https://doi.org/10.4172/2161-0495.1000326.

Fecchio, D. *et al.* (1990) 'Studies on inflammatory response induced by Ehrlich tumor in mice peritoneal cavity', *Inflammation*, 14(1), pp. 125–132. Available at: https://doi.org/10.1007/BF00914035.

Gautam, A.K. *et al.* (2020) 'Lead removal efficiency of various natural adsorbents (Moringa oleifera, Prosopis juliflora, peanut shell) from textile wastewater', *SN Applied Sciences*, 2(2). Available at: https://doi.org/10.1007/s42452-020-2065-0.

Malik, S.K., Ahmed, M. and Khan, F. (2018) 'Identification of novel anticancer terpenoids from Prosopis juliflora (Sw) DC (Leguminosae) pods', *Tropical Journal of Pharmaceutical Research*, 17(4), pp. 661–668. Available at: https://doi.org/10.4314/tjpr.v17i4.14.

Mani, P. (2017) 'PHYTOCHEMICAL AND PHARMACOLOGICAL ANALYSIS OF ETHANOLIC EXTRACTS OF PROSOPIS JULIFLORA (SW.)', *World Journal of Pharmacy and Pharmaceutical Sciences*, pp. 2059–2069. Available at: https://doi.org/10.20959/wjpps20179-10109.

Preeti, K., Ram Avatar, S. and Mala, A. (2015) 'Pharmacology and Therapeutic Application of Prosopisjuliflora: A', *Review. Journal of Plant Sciences*, 3(4), pp. 234–240. Available at: https://doi.org/10.11648/j.jps.20150304.20.

Priya, N.P. *et al.* (2015) 'Cytotoxic and antitumour studies of acetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone and its transition Metal Complexes', *Indian Journal of Pharmaceutical Sciences*, 77(6), pp. 655–660. Available at: https://doi.org/10.4103/0250-474X.174981.

Redfern, J. *et al.* (2014) 'Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties', *Journal of Microbiology & Biology Education*, 15(1), pp. 45–46. Available at: https://doi.org/10.1128/jmbe.v15i1.656.

dos Santos, E.T. *et al.* (2013) 'Antibacterial activity of the alkaloid-enriched extract from Prosopis juliflora pods and its influence on in Vitro ruminaldigestion', *International Journal of Molecular Sciences*, 14(4), pp. 8496–8516. Available at: https://doi.org/10.3390/ijms14048496.

Sathiya, D.C. and Muthuchelian, M. (2008) *Investigation of Phytochemical Profile and Antibacterial Potential of Ethanolic Leaf Extract of Prosopis juliflora DC.*, *Ethnobotanical Leaflets*.

Segura, J.A., Barbero, L.G. and Márquez, J. (2000) 'Ehrlich ascites tumour unbalances splenic cell populations and reduces responsiveness of T cells to Staphylococcus aureus enterotoxin B stimulation', *Immunology Letters*, 74(2), pp. 111–115. Available at: https://doi.org/10.1016/S0165-2478(00)00208-X.

Shiferaw, H. *et al.* (2004) 'Some biological characteristics that foster the invasion of Prosopis juliflora (Sw.) DC. at Middle Awash Rift Valley Area, north-eastern Ethiopia', *Journal of Arid Environments*, 58(2), pp. 135–154. Available at: https://doi.org/10.1016/j.jaridenv.2003.08.011.

Tzanova, M. *et al.* (2020) 'Selectivity of current extraction techniques for flavonoids from plant materials', *Processes*, 8(10), pp. 1–30. Available at: https://doi.org/10.3390/pr8101222.

Ukande, M.D. *et al.* (2019) 'Review on Pharmacological potentials of Prosopis juliflora', *Journal of Drug Delivery and Therapeutics*, 9(4-s), pp. 755–760. Available at: https://doi.org/10.22270/jddt.v9i4-s.3372.

Utage, B.G. *et al.* (2018) *Prosopis juliflora (Sw.), DC induces apoptosis and cell cycle arrest in triple negative breast cancer cells: in vitro and in vivo investigations*. Available at: www.oncotarget.com.

Zhou, J., Yu, G. and Huang, F. (2017) 'Supramolecular chemotherapy based on host-guest molecular recognition: A novel strategy in the battle against cancer with a bright future', *Chemical Society Reviews*, 46(22), pp. 7021–7053. Available at: https://doi.org/10.1039/c6cs00898d.