E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/16.01.117

Research Article

In Vivo Study on the Anticancer Property of Pugasaram: An Arecanut Based Polyherbal Gel in Oral Cell Carcinoma

Jeddu Ganapathi Bhat[1], S. Keshava Bhat[2], Sukesh Bhat[3], D Shobha[4*], S Mythri[5]
[1]Director, Jeddu Ayurveda Research Centre, Alike: 574 235, Dakshina Kannada, Karnataka, India.
[2]Executive Officer and Scientist, Arecanut Research and Development Foundation, Varanashi Towers, Mission Street, Mangalore: 575 001, Karnataka, India
[3]Assistant Professor, Kanachur Institute of Medical Sciences, Deralakatte: 575018, DK, Karnataka, India.
[4*]Associate Professor, Centre for systems Biology and Molecular Medicine, Yenepoya Research Centre, Deralakatte, 575 018, DK, Karnataka, India.
[5]Associate Professor, Century International Institute of Dental Science and Research Centre, Poinachi,671 541, Kasaragod, Kerala, India.
Corresponding Author: [4*]D Shobha
Received: 30.01.26, Revised: 10.02.26, Accepted: 13.03.26

ABSTRACT
Cancer is one of the principle causes of human mortality in all countries. Surgery, chemotherapy and radiotherapy are the usual therapeutic remedies for this disease. As there are several side effects of such treatments, the researchers are constantly in search of certain alternate remedial measures using promising herbal products. Several plants are already known for their anticancer properties. In Ayurvedic system of medicine, preparations using several herbs, called polyherbal products, are generally preferred over single herb preparation. In the present study, the anticancer property of a polyherbal gel known as Pugasaram, prepared mainly by using arecanut and betel leaf as the main ingredients along with ten other plants / plant products, was studied in cancer induced mice. The preliminary toxicity study revealed that there was no change in the behaviour of mice fed with this gel at a concentration of 2000mg/kg body weight. The anticancer efficacy profile of Pugasaram showed a significant reduction in the size of tumors and their progression compared to the untreated DMBA applied mice and showed even better than that of cisplatin treated group.

Keywords: Arecanut; Betel Leaf; Pugasaram; Anticancer; Polyherbal Gel.

INTRODUCTION

Cancer is an important cause of mortality worldwide irrespective of the status of human development[1]. This disease is characterised mainly by uncontrolled cell multiplication and proliferation. If not treated properly it spreads to other tissues and organs leading to multiple complications. The conventional treatments such as surgery, chemotherapy and radiotherapy often pose severe side effects. Hence, researchers are in search of certain alternate chemotherapeutic agents from plant sources with less side effects.Since the beginning of human civilization plants are being used for treating several diseases[2,3]. In Ayurvedic system of medicine, herbal preparations using multiple plants (polyherbal products) are generally prescribed as the remedy for treating such diseases[4]. The concept of polyherbal remedy, exploiting the combined therapeutic values of different plants, originated long back[5]. Compared to the single herbal product, the polyherbal preparations have several advantages such as lesser side effects, lower toxicity, synergistic activities and better results[6]. Because of these advantages, polyherbal formulations are now increasingly becoming popular over conventional medicines. In the present study, 12 plants / plant parts or products, namely the dried nuts of *Areca catechu* 20%, the dried leaf of *Piper betle* 20%, dried barks of *Mimusops elengi* 4%, *Areca catechu* 2%, *Azadirachta indica* 2%, *Ficus bengalensis* 2% and *Pongamia pinnata* 2%, dried rhizomes of *Curcuma longa* 2% and *Alpinia calcarata* 2%, dried flower buds of *Syzygium aromaticum* 2%, dried fruits of *Piper longum* 2% and honey 40%, already known for their anticancer properties, are used to prepare the gel Pugasaram. Arecanut (the fruit of areca palm, *A. Catechu* L. of Arecacea family) and betel leaf (the leaf of betel vine *P. betle* L. of Piperaceae family) are the two ubiquitous

ingredients of betel quid, a popular chewing mixture in India and several other countries[7]. Both these plant parts exhibit several medicinal properties including anticancer effects[8-13]. Considering this knowledge and using arecanut and betel leaf as the main ingredients, the polyherbal gel Pugasaram was prepared and its anticancer property was studied in oral cancer induced mice.

MATERIALS AND METHODS

Formulation preparation

A polyherbal preparation, was prepared using 12 different plant products, including areca nut (endosperm of unripe fresh fruit) and betel leaf (fresh green leaf) as the main ingredients[14]. The whole unripe endosperm of areca fruit and fresh betel leaf are macerated together using distilled water, strained, and the juice was obtained. Other herbs are powdered individually and mixed with the above juice, ground well and dried in the shade. After proper drying, the mixture is minced and macerated well with honey into a thick emulsion called 'Pugasaram'. The authentication of the medicinal plants was done at Jeddu Ayurveda Adhyayana evamAnusandhana Samstha, Alike - 574235, Dakshina Kannada, Karnataka, India.

Animals

Male Swiss albino mice, 8-10 weeks old and weighing 25-30 g, procured from Spring Labs, Tumakuru, India were used in the study, after obtaining Institutional animal ethics committee approval (YU/IAEC/P11/2024). The animals were housed in polypropylene cages under a 12 h dark/light cycle with feed and water *ad libitum*. They were acclimatized in the central animal facility in the Department of Pharmacology, Yenepoya Medical College, Yenepoya (Deemed to be University).

Acute Toxicity Test

The test was performed as per OECD 425 guidelines for the determination of acute oral toxicity. Pugasaram was administered orally to mice (n=5) one time at 2000mg/kg bodyweight and the animals were monitored upto 14 days for behavioural signs of toxicity.

Induction of Oral Cancer in Animals and Assessment of Anticancer Efficacy

DMBA-induced oral squamous cell carcinoma (OSCC) was used to evaluate the anticancer efficacy of Pugasaram, in comparison with the standard chemotherapeutic drug, cisplatin. The experiment was conducted in four groups of mice (n=8 in each group) as shown in table1:

Table 1. Experimental Design

Group	Name	Treatment Protocol	Duration
Group 1	Control Group	Healthy mice receiving no treatment	Entire study period
Group 2	DMBA-Induced Oral Cancer Group	Topical application of 0.5% 7,12-dimethylbenz[a]anthracene (DMBA) in acetone to the lower lip three times per week to induce oral squamous cell carcinoma (OSCC)	8 weeks (Week 1–8)
Group 3	DMBA+Cisplatir Treatment Grou	Mice received DMBA as in Group 2, followed by oral cisplatin 3 mg/kg body weight once every two weeks starting from week 9	8 weeks (Week 9–16)
Group 4	DMBA + Pugasaram Treatment Group	Mice received DMBA as in Group 2. Pugasaram gel (200 mg/kg body weight) applied topically to the tumor area once daily starting from week 9	8 weeks (Week 9–16)

At the end of the experimental period, all animals were sacrificed by euthanasia. Tumor incidence, number, and size were recorded. Oral tissues from the different experimental groups were fixed in formalin, dehydrated using different grades of alcohol, and then paraffin-embedded. The blocks were cut into sections of 5 µm thickness and stained using hematoxylin-eosin. Photomicrographs were captured using a brightfield microscope. Histopathological analyses were conducted according to standard protocols to assess morphological changes, degree of dysplasia, and carcinoma progression.

RESULTS

Acute toxicity

Feeding of Pugasaram at 2000mg/kg body weight did not induce any toxicity in mice.

There was no change in the behaviour of such mice even up to 14 days of observation and the treated mice were all found normal and healthy.

Anticancer activity

After 8-weeks of DMBA application, visible tumors began to appear on the lower lips of mice, indicating successful tumor induction. On observation it was noticed that treatment of Pugasaramon DMBA applied group led to a significant reduction in both tumour size and progression compared to the untreated DMBA applied group. Further, hair regrowth was observed in the tumour-affected region following Pugasaram administration (Figure 1). Additionally, the anticancer efficacy profile of Pugasaram showed better than that of cisplatin. Although cisplatin effectively suppressed tumour growth, several mice in this group showed signs of metastasis. In contrast, no metastasis was observed in the Pugasaram treated mice. Further, Pugasaram treated mice demonstrated improved epithelial integrity and reduced histopathological markers of malignancy.

Histopathological examinations of oral cancer tissues induced by DMBA application showed invasive squamous cell carcinoma with disturbed basement membrane architecture, hyperkeratosis, and substantial epithelial dysplasia (Figure 2). Reduced mitotic figures and partial restoration of epithelial integrity were noted in cisplatin-treated animals, suggesting modest anticancer efficacy. Significant histological improvements, such as re-epithelialization, decreased nuclear pleomorphism, and low keratin pearl formation, were observed in mice treated with the Pugasaram, indicating strong chemo preventive potential. Angiogenesis and inflammatory infiltration were significantly reduced in the Pugasaram treated animals.

Figure 1. In vivo anticancer activity of Pugasaram in Swiss albino mice

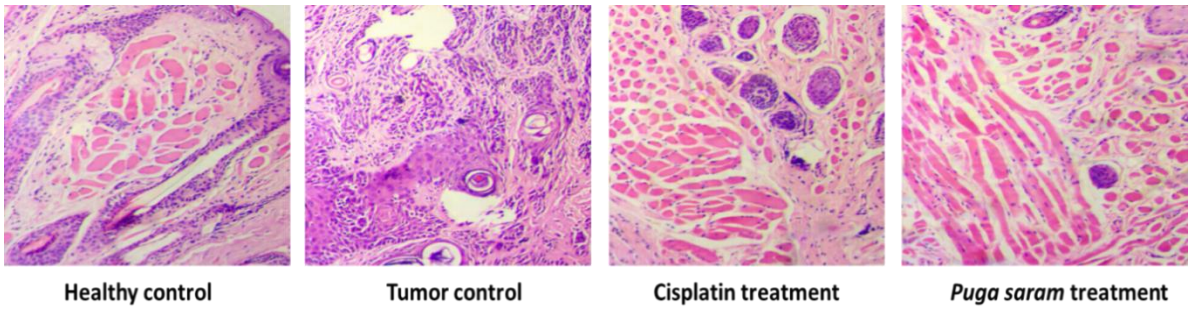

Figure 2. Photomicrographs of H and E-stained oral tissues of mice from different treatment groups

DISCUSSION

Earlier experiments conducted on the toxicity of arecanut and betel leaf reported that both these plant parts were safe to animals[15,16]. The present observation is in conformity with this. The gel prepared by using arecanut and betel leaf as the main ingredients was found nontoxic and well tolerated by mice at a dose of 2000mg/kg body weight. *In vitro* studies conducted earlier revealed that Pugasaram was anticancerous against human lung cancer (A549) and oral squamous cell carcinoma

(CAL-27) cells with IC50 values of 109.32 µg/ml and 500.00 µg/ml, respectively [14,17]. The present *in vivo* study also showed similar results. Further, in the present observation, the efficacy of Pugasaram was found even better than that of the conventional chemotherapy drug cisplatin against OSCC cells.Arecanut and betel leaf are the two main plant parts used in the preparation of Pugasaram. Further, both these plant parts are the main ingredients of the common chewing mixture called betel quid (BQ) which is chewed mainly for its useful properties [18].

Several *in vivo* studies carried out on BQ containing arecanut, betel leaf and lime (calcium hydroxide) but without tobacco, also reported anticancer properties. It was observed that in mice, the extracts of arecanut as well as BQ exhibited inhibitory effects on the development and growth of tumors induced by chemical carcinogen 3:4, benzpyrene[19]. In another *in vivo* study, application of the chemical carcinogen, DMBA, for six months on the cheek pouches of hamsters, 70% animals produced carcinoma, but when DMBA was applied even up to 10 weeks after the application of BQ for 36 weeks no cancer development was noticed[20].

There are evidences to show that chewing of traditional form of BQ or `*tambula*' without tobacco is a healthy practice[21,22]. In a survey conducted on 917 chewers and non-chewers of BQ, it was noticed that among 292 BQ chewers without tobacco, incidence of cancer was not reported, whereas among 232 non-chewers the incidence of cancer was 0.86% and among 393 chewers of BQ with tobacco (BQT) it was 0.25%. Similarly, overall health problems reported were 13.70% in BQ chewers, 31.03% in non-chewers and 18.07% in BQT chewers[23].

Sole arecanut was also reported to be anticancerous. In hepatocellular carcinoma (HCC) xenograft mice models, injection of arecanut extract at 20mg/kg body weight inhibited proliferation of HCC cells[24]. In a study conducted on rats, feeding of chemical carcinogen, 4NQO in drinking water for 12 weeks produced OSCC in 71.43% of the animals, but, when arecanut extract was fed at 500mg/kg body weight for 22 weeks after feeding 4NQO for 12 weeks no OSCC was detected[25]. Even Arecoline, the most active chemical constituent of arecanut, was reported to arrest the growth of diverse human cancer cells such as leukemia (K562) and lung carcinoma (H 1299) in xenograft mice models at the concentration of 50mg/kg bodyweight[26]. Betel leaf is another major ingredient of Pugasaram. Several reports say that this leaf also exhibits effective anticancer property. In a study conducted on Ehrlich ascites carcinoma (EAC) xenograft mice, it was reported that the methanolic extract of betel leaf exhibited significant anti-tumor activity[27]. Similarly, oral feeding of betel leaf extract at 400 mg/kg body weight daily for six weeks showed a remarkable recovery in human prostate tumor xenograft mice[28]. Hydroxychavicol (HC), the major chemical compound found in betel leaf exhibits even better anticancer properties than its leaf extract. The reports show a remarkable inhibition of prostate tumors in xenograft mice by 72% by daily oral administration of 150 mg/kg bodyweight of HC for six weeks[29]. In another study conducted on human chronic myelogenous leukemia (CML) xenograft mice models, oral administration of NPB001-05, an active component derived from betel leaf, also showed anti-tumor activity[30].

Likewise, there are reports to show that all other ingredients of Pugasaram such as dried barks of *M.elengi*, *A. catechu*, *A. indica*, *F. bengalensis* and *P. pinnata*, dried rhizomes of *C. longa* and *A.calcarata*, dried flower buds of *S. aromaticum*, dried fruits of *P. longum* and honey also exhibit anticancer properties[31-40]. All these together improved the effectiveness of Pugasaram over the conventional chemotherapy medicationCisplatin.

CONCLUSION

The present study provides first evidence-based support and molecular legitimacy of using this polyherbal ayurvedic preparation Pugasaram in cancer therapy. Treatments using this ayurvedic gel might offer an alternative choice besides chemotherapy and radiotherapy. This study provides the scientific evidence to conduct clinical trials on oral cancer patients.

ACKNOWLEDGEMENT

The Central Arecanut & Cocoa Marketing & Processing Cooperative Limited, Mangaluru, Karnataka, India provided financial support for this study.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185

countries. CA: A Cancer Journal for Clinicians. 2018; 68(6): 394-424. https://doi.org/10.3322/caac.21492.

2. Kirtikar KR, Basu BD, An ICS: *Areca catechu*. In: Indian Medicinal Plants. (Eds: Blatter E, Caius JF, Mhaskar KS) Bishen Singh Mahendra Pal Singh, Dehra Dun, India. 1918; p. 2547-2591.
3. Tavera PDTH. The medicinal plants of the Phillippines. P. Blakiston's Son & Co., 1012 Walnut Street, Philadelphia. 1901; p. 234-236.
4. Sujatha S, Shalin JJ. Complementary Therapeutic Potential: A Focus on Polyherbal Products for Hyperglycemia. Asian Journal of Scientific Research, 2012; 5: 1-13.
5. Srivastava S, Lal VK, Pant KK. Polyherbal formulations based on Indian medicinal plants as antidiabetic phytotherapeutics. Phytopharmacology, 2012; 2(1) 1-15.
6. Rooney D. Betel chewing traditions in South-East Asia (Oxford University Press, Kuala Lumpur, Malaysia), 1993; 59pp.
7. WHO. Monographs on the evaluation of carcinogenic risks to humans. Betel quid and arecanut chewing and some arecanut derived nitrosamines.2004; Vol 85. IARC, Lyon, France. 349pp.
8. Jaiswal P, Kumar P, Singh VK, Singh DK. *Areca catechu* L.: A valuable herbal medicine against different health problems. Res J Med Plants 2011; 5(1): 145-152.
9. Keshava Bhat, S. 2019. Arecanut (*Areca catechu* L): A storehouse of medicines. Indian Journal of Arecanut, Spices & Medicinal Plants 21(1): 40-55.
10. Salehi B, Konovalov DA, Fru P, Kapewangolo P, Peron G, Ksenija MS. et al. *Areca catechu*- From farm to food and biomedical applications. Phytother Res. 2020; 34(9):2140-2158.
11. Rai MP, Ramdas KT, Palatty PL, Rao P, Rao S, Bhat HP, Baliga MS. *Piper betle* Linn (Betel Vine), the maligned Southeast Asian medicinal plant possesses cancer preventive effects: Time to reconsider the wronged opinion. Asian Pacific J Cancer Prev. 2011; 12, 2149-2156.
12. Sushma R, GundalaSR, Aneja R. *Piper betle* Leaf: A reservoir of potential xenohormetic utraceuticals with cancer-fighting properties. Cancer Prev Res.2014;7(5): 477-486.
13. Alam B, Majumder R, Akter S, Lee SH. *Piper betle* extracts exhibit antitumor activity by augmenting antioxidant potential. Oncology letters 2015; 9: 863-868.
14. Ganapathi Bhat, J., Manorama Bhat, G., Keshava Bhat, S., Sukesh Bhat, Pujari G.R. Anticancer property of an ayurvedic polyherbal preparation chiefly containing arecanut and betel leaf. International Journal of Research in Ayurveda and Pharmacy2023; 14(1): 13-16.
15. Hindayani S, Jenie RI, Susidarti RA. The safety of areca seed ethanolic extract as potential chemopreventive agent is proven by acute toxicity test. Indonesian Journal of cancer chemoprevention2012;3(1): 345-350.
16. Choudhary D, Kale.RK. Antioxidant and non-toxic properties of *Piper betle* leaf extract: in vitro and in vivo studies. Phytotherapy Research2002; 16(5): 461-466.
17. Ganapathi Bhat J, Shobha D, Keshava Bhat S, Sukesh Bhat, Mythri S. In vitro evaluation of anticancer property of Pugasaram: a polyherbal gel containing mainly arecanut (*Areca catechu*) and betel (*Piper betle*) leaf on oral squamous cell carcinoma. International Journal of Ayurvedic and Herbal Medicine 2025; 15 (5) 5130-5138.
18. Shrihari JSB, Blank MD, Balster RL, Mimi N, Mark N. Areca nut dependence among chewers in a South Indian community who do not also use tobacco. Addiction 2010; 105: 1303-1310.
19. Kumari HL, Sirsi M, Bhargava MK. Inhibitory activity of *Areca catechu* on the development of mouse skin tumors induced by the chemical carcinogen 3.4, benzpyrene. J Plantn Crops 1974; 2(1): 23-29.
20. Wong TY, Jin YT, Chen HO, Lin LM. Studies on Taiwan betel quid carcinogenicity in hamster cheek pouch. Chinese Dental Journal 1992; 11(4): 155-162.
21. Akhilesh S, Shukla A, Baghel AS, Vyas M. Ayurvedic Tambula Sevana- A healthy traditional practice. International Journal of Herbal Medicine 2015; 3(1): 40-44.
22. Keshava Bhat S, Sukesh Bhat, Jagadish, Khandige SN, Kumar P. Chewing farm fresh Betel Quid: a healthy practice in

major areca nut (*Areca catechu* L.) growing region of South India. IOSR J Pharmacy Bio Sci. 2020; 15(5): 10-21.

23. Jose CT, Keshava Bhat S, Chandran KP, Jayasekhar S, Gowda A. Traditional chewing of areca nut / betel quid and human health – a survey report. Indian J Areca nut, Spices and Med Plants 2020; 22(1): 3-15.
24. Wei PL, Hung CS, Lu HH, Batzorig U, Huang CY, Chang YJ. Areca nut extract (ANE) inhibits the progression of hepatocellular carcinoma cells via activation of ROS production and activation of autophagy. Int. J. Med. Sci. 2021; 18(15): 3452-62. doi: 10.7150/ijms.6157.
25. Sari LM, Novita CF, Andriany P, Sari DK. The chemotherapeutic activity of areca nut extract increased stromal tumor-infiltrating lymphocytes in 4 nutriquinoline-1-oxide-tumor-induced Sprague-Dawley rats F1000Research 2023; 11:1571.
26. Fan J, Lin R, Xia S, Chen D, Elf SE, Liu S, et al. Tetrameric acetyl-CoA acetyltransferase 1 is important for tumor growth. Molecular Cell 2016; 64(5): 859-874.
27. Alam B, Majumder R, Akter S, Lee SH. *Piper betle* extracts exhibit antitumor activity by augmenting antioxidant potential. Oncology letters 2015; 9: 863-868.
28. Paranjpe R, Gundala SR, Lakshminarayana N, Sagwal A, Asif G, Pandey A, et al. *Piper betle* leaf extract: anticancer benefits and bio-guided fractionation to identify active principles for prostate cancer management. Carcinogenesis 2013; 34 (7): 1558–1566.
29. Gundala SR, Yang C, Mukkavilli R, Paranjpe R, Brahmbhatt M, Pannu V, et al. Hydroxychavicol, a betel leaf component, inhibits prostate cancer through ROS-driven DNA damage and apoptosis. Toxicol Appl Pharmacol. 2014; 280(1): 86–96. doi:10.1016/j.taap.2014.07.012.
30. Wagh V, Mishra P, Thakkar A, Shinde V, Sharma S, Padigaru M, Joshi K. Antitumor activity of NPB001-05, an orally active inhibitor of Bcr-Abl tyrosine kinase. Frontiers in Bioscience Elite edition, 2011; 3(4): 1349-1364. doi: 10.2741/e338.
31. Ganesha G, Abhishek T, M. Saurabh M, Sarada NC. Cytotoxic and apoptosis induction potential of *Mimusopselengi* L. in human cervical cancer (SiHa) cell line. Journal of King Saud University – Science 2014; 26(4); 333-7. doi:10.1016/j.jksus.2013.10.002.
32. Widyananda MH, Puspitarini S, RohimA, Khairunnisa1 FA, Jatmiko1 YD, Masruri M, et al. Anticancer potential of turmeric (*Curcuma longa*) ethanol extract and prediction of its mechanism through the Akt1 pathway. F1000Research, 2022; 11: 1000.
33. Lakshmi T, Ezhilarasan D, Vijayaragavan R, Bhullar SK, Rajendran R. *Acacia catechu* ethanolic bark extract induces apoptosis in human oral squamous carcinoma cells. J Adv Pharm Technol Res. 2017; 8(4):143–149. doi: 10.4103/japtr.JAPTR73 17.
34. Sawhney SS, Painuli RM, Chauhan N. Evaluation of bactericidal and anticancer properties of fruits of *Piper longum*. Int J Pharm Pharm Sci, 2011; Vol 3, Suppl 5, 282-287.
35. Islam M, Yesmin R, Ali H, Karmakar PC, Habib R, Satter MA, Yeasmin T. Chemical composition and anticancer properties of *Alpinia calcarata* Rosc. rhizome. J Pharmacognosy Phytochemistry 2017; 6(5): 2491-2494.
36. Karimi A, Moradi MT, Lorigooini Z, Hashemi L, Alidadi S, Saeedi M. Antiproliferative and apoptotic effect of dried flower buds of *Syzygium aromaticum* L. extract on human cervical cancer (Hela) cells. J Reports in Pharmaceutical Sciences 2018; 7(2), 130-137.
37. Kumar, S., Mulchandani, V, Sarma, DJ. Methanolic neem (*Azadirachta indica*) stem bark extract induces cell cycle arrest, apoptosis and inhibits the migration of cervical cancer cells in vitro. BMC Complement Med Ther. 2022; 22: 239. https://doi.org/10.1186/s12906-022-03718-7.
38. Tulasi CDSLN, Narasu ML, Saida L. Cytotoxic effect of *Ficus religiosa* and *Ficus benghalensis* latex extracts on MCF-7 cell line. Int. J. Sci. Res. in Biological Sciences 2018; 5(6): 96-100.
39. Karamala LN, Karthik Y, Raghu M, Aditi N, Rachana V, Prasanna A, et al. Exploring the therapeutic potential

of *Pongamia pinnata* plant extract against skin cancer: in-silico and in-vitro study. J Ethnopharmacol. 2025; 337(Pt 3):118964. doi:10.1016/j.jep.2024.118964.

40. Kudva AK, Rao S, Rao P, Baliga MS, Pais MLJ, Adnan M, et al. Evidence for anticancer properties of honey with emphasis on mechanistic overview. In: Functional Foods in Cancer Prevention and Therapy 2020: (pp. 121-135). Elsevier. https://doi.org/10.1016/B978-0-12-816151-7.00007-7