

REVIEW ON MULTIFACETED ANTICANCER ACTIVITY OF *OCIMUM SANCTUM* AND ITS CONSTITUENTS: PAST, PRESENT AND FUTURE

Rahul Banerjee, Sudeepa Ghosh and Avik Acharya Chowdhury*

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Abstract

Ocimum sanctum Linn (OS), is the medicinal herb belonging to the Lamiaceae family, well-known as the 'Holi Basil' or 'Tulsi'. It has been a part of ayurveda and Indian culture for its tremendous ethnomedicinal significance as it is employed to cure common cough and cold along with bronchitis, skin diseases, insect bite etc. The leaves of OS in particular are widely used for the treatment of cancer. Approximately 15 different types of biologically active chemicals have been extracted, purified and identified from this medicinal herb. The anticancer properties of the crude and purified products from OS have been studied extensively over decades. They showed tumor suppressive, radioprotective, chemopreventive, anti-metastatic, anti-angiogenic and apoptotic effect on various types of cancer. This review summarizes the anticancer effect and mechanism of the purified products and extract obtained from OS on various cancers like breast, colon, lung, liver etc. The summarized report would help to fine-tune our understanding on the anti-cancer properties of OS that might open up new horizon in developing natural product-based cost- efficient cancer drug.

Keywords: Anticancer properties; Cancer; Mechanism of action; *Ocimum sanctum*

Introduction

Plant-based natural products have been abundantly used in medicinal purpose for ages. Phytochemicals are, currently the blooming field of various alternative medical treatments. *Ocimum sanctum* Linn (OS), commonly known as the 'Holy Basil' and 'Tulsi', is an aromatic perennial shrub, belongs to the Lamiaceae family. Apart from being the most sacred plant of India, OS has tremendous health benefits. This plant is found not only in the Indian subcontinent, but also throughout the Southeast Asian tropics. Several studies have enlightened our wisdom with the fact that OS extract and its oil exert

innumerable pharmacological effects. *Ocimum sanctum* has analgesic, anti-inflammatory, anti-pyretic, anti-carcinogenic, hepatoprotective and immunomodulatory effects (Singh *et al.*, 1996; Uma Devi, 2001; Gupta *et al.*, 2002; Prakash and Gupta, 2005; Singh *et al.*, 2007). The phytochemicals present in OS also have a cumulative effect in its medicinal properties. This review extensively and exclusively focuses on the preclinical activities of OS and its constituents as a potential anticancer agent.

Department of Biosciences, JIS University, 81, Nilgunj Road, Agarpara, Kolkata- 700109, West Bengal, India.,
E-mail: acharyaavik@gmail.com; avikacharya@jisuniversity.ac.in

* Corresponding Author

Literature review process

Scientific literatures were searched on Pubmed, Google Scholar, Elsevier, Science Direct. The authors first searched with the combination of words “*Ocimum sanctum*, cancer”. The output results were several literatures with *Ocimum sanctum* and other sources with their anticancer activity. The literatures only with *Ocimum sanctum* having anticancer activity were selected manually and after thorough reading. Major phytochemicals from *Ocimum sanctum* were selected. They are vicenin, luteolin, eugenol, apigenin, carnosic acid, rosmarinic acid, cosmosiin, orientin, molludistin, nepetoidin, hymenoxin, gardenin B. Then individually each phytochemical was selected and searched as keywords “vicenin, cancer”, “luteolin, cancer”, “eugenol, cancer” etc. The results again showed several literatures consisting studies on those phytochemicals from *Ocimum sanctum* and from different sources. The authors carefully have gone through each literature and selected only those literatures which have work on those phytochemicals from *Ocimum sanctum*. The literature review process is summarized in Figure 1.

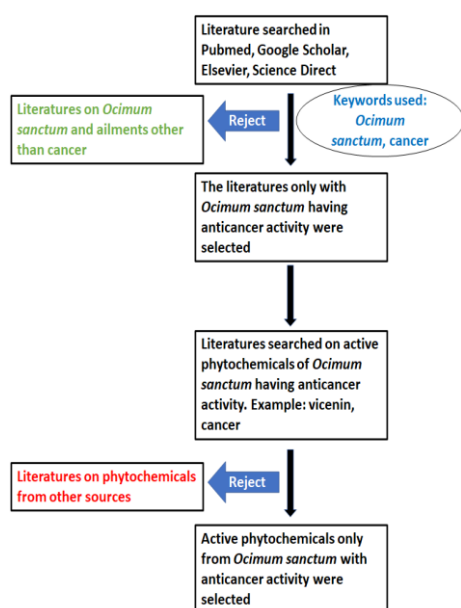


Figure 1. Literature review methodology flow chart

Ocimum sanctum: a reservoir of potential phytochemicals

Phytochemicals are biologically active compounds synthesized and found in plants. They carry out specific biological activities in plants including defense against foreign pathogens.

Phytochemicals are shown to exert cytotoxic or apoptotic effect towards cancer cells, both in purified forms alone and/or in combination. The LC-ESI-MS/MS analysis of OS revealed that it contains active metabolites like luteolin, cosmosiin, orientin, eugenol, apigenin, eupalitin, ocimarin and carnosic acid. The presence of molludistin, nepetoidin, luteolin 5-O-beta-glucopyranoside, hymenoxin, gardenin B, rosmarinic acid have also been reported (Venuprasad *et al.*, 2017). All the phytochemicals of OS have several important functions. For instance, Luteolin, a tetrahydroxyflavone, has an important role as an antioxidant. Cosmosiin, on the other hand, has potent antibacterial and anti-inflammatory effects. In addition to that, phytochemicals like carnosic and rosmarinic acid in OS has anti-inflammatory and anti-oxidant activities. This signifies that the phytochemicals of OS are a reservoir of multifunctional biologically active molecules (Figure 2).

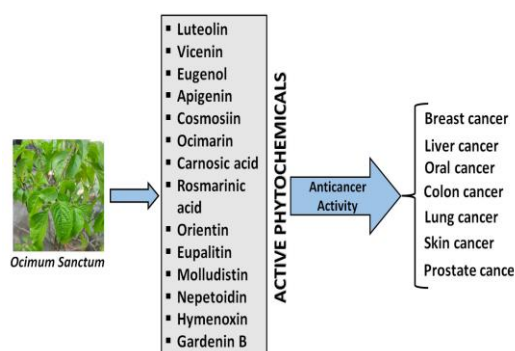


Figure 2. Major phytochemicals present in *Ocimum sanctum* having anti-cancer activity.

Ocimum sanctum as an anti-cancer agent Breast cancer

From a global point of view, breast cancer is the most common cancer in women and one of the major causes of cancer-related deaths. The *in vitro* breast cancer model has demonstrated an inhibitory activity of *Ocimum sanctum* extract on the migration and proliferation of breast cancer cells (Nangia-Makker *et al.*, 2007). OS extract prevents capillary tube development and reduces formation of blood vessels. In further studies with MDA-MB-231, a breast cancer cell line, demonstrated that a TPA-induced increase in levels of COX-2 was inhibited by OS extract (Nangia-Makker *et al.*, 2007). So, in a nutshell, the aforementioned studies confirms that the leaf extract of *Ocimum sanctum* possesses certain anti-tumorigenic as well as anti-angiogenic properties (Table 1).

Table 1. Anticancer activities of phytoconstituents isolated from *Ocimum sanctum*.

Phytoconstituents	Anti-cancer effect	Cancer type/cell line	Reference(s)
Eugenol	Inhibits cellular proliferation in MCF-7 cell line	MCF-7 (Breast cancer)	Vidhya and Niranjali 2011
Luteolin	1. Induces apoptosis by regulating several cell cycle pathway genes including CCNA2, PCNA, CSKN1, CCND1, and PLK1 2. Bring down histone deacetylase activity, cellular growth and invasiveness of breast cancer 3. Inhibits the proliferation and migration of human lung cancer cells	Different human breast cancer cell lines Human lung cancer cells	Attoub <i>et al.</i> 2011; Markaverich <i>et al.</i> , 2011; Kim <i>et al.</i> , 2012 Zhao <i>et al.</i> , 2011; Chen <i>et al.</i> , 2012
Apigenin	1. Inhibits proliferation and induces apoptosis in breast cancer cell lines like MCF-7, MDA-MB-435 and SK-BR-3 2. Inhibits tumor cell invasion, migration and proliferation in MDA-MB-453 cells 3. Induces apoptosis in cultured cells as well as in mice xenograft model 4. Prevention of mammary tumors developed in rats by DMBA and MPA 5. Apoptosis induction in BT-474 xenograft tumor model 6. Chemo-preventive effects against oral carcinogenesis induced by DMBA	Breast cancer cell lines like MCF-7, MDA-MB-435 and SK-BR-3 Mouse xenograft model Rats BT-474 xenograft tumor model	Lindenmeyer <i>et al.</i> , 2001; Choi and Kim, 2009; Seo <i>et al.</i> , 2012 Lindenmeyer <i>et al.</i> , 2001; Chen <i>et al.</i> , 2007 Mafuvadze <i>et al.</i> , 2011 Mafuvadze <i>et al.</i> , 2012 Silvan <i>et al.</i> , 2011
Carnosic acid	1. Inhibits proliferation of two breast cancer cell lines, MDA-MB-231 and MCF-7 2. Inhibits the growth of NCI-H82 lung cancer cells	MDA-MB-231 and MCF-7 NCI-H82	Yesil-Celiktas <i>et al.</i> , 2010; Baliga <i>et al.</i> , 2013
Rosmarinic acid	1. Inhibits proliferation of two breast cancer cell lines, MDA-MB-231 and MCF-7 2. Inhibits the growth of NCI-H82 lung cancer cells	MDA-MB-231 and MCF-7 NCI-H82	Yesil-Celiktas <i>et al.</i> , 2010; Baliga <i>et al.</i> , 2013 Yesil-Celiktas <i>et al.</i> , 2010
Vicenin-2	1. Inhibits of cellular proliferation in colon cancer and can induce apoptosis in HT-29 cells 2. Inhibits Wnt/ β -catenin signaling and while acting against colon and colorectal cancer 3. Reduces the proliferation of NCI-H23 cell line 4. Induces anti-proliferative and anti-angiogenic effects along with pro-apoptotic effects in several CaP cells lines like PC-3, DU-145 and LnCaP 5. In combination with DTL is reported to have synergistic effect on the growth of prostate tumors <i>in vivo</i>	HT-29 cells, colon cancer colon and colorectal cancer NCI-H23 PC-3, DU-145 and LnCaP <i>In vivo</i> model	Yang <i>et al.</i> , 2018 Baruah <i>et al.</i> , 2018 Nagaprashantha <i>et al.</i> , 2011 Nagaprashantha <i>et al.</i> , 2011
Ethanol extract of OS	1. Has preventive effect against DMBA-induced buccal pouch carcinogenesis in hamster 2. Apoptosis induction in human A549 lung cancer cells 3. Suppresses the tumor growth in the <i>in vivo</i> lung cancer model 4. Efficiently inhibits the tumor growth in Lewis lung carcinoma 5. Has inhibitory and anti-metastatic action on lung cancer cells 6. Protective effect against carcinogenic smoke-induced damage in lung and neuronal tissue 7. Significantly prevent tumor growth in <i>in vivo</i> hepatoma xenograft model 8. Reduce tumor volume in B16F1 melanoma bearing mice 9. Apoptosis induction ability in LnCaP prostate cancer cell lines. 10. Exerts cytotoxic and apoptosis inducing effects in human fibrosarcoma cells 11. Mitigates tumor volume significantly in sarcoma-180 bearing animals	Hamster A549 lung cancer cells <i>in vivo</i> lung cancer model, C57BL/6 mice Lewis lung carcinoma, mice model NCI-H460 cells Rats <i>in vivo</i> hepatoma xenograft model B16F1 melanoma bearing mice LnCaP Human fibrosarcoma cells Sarcoma-180 bearing animals	Karthikeyan, <i>et al.</i> , 1999 Magesh <i>et al.</i> , 2009 Magesh <i>et al.</i> , 2009 Kim <i>et al.</i> , 2010 Kwak <i>et al.</i> , 2014 Venuprasad <i>et al.</i> , 2017 Manikandan <i>et al.</i> , 2007 Monga <i>et al.</i> , 2011 Dhandayuthapani <i>et al.</i> , 2015 Karthikeyan <i>et al.</i> , 1999 Karthikeyan <i>et al.</i> , 1999; Baliga <i>et al.</i> , 2013

Eugenol, a potent phytochemical present in OS, in a dose-dependent manner, can inhibit cellular proliferation in the MCF-7 cell line. The growth inhibition is dependent on the depletion of intracellular GSH levels and the increment of the lipid peroxidation level (Vidhya and Niranjali, 2011). Various studies with different human breast cancer cell lines have demonstrated that molecules like luteolin, which isolated from OS, induces apoptosis by regulating several cell cycle pathway genes including CCNA2, PCNA, CSKN1, CCND1, and PLK1 (Markaverich *et al.*, 2011; Kim *et al.*, 2012). Luteolin has been reported to bring down histone deacetylase activity, cellular growth and invasiveness of breast cancer (Attoub *et al.*, 2011). The flavonoid apigenin, present in OS, inhibits proliferation and induces apoptosis in breast cancer cell lines like MCF-7, MDA-MB-435 and SK-BR-3. It also has effect on HER-2-overexpressing breast cancer cells (Choi and Kim, 2009; Seo *et al.*, 2012). Apigenin was also reported to have inhibited tumor cell invasion, migration and proliferation in MDA-MB-453 cells (Lindenmeyer *et al.*, 2001). Studies showed significant role of apigenin in inducing apoptosis in cultured cells as well as in mice xenograft model (Lindenmeyer *et al.*, 2001; Chen *et al.*, 2007). Mafuvadze *et al.* demonstrated that mammary tumors developed in rats by DMBA and MPA is prevented by apigenin (Mafuvadze *et al.*, 2011). Studies also reported that in BT-474 xenograft tumor model, apigenin is responsible for apoptosis induction (Mafuvadze *et al.*, 2012). *In-vitro* studies exhibited that proliferation of two breast cancer cell lines, MDA-MB-231 and MCF-7, can also be inhibited by carnosic and rosmarinic acid, the phytochemicals present in OS (Yesil-Celiktas *et al.*, 2010; Baliga *et al.*, 2013). *Ocimum sanctum* Essential Oil (OSEO) as a whole is also a subject of immense study. Studies demonstrated that OSEO has the potential to induce apoptosis in human breast cancer cells (Manaharan *et al.*, 2016). OSEO were extracted by leaf hydro-distillation. OSEO was found to be responsible for inhibition of MCF-7 breast cancer cells proliferation. Further studies revealed that OSEO has the ability of up-regulating the apoptotic genes p53 and elevating Bax/Bcl-2 ratio (Manaharan *et al.*, 2016) (Table 1)

Oral cancer

The ethanolic extract of OS has preventive effect against DMBA-induced buccal pouch carcinogenesis in hamster (Karthikeyan *et al.*, 1999). Phytochemical analysis of OS and investigative research has demonstrated chemopreventive effects of flavonoid apigenin against oral carcinogenesis induced by DMBA (Silvan *et al.*, 2011). On the other hand, Luteolin treatment has

been shown to reduce the viability of SCC-4 cells and induce apoptosis by decreasing the expression of CDKs and cyclins along with the phosphorylation level of retinoblastoma protein (p-Rb). Apart from the effect on cell cycle regulatory proteins, luteolin increases the expression of proapoptotic proteins and activates caspase 3 and PARP (Yang *et al.*, 2008) (Table 1). On account of contemporary research, it is conclusive that oral carcinoma which is induced by DMBA, can be prevented by both rosmarinic acid and carnosic acid (Manoharan *et al.*, 2010; Anusuya and Manoharan, 2011). Both the phytochemical components increase the detoxification enzymes activity and level of antioxidants and decrease lipid peroxidation levels to exert protective effect. Further research has dawned upon us the fact that rosmarinic acid down-regulated the expression of tumor suppressor protein p53 and anti-apoptotic protein Bcl-2 (Manoharan *et al.*, 2010; Anusuya and Manoharan, 2011).

Colon cancer

Colon cancer is a form of cancer that is becoming quite widespread across the globe. As per the preferences are concerned, naturally occurring dietary substances are more preferable over synthetic products for the prevention of colon cancer. This is exactly the drive behind the investigation of OS and its phytochemicals against colon cancer.

Vicenin-2, a phytochemical of OS, is the molecule of real interest against colon cancer. It is reported that Vicenin-2 has demonstrated antioxidant and anti-inflammatory activity (Yang *et al.*, 2018). Anti-carcinogenic and hepatoprotective activities are also shown by Vicenin-2. The same study showed that Vicenin-2 decreases the phosphorylation of glycogen synthase kinase-3 β along with cyclin D1 and non-p- β -catenin. Vicenin-2 found in OS, inhibited the cell cycle progression at G2/M phase of HT-29 colon cancer cells and induces apoptosis. This molecule also enhances the expression of cytochrome C, Bax and caspase-3, simultaneously suppressing Bcl-2 expression. These results were conclusive of the fact that Vicenin-2, which is present in OS, can act as an inhibitor of cellular proliferation in colon cancer and can induce apoptosis in HT-29 cells. It also inhibits Wnt/ β -catenin signaling and while acting against colon and colorectal cancer (Yang *et al.*, 2018) (Table 1).

Lung cancer

Globally, nearly a million cases of lung cancer are detected each year (Jemal *et al.*, 2011). Apoptosis induction has been demonstrated by ethanolic extract of OS in human A549 lung cancer cells (Magesh *et al.*, 2009). When Lewis lung

carcinoma is developed in C57BL/6 mice to evaluate the effect of ethanolic extract of OS, it is observed that the ethanolic extract suppressed the tumor growth in the in vivo lung cancer model. OS extract showed a concentration-dependent apoptotic effect in A549 lung cancer cells. Mechanistic studies pointed out the fact that phosphorylation of the survival pathway proteins, Akt and ERK, were decreased by OS extract. This extract is thought to extend its apoptotic effect by releasing cytochrome C in the cytosol from mitochondria, which sequentially lowers the expression of anti-apoptotic protein Bcl-2. Magesh *et al.* showed that 50 and 100mg/kg doses of OS extract can efficiently inhibit tumor growth in Lewis lung carcinoma (Magesh *et al.*, 2009). In-vitro studies revealed that the extract had the ability to inhibit cell adhesion and invasion by decreasing Matrix metalloproteinase-9 (MMP-9). Further studies brought to light the fact that OS extract can reduce tumor of Lewis lung carcinoma in mice model (Kim *et al.*, 2010). Two potential phytochemicals obtained from OS, rosmarinic acid and carnosic acid, inhibit the growth of NCI-H82 lung cancer cells (Yesil-Celiktas *et al.*, 2010). It has also been discovered that luteolin induces G2/M cell cycle arrest (Cai *et al.*, 2011). Luteolin induces apoptosis via mitochondrial pathway and inhibits the proliferation and migration of human lung cancer cells (Zhao *et al.*, 2011; Chen *et al.*, 2012). Baruah *et al.* investigated the action of another phytochemical of OS, Vicenin-2, on non-small cell lung cancer (NSCLC) cells (Baruah *et al.*, 2018) (Table 1). NSCLC, which is a major form of lung cancer, is extremely resistant to chemotherapy and radiotherapy. In the investigation, a NSCLC cell line NCI-H23 were treated with Vicenin-2 and X-ray alone and in combination. Results revealed that Vicenin-2 in combination with radiation reduce the proliferation of cancer cells by increasing the level of Rad-50 and by lowering the level of Matrix metalloproteinase-2 (MMP-2) and p21 proteins. Simultaneously this pre-clinical treatment is nontoxic and radioprotective to the normal fibroblast cells. Thus, from the study, it was conclusive that Vicenin-2 is a potential radiosensitizer of NSCLC cells (Baruah *et al.*, 2018).

Studies have also revealed that ethanolic extract of *O. sanctum* (EEOS) significantly inhibited cellular adhesion and invasion in NCIH460 cells after treatment with osteopontin (OPN). Osteopontin (OPN) is an important molecular target in cancer progression which is a mediator of cancer metastasis. Results revealed that EEOS did not show any toxicity at 200 µg/ml, but at higher doses, effectively decrease OPN and CD44 expression and simultaneously suppress the expression of phosphatidylinositol 3-kinase (PI3K) and COX-2

(Kwak *et al.*, 2014). The same study reported that EEOS decreases Akt phosphorylation in OPN-treated NCI-H460 cells. Furthermore, it has been observed that ethanolic extract resulted in decreased expression of EGFR and VEGF, which are important in tumour growth and proliferation, in OPN-treated cells (Kwak *et al.* 2014). These are indicative of the fact that EEOS as a whole has inhibitory and anti-metastatic action on lung cancer cells.

EEOC also has a significant protective effect against carcinogenic smoke-induced damage in lung and neuronal tissue (Venuprasad *et al.*, 2017). The level of several antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) in smoke-induced tissues decreased when compared with control, which signifies that smoke exposure depletes the antioxidant level (Attoub *et al.*, 2011). In continuation to this work, EEOC administration at 150 mg/kg and 300mg/kg dose increased the level of antioxidant enzyme. EEOC was also responsible for the regulation of nitric oxide production and neurotransmitter levels in the brain of rats. Smoke exposure significantly decreases AchE activity. EEOC restored the morphological damage of brain as well as lung caused by smoke stress by regulating HSP-70, inducible nitric oxide synthase (i-NOS) and expression of caspase-3 (Venuprasad *et al.*, 2017).

Liver cancer

Liver cancer is one of the most frequent cause of cancer related deaths in men and women (Jemal *et al.*, 2011). The ethanolic extract of OS leaf extract can inhibit the carcinogenic activity of DMBA. In primary hepatocytes pre-treatment with 20-100µg/ml of OS ethanolic extract followed by DMBA exposure, showed a significant reduction in DMBA-DNA adducts formation (Prashar *et al.*, 1998; Baliga *et al.*, 2013). The pronounced effects were observed at lower doses of OS. Aruna and Sivaramakrishnan showed that in in vivo hepatoma xenograft model, which was induced by 3'-MeDAB, the administration of OS can significantly prevent tumor growth (Aruna and Sivaramakrishnan, 1992). OS also mediates the protective effects as revealed by mechanistic studies. It also exerts protective effects by lowering protein oxidation level along with the level of lipids (Manikandan *et al.*, 2007) (Table 1).

Skin cancer

Skin cancer still remains one of the most common forms of cancer. Certain xenobiotic agents and their constant exposure primarily induce skin cancer (Ferlay *et al.*, 2010). Early studies have

confirmed that when ethanolic extract of OS is applied topically, it decreases DMBA-induced skin papilloma development (Prashar *et al.*, 1994). Successive research has documented that alcoholic leaf extract of OS was effective against skin cancer caused by different skin carcinogens (Rastogi *et al.*, 2007). In contemporary research with hydroalcoholic extract of OS and melanoma, it was revealed that hydroalcoholic extract of OS can reduce tumor volume in B16F1 melanoma bearing mice (Monga *et al.*, 2011). Apart from leaf, oil from OS seeds has also been found to be effective in reducing MCA- induced skin carcinogenesis and tumor volume in mice (Prakash and Gupta, 2000). The protective effects of OS are imparted by the elevation of antioxidants like glutathione (GSH), superoxide dismutase (SOD) and Catalase (CAT). Concomitant decrease in the activity of ODC, Cytochrome p450 and lipid peroxidation levels were also seen in cells treated with OS (Prashar *et al.*, 1994; Prakash and Gupta, 2000; Rastogi *et al.*, 2007; Monga *et al.*, 2011). Several studies showed that the dermatotoxic effect of xenobiotics and UV radiation can be nullified by eugenol, apigenin and luteolin (Sukumaran *et al.*, 1994; Kaur *et al.*, 2010; Pal *et al.*, 2010).

Prostate cancer

Carcinoma of Prostate (CaP) occupies the top end of the list of leading causes of cancer related deaths. Over the last few decades, plant extracts and their bioactive components have been extensively studied for their use in cancer therapeutics. Thus, natural products that have apoptosis inducing abilities through induction of several signaling pathways are nowadays studied with real zest (Nagaprashantha *et al.*, 2011; Dhandayuthapani *et al.*, 2015). Studies revealed that, treatment of LnCaP prostate cancer cells with Ethanolic Extract of OS (EET) for a period of 24 hours resulted in cell shrinkage and irregularly shaped cells, a typical feature of early apoptosis. EET treatment in dose-dependent manner, enhanced the number of apoptotic cells as confirmed by annexinV/PI staining and flow cytometric analysis. Further, EET-treated LnCaP prostate cancer cells exhibited significant disruption of the mitochondrial membrane potential (Dhandayuthapani *et al.*, 2015). On comparison with control cells, EET treated cells demonstrated higher levels of cleaved PARP and lower levels of Bcl-2 after 24 hours, which are indeed signs of apoptotic activities. Thus, the results were conclusive that EET had apoptosis induction ability in LnCaP prostate cancer cell lines.

A cytoskeletal targeted chemotherapeutic drug called Docetaxel (DTL) is often used for metastatic CaP. Docetaxel is fairly effective but the patients

quickly develop resistance rendering the drug ineffective. Therefore, novel strategies to enhance DTL efficacy is under investigation. With respect to OS against prostate cancer, one of the most extensively studied molecules is Vicenin-2, an important phytochemical of OS. Nagaprashantha *et al.* investigated the effects of Vicenin-2 as a single agent and as a combinational agent along with docetaxel (DTL), and reported that Vicenin-2 is able to induce anti-proliferative and anti-angiogenic effects along with pro-apoptotic effects in several CaP cells lines like PC-3, DU-145 and LnCaP (Nagaprashantha *et al.*, 2011) (Table 1). The effect of this combination is irrespective of the p53 status or androgen responsiveness of prostate cancer cell lines. Vicenin-2, as a single agent was able to inhibit EGFR/Akt/mTOR/p70S6k pathway. The expression of c-Myc, cyclin D1, CDK4, cyclin B1, PCNA and hTERT is decreased by Vicenin-2 in vitro. On the other hand, Vicenin-2, in combination with DTL is also reported to have synergistic effect on the growth of prostate tumors in vivo. The combination of Vicenin-2 and DTL decreases the level of the androgen receptor, Akt phosphorylation, pIGF1R, PCNA, cyclin D1, Ki67, CD31 and increase in the level of E-cadherin (Nagaprashantha *et al.*, 2011). These findings could open a plethora of possibilities in the fight against prostate cancer.

Fibrosarcoma carcinogenesis

Fibrosarcoma is a rare and malignant neoplasm originated from mesenchymal cells. The cytotoxic and apoptosis inducing effects of Ethanolic Extract of OS (EET) in human fibrosarcoma cells have been extensively studied in the recent past (Karthikeyan *et al.*, 1999). In human fibrosarcoma cells, EET does not have significant cytotoxic effect at lower concentration which is less than 50µg/ml. But with an increasing concentration, an exponential increase in the cytotoxic effects can be observed. EET was found to be responsible for decreasing the free GSH levels and concomitantly increasing lipid peroxidation product levels. In sarcoma-180 bearing animals, administration of both aqueous and ethanolic extract of OS mitigate tumor volume significantly (Karthikeyan *et al.*, 1999; Baliga *et al.*, 2013). Thus, from contemporary investigative studies, it can be said that OS has potential cytotoxic effects against deadly fibrosarcoma carcinogenesis.

Clinical Trials

Ocimum sanctum evolved as a promising anticancer agent as evident from both in vitro and in vivo studies. Clinical studies on cancer involving human subjects are still very limited in case of *Ocimum sanctum*. As flavonoids from OS showed significant results as radioprotector in rodents, the

effect of *Ocimum sanctum* flavonoids (OF) on the erythrocyte antioxidants in oral cancer in humans were also investigated. The patients were concurrently treated with radiation and OS flavonoids. Dividing the patients in two groups, A and B, group A were only exposed to radiation alone whereas group B received radiation and OF. Remarkably, it was also noted that OF-treated group B patients exhibited a reduced glutathione levels compared to group A. From the study it is conclusive that erythrocytes of cancer patients have elevated glutathione levels as a response to oxidative stress. OF can scavenge free radical and nullify the effect of reactive free radicals (Reshma *et al.*, 2005). So, it is quite understandable that OS might exert a significant effect against oral carcinogenesis.

Conclusions

Plant-based food and food supplements have been in practice for centuries. As these products are safe for human consumption, the efficacy of these biologically active phytoconstituents against various human diseases has become a field of emerging modern sustainable medical research. Plant-derived phytoconstituents have been explored for cancer prevention and therapy purpose as these natural products pose no threat to human health. The available studies and findings on the effects of OS extract and isolated molecules on different types of cancer have been documented here, although their mode of action and specificity remains an unexplored research region.

The preclinical studies documented in this review, authenticates that OS possesses chemo preventive, radioprotective and anti-carcinogenic activities. In spite of studies concluding that OS has certain anti-carcinogenic effects, countless opportunities of investigation and innovative revelation still remains. Clinical studies revealed radioprotective effect of OS (Baliga *et al.*, 2013). Manikandan *et al.* elucidated that OS can be used in combinational drug therapy to magnify its chemo-preventive and anti-carcinogenic effects (Manikandan *et al.*, 2008). The combination of *Azadirachta indica* (AI) and *O. sanctum* (OS) showed chemo-preventive efficacy against N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastric carcinogenesis. It revealed that combinational therapy can indeed be very useful in chemoprevention. In addition to that, Bhagat *et al.* reported about 63 endophytic fungal isolates from OS which can exert cytotoxic effects on different cancer cell lines like breast, lung, ovary, colon and prostate (Bhagat *et al.*, 2012). The isolated fungal

strains were further studied to arrive at a conclusion that they exerted cytotoxic effects on different cancer cell lines of lung, ovary, breast, prostate and colon. Flegkas and colleagues has reported that Rabdosiin, a phytochemical primarily found in OS, had the greatest cytotoxicity among other phytochemicals against most human cancer cell lines (Flegkas *et al.*, 2019). Hence, Rabdosiin has shown a lot of promise as a future anticancer drug. Other phytoconstituents isolated from OS extract remains the molecules of interest in cancer research field and their combinatorial effects are still left for the future scientists to explore. The most common type of solvent used for OS extraction preparation is distilled water, so the other extraction solvents and the different efficacy of these solvent extractions remains a field of concern.

These studies are just instances which give us an idea about the endless future possibilities involving OS against cancer. Evidently, OS and its biologically active molecules are in demand for natural product-based anti-cancer treatment. But studies need to be carried out to further elucidate the exact mechanisms and targets in cancer prevention. Future studies on combination of OS extract with individual phytochemicals will help investigators to understand their synergistic or combinatorial effects for their anti-cancer activity.

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