Introduction

Reactive oxygen species (ROS) are tumorigenic by their ability to increase cell proliferation, survival, and cellular migration. Ethnobotany, the study of traditional human uses of plants, is recognized as an effective way to discover future medicines [1]. Antioxidants have the potential to suppress cancer and to reduce the risk of cancer development by scavenging ROS [2]. Intake of Vitamin E, C, and A has been reported to reduce lung cancer risk because of their roles as regulators of cell differentiation [Vitamin A], antioxidants [Vitamins C and E], and modulators of DNA synthesis, methylation, and repair [3]. Berries are said to be vital sources of natural chemopreventive agents comprising Vitamins A, C, and E, selenium, carotenoids, anthocyanins, flavonols, proanthocyanidins, ellagitannins, and phenolic acids that have anticancer effects [4].

Berry fruits have been widely consumed in our diet and have attracted much attention due to their potential health benefits [5]. Berries contain a diverse range of phytochemicals with properties including antioxidant, anticancer, and anti-inflammatory works [6]. Berries possess many cancer-preventive substances in them including vitamins such as A, C, E, folic acid, as well as minerals, such as calcium, selenium, and zinc [7]. Berry bioactives are known to have many roles in cancer prevention. Inhibition of the formation of carcinogen-induced DNA adducts, inhibition of carcinogen-induced tumorigenesis in animals, and modulation of signaling pathways involved with cellular proliferation, apoptosis, inflammation, angiogenesis, and cell cycle arrest include in the many roles it plays [8,9].

Cancer till today remains the leading cause of death in both developed and developing countries [10]. Plants have been beacon of therapeutic sources for curing diseases from times immemorial [11]. Strawberry, one of the most commonly berries consumed in the Mediterranean diet, is a rich source of antioxidants and phenolic compounds that exert beneficial effects on human health [12]. However, to date, there is a lack of investigation about its cytotoxic effects [13,14]. Berries including strawberry, Korean raspberry, and mulberry are noted to have beneficial effects against diseases such as cancer [15]. Hence, the present study aimed at evaluating the cytotoxic activity of strawberry extract on oral cancer cell line.

Methods

Extract preparation

For the preparation of strawberry methanolic extract (SBE), Indian strawberry fruits were purchased from the local markets, cut into small pieces and dried in shadow. The powdered strawberry was stored at room temperature under sterile conditions until further use.

Maintenance of cell lines

KB cell lines were procured from National Centre for Cell Sciences, Pune. The cells were maintained in Minimal Essential Medium enhanced with 10% FBS, streptomycin (100 μg/ml), and penicillin (100 U/ml) in a humidified atmosphere of 50 μg/ml CO at 37°C. The vial containing the KB cell lines acquired from ATCC (CCL-17) was removed from the liquid nitrogen freezer and immediately placed in a 37°C water bath. It was agitated continuously until the medium thawed. Then, it was centrifuged for 10 min at 150–200×g room temperature. Supernatant was discarded and cells were washed with fresh medium to remove residual dimethyl sulfoxide (Table 1).

(3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (MTT) assay

MTT is a water-soluble tetrazolium salt, which on cleavage by succinate dehydrogenase is converted to an insoluble purple formazan by cleavage of the tetrazolium ring within the mitochondria. The formazan product, thus formed is impermeable to the cell membranes, and therefore, it accumulates in healthy cells [16]. The KB cells were routinely grown and subcultured as monolayers in Dulbecco’s Minimal Essential Medium supplemented with 10% newborn calf serum. The experiments in this
Table 1: Cell viability assay of SBE-treated KB cell lines

| S. No | Treatment          | Concentration (μg/ml) | Absorbance 540 nm | % Cell viability |
|-------|--------------------|-----------------------|-------------------|-----------------|
| 1     | KB untreated cells |                      | 0.318             | 100±5.9         |
| 2     | SBE treated        | 25                    | 0.267             | 83.9±7.1*       |
| 3     |                    | 50                    | 0.221             | 69.4±5.8*       |
| 4     |                    | 100                   | 0.205             | 64.4±4.1*       |
| 5     | Cyclophosphamide   | 100                   | 0.097             | 50.5±2.1*       |

Results are expressed as Mean±SEM (n=3). *p<0.001 statistically different in comparison with KB-untreated cells. SEM: Standard error of the mean, SBE: Strawberry methanic extract.
Fig. 1: Cell viability in strawberry methanolic extract-treated KB bells using neutral red incorporation. The results are expressed as meanstandard error of the mean (n=3)

CONFLICTS OF INTEREST
The authors declare that there are no conflicts of interest regarding the publication of this article.

REFERENCES
1. Packyanathan JS, Gayathri R, Vishnupriya V. Preliminary phytochemical analysis and cytotoxicity potential of *Bacopa monnieri* on oral cancer cell lines. Int J Pharm Sci Res 2016;39:4-8.
2. Rahman M, Islam SN. Effect of serum antioxidants (vitamin E, C and A) in lung cancer patients. Int J Pharm Sci Res 2014;6:578-80.
3. Weisburger JH. Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea. Food Chem Toxicol 1999;37:943-8.
4. Fuchs-Tarlovsky V. Role of antioxidants in cancer therapy. Nutrition 2013;29:1521.
5. Masters RW. Animal cell culture, cytotoxicity and viability assays. 3rd ed. Oxford; Oxford University; 2000. p. 202-203.
6. Tau sigma SJ, Batkin S. Bromelain, the enzyme complex of pineapple (*Ananas comosus*) and its clinical application. An update. J Ethnopharmacol 1988;22:191-203.
7. Kresty LA, Morse MA, Morgan C, Carlton PS, Lu J, Gupta A, et al. Chemoprevention of esophageal tumorigenesis by dietary administration of lyophilized black raspberries. Cancer Res 2001;61:6112-9.
8. Eberhardt MV, Lee CY, Liu RH. Antioxidant activity of fresh apples. Nature 2000;405:903-4.
9. Sun J, Chu YF, Wu XZ, Liu RH. Antioxidant and anti proliferative activities of vegetables. J Agric Food Chem 2002;50:6910-16.
10. Steinmetz KA, Potter JD. Vegetables, fruit and cancer. II mechanisms. Cancer Causes Control 1991;2:427-42.
11. Willett WC. Diet, nutrition, and avoidable cancer. Environ Health Perspect 1995;103:165-70.
12. Block G. Fruit, vegetables and cancer prevention: A review of the epidemiological evidence. Nutr Cancer 1992;18:1-29.
13. Ames BN. Endogenous mutagens and the causes of aging and cancer. Mutat Res 1991;250:3-16.
14. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. AICR, Washington, DC; 2007.
15. Wedge DE, Mesquaglia KM, Magee JB, Smith SH, Huang G, Larcom LL. Anticarcinogenic activity of strawberry, blueberry, and raspberry extracts to breast and cervical cancer cells. J Med Food 2004;4:49-51.
16. Fotakis G, Timbrell JA. In vitro cytotoxicity assays: Comparison of LDH, neutral red, MTT and trypan blue assays in hepatoma cell lines following exposure to cadmium chloride. Toxicol Lett 2006;160:171-7.
17. Menon A, Vishnupriya V, Gayathri R. Preliminary phytochemical analysis and cytotoxicity potential of pineapple extract on oral cancer cell lines. Asian J Pharm Clin Res 2016;9:140-3.
18. Chen T, Rose ME, Hwang H, Nines RG, Stoner GD. Black raspberries inhibit N-nitroso methylbenzylamine (N MBA)-induced angiogenesis in rat esophagus parallel to the suppression of COX-2 and iNOS. Carcinogenesis 2006;27:2301-7.
19. Hong JY, Song SH, Park HJ, Cho YJ, Pyee JH, Lee SK. Antioxidant, antiinflammatory, and ant proliferative activities of strawberry extracts. Biomol Ther 2008;16:286-92.
20. Carlton PS, Kresty LA, Siglin JC, Morse MA, Lu J, Morgan C, et al. Inhibition of N-nitro so methyl benzyl amine-induced tumorigenesis in the rat esophagus by dietary freeze-dried berries. Carcinogenesis 2001;22:441-6.
21. Prashanth NV, Dilip C, Dev KT, Augustine L, Saraswathi R. Research Article Evaluation of cytotoxic, in vitro antioxidant activities of *Ipomea batatas*. Int J Pharm Pharm Sci 2010;2:91-2.
22. Rao SI, Ramesh CK, Mahmod R, Jamuna KS, Prabhakar BT. Antitumor activity of two species of mulberry against eat cell lines in mice. World J Pharm Res 2015;4:1934-43.
23. Zhang Y, Seeram NP, Lee R, Feng L, Heber D. Identification and isolation of strawberry phytonutrients with antioxidant and human cancer cell antiproliferative properties. J Agric Food Chem 2008;56:670-5.
24. Seeram NP, Lee R, Scheuller HS, Heber D. Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionization mass spectroscopy. Food Chem 2006;97:1-11.
25. Xue H, Aziz RM, Sun N, Cassady JM, Kamendulis LM, Xu Y, et al. Inhibition of cellular transformation by berry extracts. Carcinogenesis 2001;22:351-6.
26. Arfan M, Khan R, Rybarczyk A, Amarowicz R. Antioxidant activity of mulberry fruit extracts. Int J Mol Sci 2012;13:2472-80.
27. Stoner GD, Wang LS, Casto BC. Laboratory and clinical studies of cancer chemoprevention by antioxidants in berries. Carcinogenesis 2008;29:1665-74.
28. Hasan S, Elongovan S. Conventional and advanced diagnostic aids on oral cancer screening-the journey so far. Int J Pharm Pharm Sci 2015;7:29-33.
29. Umesh TG. Preliminary phytochemical analysis and cytotoxicity potential of *Pimenta dioica* leaves. Int J Pharm Pharmaceut Sci 2010;2:91-2.
30. Hasnain S, Elongovan S. Conventional and advanced diagnostic aids in oral cancer screening-the journey so far. Int J Pharm Pharm Sci 2015;7:29-33.
31. Umesh TG. Preliminary phytochemical analysis and cytotoxicity potential of *Pimenta dioica* leaves. Int J Pharm Pharmaceut Sci 2010;2:91-2.
32. Hasnain S, Elongovan S. Conventional and advanced diagnostic aids in oral cancer screening-the journey so far. Int J Pharm Pharm Sci 2015;7:29-33.
33. Hasnain S, Elongovan S. Conventional and advanced diagnostic aids in oral cancer screening-the journey so far. Int J Pharm Pharm Sci 2015;7:29-33.