E3 Journal of Medical Research Vol. 1(4). pp. 038-043, May, 2012
Available online @ http://www.e3journals.org

Full length research paper

Antiproliferative activity of primates-consumed plants against MCF-7 human breast cancer cell lines

Anas Subarnas[1*], Ajeng Diantini[1], Rizky Abdulah[1], Ade Zuhrotun[1], Chiho Yamazaki[2], Mintao Nakazawa[2], Hiroshi Koyama[2]

[1]Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, Sumedang, Indonesia.
[2]Department of Public Health, Gunma University Graduate School of Medicine, Japan.

Accepted 17 April, 2012

Primate-consumed plants are assumed to be a promising source of therapeutic agents since primates can survive and be cured from any disease by their daily consumed food. In the course of our study to search for anticancer agents, we evaluated 42 species of plants usually consumed by primates for their antiproliferative activity against cell lines of human breast adenocarcinoma (MCF-7). In this study, crude ethanol extracts of the plants were tested using MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay. The results showed that four extracts of *Dysoxylum caulostachyum, Eugenia aquea, Garcinia celebica,* and *Psychotria valentonic* leaves strongly inhibited the MCF-7 cell proliferation with IC_{50} values of 12, 58, 87, and 87 µg/ml, respectively. Further examination on the fractions of the four extracts indicated that the ethyl acetate fraction of *D. caulostachyum*, the n-hexane fractions of *E. aquea* and *G. celebica,* and the water fraction of *P. valentonic* were the most active fractions with the IC_{50} of 78 , 24, 60, and 23 µg/ml, respectively. These results suggest that primate-consumed plants might have potential as a source of anticancer agents.

Key words: Anticancer; primate; cell lines; proliferation

Introduction

Cancer known as one of the most malignant diseases worldwide (Diantini, *et al*., 2012) is characterized by uncontrolled growth and local tissue invasion with sometimes distant metastates of abnormal form of body's cells (Dashora, *et al*., 2011). Among the various cancer types, breast cancer contributes to more than 1.2 million new cases and 0.5 million mortalities annually, making it the most malignant form of cancer among women (Ferlay, *et al*., 2010). Unfortunately that currently available chemotherapeutic agents for cancer diseases including breast cancer give serious side effects and cause excessive damage to normal cells (Sakarkar and Deshmukh, 2011).

It has been known that plants have a long history of use in the treatment of cancer (Cragg and Newman, 2006), and herbal medicines have a vital role in the prevention and treatment of cancer (Sakarkar and Deshmukh, 2011). Most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer (Newman et al., 2003; Butler, 2005; Cragg and Newman, 2006).In searching for anticancer agents from plant origin, we have carried out investigations on edible plants for primates (Koshimizu *et al*., 1998). Primates are known to have very close anatomy and physiology to human; hence their diseases might be also similar. Since primates only depend on their daily consumed food, thus, primates-consumed food is assumed to contain active therapeutic compounds which can be used in human disease management, including cancer. In our previous study, we have tested 19 primate-consumed plants for their anti-tumor promoting activity and some of them have prominent activity (Koshimizu *et al*., 1998). In further investigations, we isolated kaempferol-3-*O*-rhamnoside as an active compound from leaves of *Schima walichii* Korth, a plant commonly consumed by primates, and the compound inhibits MCF-7 breast cancer cell proliferation

*Corresponding Author Email: aasubarnas@yahoo.co.id

Table 1. A list of primates-consumed plants collected in Pangandaran Beach Conservation Area of West Java, Indonesia.

Name of plants	Part of plants collected
Acronychya laurifolia	Leaves
Amoora aphanamimixis	Leaves
Antidesma bunius	Leaves
Baccaurea javanic	Leaves
Barringtonia macrocarpa	Leaves
Buchanania arborescens	Leaves
Cinnamomum iners	Leaves
Cynometra ramiflora	Leaves
Dalbergia latifolia	Leaves
Decaspermum fruticosum	Leaves
Dysoxylum caulostachyum	Leaves
Elaecocarpus glabra	Leaves
Eugenia aquea	Leaves
Ficus annulata	Leaves
Ficus benyamina	Leaves
Ficus pubinervis	Leaves
Ficus septica	Leaves
Ficus sp.	Leaves
Ficus sumatrana	Leaves
Ficus veriegata	Leaves
Flacourtia rukem	Leaves
Garcinia celebica	Leaves
Heritiera sp	Leaves
Heritiera littoralis	Leaves
Hernandia peltata	Leaves
Kiara kebo	Leaves
Kleinhovia hospital	Leaves
Leea angulata	Leaves
Leea sambucina	Leaves
Litsea mappaceae	Leaves
Lygodium circinatum	Leaves
Melastoma polyantum	Leaves
Microcos tomentosa	Leaves
Neonauchea calycina	Leaves
Pandanus nitidus	Leaves
Phanera fulva	Leaves
Psychotria valentonic	Leaves
Pterospermum diversifolium	Leaves
Rhodamnia cinerea	Leaves
Schleitsera oleosa	Leaves
Stelechocarvus burahol	Leaves
Vitex heterophylla	Leaves

through activation of the caspase cascade pathway (Diantini, *et al*., 2012). In the series of our investigations, we have currently evaluated 42 species of Indonesian primate-consumed plants for their antiproliferative activity against MCF-7 human breast cell lines using a MTT bioassay. The results showed that some extracts had strong inhibitory activity against the MCF-7 cell proliferation.

Materials and Methods

Plant materials

Plant materials used in this research were leaves of primate-consumed plants collected in Pangandaran Beach Conservation Area of West Java, Indonesia. The leaves were dried on an open air away from the direct sunlight. The list of plant materials collected is shown in Table 1.

Extract and Fraction Preparation

Dried leaves of 42 species of plants were powdered and extracted with ethanol 95% (3 x 24 hrs) at a room temperature and the solvent was evaporated under reduced pressure at 50^0 C to yield concentrated extracts. The extracts which showed strong inhibitory activity against the MCF-7 cell proliferation in a MTT bioassay were partitioned with a mixture of n-hexane-water (3 : 1) to afford an hexane and water layers, and the water layer was further extracted with ethyl acetate to yield ethyl acetate and water fractions. The concentrated n-hexane, ethyl acetate, and water fractions were then tested for their inhibitory activity against the MCF-7 cell proliferation.

Cell culture and drug sensitivity assays

MCF-7 human breast cancer cell lines were purchased from the American Type Culture Collection (VA, USA). The cell lines were cultured in RPMI-1640 medium (Sigma, MO, USA) supplemented with 10% fetal bovine serum and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). Cell proliferation analysis was performed with cells in the presence of various concentrations of primates-consumed plant extracts by a MTT assay following the methods of Abdulah and co-workers (Abdulah, 2009). Brieftly, 2×10^4 of cells in 50 µl/well) cells were plated in 96-well plates. After the initial cell seeding, different concentrations of primates-consumed plants extracts were added and incubated for 24 hours. Ten microliters of WST-8 assay cell-counting solution (Dojindo Lab., Tokyo, Japan) was added to each well and incubated at 37°C for 3 hours. After the addition of 100 µl/well of 1 N HCl, the cell proliferation rate was then determined by measuring the absorbance at a wavelength of 450 nm. The absorbance was read using a microtiter plate reader (Becton-Dickinson, NJ, USA).

Table 2. IC_{50} of 42 primates-consumed plant extracts in inhibiting MCF-7 human breast cancer cells proliferation after 24 hours of treatment.

Plant extracts	IC_{50} values (µg/ml)
Dysoxylum caulostachyum	12
Eugenia aquea	58
Garcinia celebica	87
Psychotria valentonic	87
Buchanania arborescens	116
Ficus benyamina	133
Stelechocarvus burahol	141
Rhodamnia cinerea	150
Baccaurea javanic	153
Decaspermum fruticosum	154
Flacourtia rukem	172
Melastoma polyantum	174
Cinnamomum iners	175
Litsea mappaceae	200
Leea sambucina	207
Acronychya laurifolia	260
Dalbergia latifolia	286
Elaecocarpus glabra	297
Cynometra ramiflora	317
Heritiera sp	350
Kleinhovia hospital	369
Ficus septica	400
Antidesma bunius	>400
Ficus annulata	>400
Ficus pubinervis	>400
Hernandia peltata	>400
Lygodium circinatum	>400
Microcos tomentosa	>400
Ficus sumatrana	>400
Amoora aphanamimixis	>400
Neonauchea calycina	>400
Leea angulata	>400
Ficus veriegata	>400
Pandanus nitidus	>400
Barringtonia macrocarpa	>400
Heritiera littoralis	>400
Vitex heterophylla	>400
Ficus sp.	>400
Phanera fulva	>400
Pterospermum diversifolium	>400
Kiara kebo	>400
Schleitsera oleosa	>400

Results

Antiproliferative properties of plant extracts on MCF-7 cells

Forty two plant extracts were tested for their 24 hours effect on MCF-7 human breast cancer cell lines using the MTT bioassay and the results are presented in Tabel 2. Among all the extracts tested, four extracts namely those of *D. Caulostachyum*, *E. Aquea*, *G. Celebica,* , and *P. Valentonic* leaves showed a strong inhibition against the MCF-7 cell lines proliferation with the IC_{50} of 12, 58, 87, and 87 µg/ml, respectively. Ten extracts had IC_{50} values of 101-200 µg/ml and the other extracts showed higher IC_{50} values which were regarded very weak cytotoxicity.

Antiproliferative properties of fractions of the highly active extracts on MCF-7 cells

A further investigation was performed on the extracts of *D. Caulostachyum*, *E. aquea*, *G. celebica* and *P. valentonic* leaves which showed strong inhibition on MCF-7 cells proliferation to explore active compounds responsible for their cytotoxicity. Each extract was fractionated with n-hexane, ethyl acetate, and water, successively, and all fractions were tested for their 24 hours effect on MCF-7 cell lines. The results are shown in Figure 1-4. The ethyl acetate fraction of the *D. caulostachyum* extract was the most promising fraction to inhibit MCF-7 cells proliferation with the IC_{50} of 78 µg/ml (Figure 1). In the *E. aquea* and *G. celebica* extracts, their *n*-hexane fractions had the highest cytotoxicity with the IC_{50} of 24 and 60 µg/ml, respectively (Figure 2 and 3). Meanwhile, the water fraction of the *P. valentonic* extract showed the most cytotoxic activity with the IC_{50} of 23 µg/ml (Figure 4).

Discussion

With the high prevalence of cancer cases, searching for naturally occurring agents that may inhibit cancer development is becoming an important objective for scientists. Primates, anatomically and physiologically similar with human, are a potential source of new drugs or lead compounds for chemoprevention or chemotherapy of human diseases. So, the search for anticancer agents on the basis of follow-up of primate uses of plants is a new approach that is highly possible to get new anticancer drugs or lead compounds of plant origin.

In this study, we showed that the extracts of plants ingested by primates inhibited the growth of MCF-7 breast cancer cell lines and some of them had strong cytotoxicity in a concentration-dependent manner. As shown in Table 2 that all tested extracts showed a variety of IC_{50} values in inhibiting MCF-7 cancer cells proliferation. These values indicated the cytotoxicity level of the extracts, the lower the IC_{50} values the higher the toxicity. So, based on the IC_{50} values, the cytotoxicity level of the extracts might be devided into strong (<100 µg/ml), moderate (101-200 µg/ml), and weak(>200µg/ml). The four extracts of *D. Caulostachyum*, *E. Aquea*,

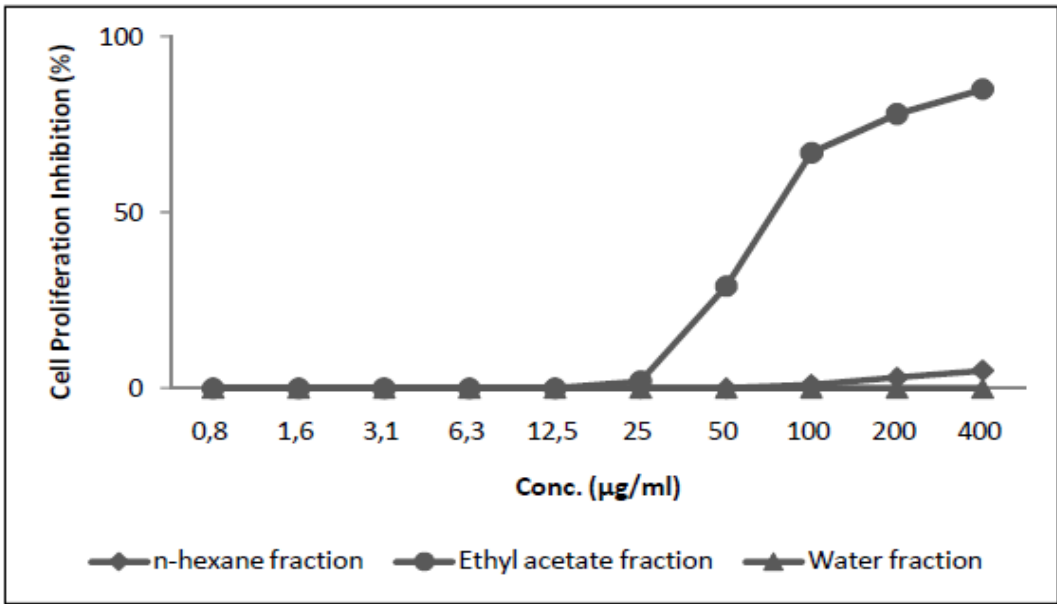

Figure 1. Effect of 24h treatment of *D. caulostachyum* leaves extract's fractions on MCF-7 human breast cancer cell lines proliferation.

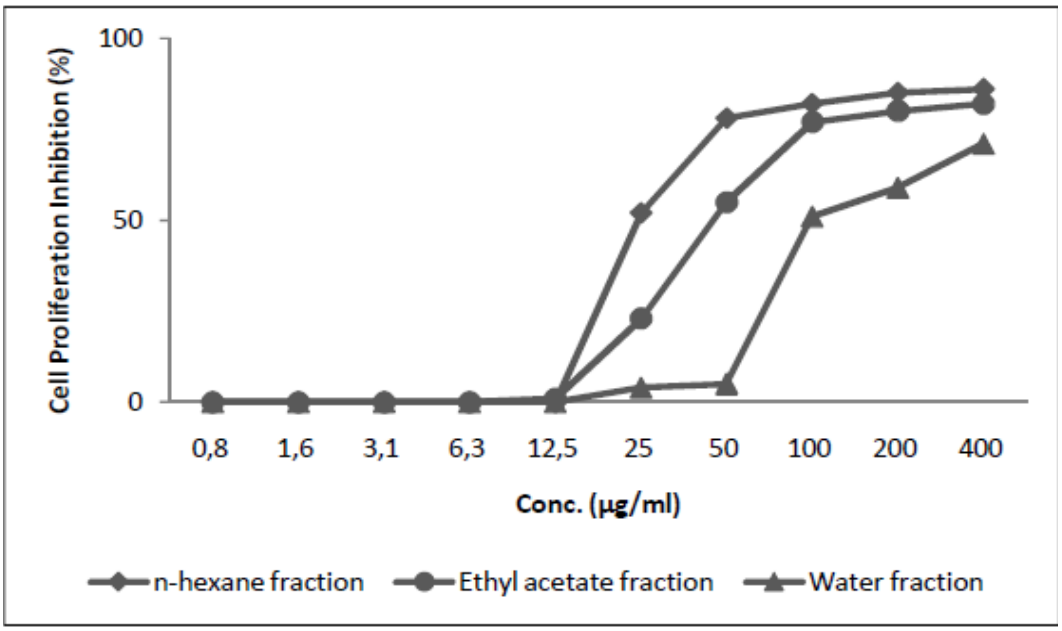

Figure 2. Effect of 24h treatment of *E. aquea* leaves extract's fractions on MCF-7 human breast cancer cell lines proliferation.

G. Celebica, and *P. Valentonic* leaves which showed a strong inhibition against the MCF-7 cell lines proliferation (the IC_{50} below 100 µg/ml) was worthy of further investigation to explore active compounds responsible for the antiproliferative activity. This was done by a means of an activity-guided fractionation based on an increasing order of solvent polarity.

The activities of the extracts or fractions are determined by secondary metabolites contained in them. Based on phytochemical screening done in our laboratory, the four

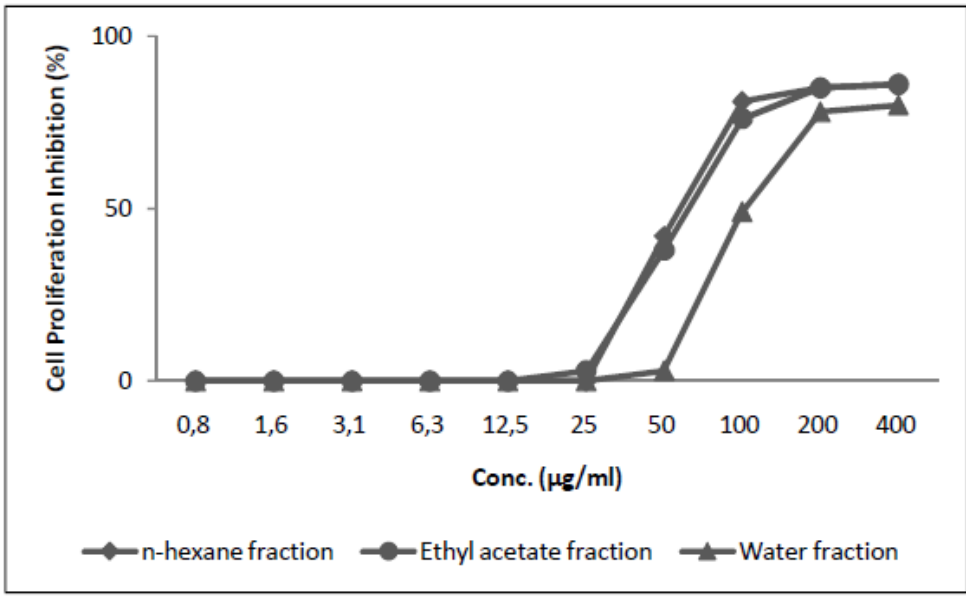

Figure 3. Effect of 24h treatment of *G. celebica* leaves extract's fractions on MCF-7 human breast cancer cell lines proliferation.

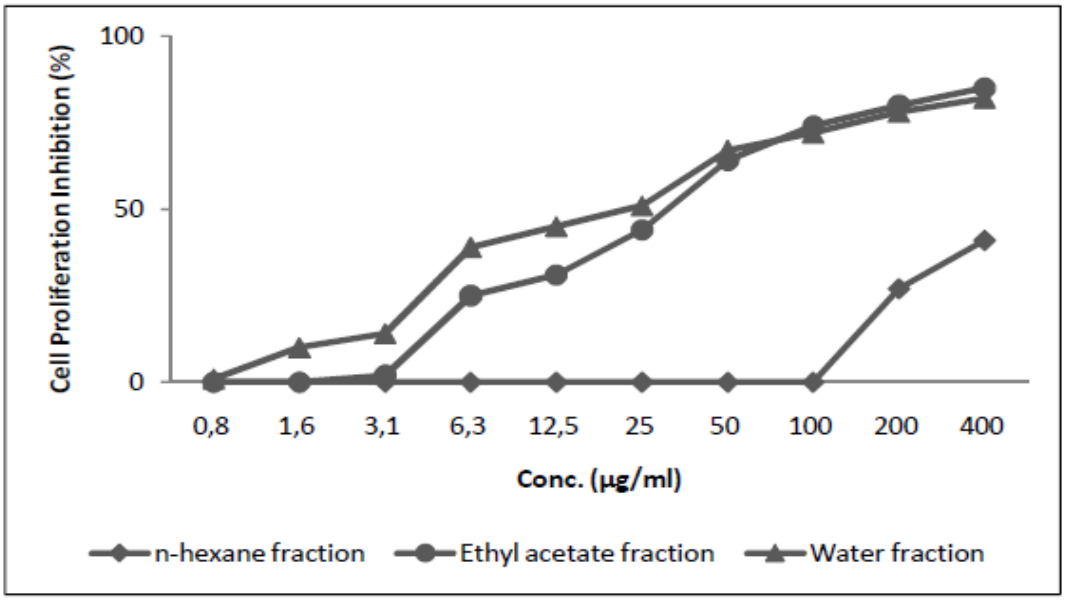

Figure 4. Effect of 24 h treatment of *P. valentonic* leaves extract's fractions on MCF-7 human breast cancer cell lines proliferation.

extracts mainly contained polyphenols and flavonoids, the compounds of which are known to have antioxidant and anticancer activity (Ren, *et al.*, 2003; Cai, *et al.*, 2004). For the *G. Celebica* extract, its inhibitory activity on the MCF-7 cell lines proliferation and its active compounds might be related to those reported for *G. mangostana*. It has been reported that *G. mangostana* pericarps contain prenylated xanthones which have

antiproliferative effects in various human cancer cells (Akao, *et al*., 2008). However, this plant probably also contain other cancer chemotherapeutic agents beyond the xanthone derivatives.

Although this study is still in preliminary stage, these results supported our hypothesis that primate consumed plants might be a promising source of anticancer agents and are possible to be used in human disease management, including cancer. This hypothesis is in line with our previous findings that the leaves of *Schima walichii*, contains an inhibitory compound against MCF-7 breast cancer cell proliferation through activation of the caspase cascade pathway (Diantini, *et al*., 2012).

A further investigation with the target of finding new active compounds responsible for antiproliferative properties from *D. caulostachyum, E. aquea, G. celebica,* and *P. valentonic* are focused on the most active fractions and the work are currently being conducted in our laboratory.

Acknowledgements

This work was financially supported by The Directorate General of Higher Education of The Ministry of National Education of Indonesia (Grand-in-Aid for The International Collaborations and Publications).

References

Abdulah, R., Faried, A., Kobayashi, K., Yamazaki, C., Suradji, E. W., Ito, K., Suzuki, K., Murakami, M., Kuwano, H. and Koyama, H. 2009. *Selenium enrichment of broccoli sprout extract increases chemosensitivity and apoptosis of LNCaP prostate cancer cells*. BMC Cancer 9: 414.

Butler, M. S. 2005. *Natural products to drugs: natural product derived compounds in clinical trial*, Nat. Prod. Rep., 22, 162-195.

Cai, Y., Luo, Q., Sun, M., Corke, H. 2004. *Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer*, Life Sciences, 74(17): 21572184.

Cragg, G. M. and Newman, D. J. 2006. *Plants as a source of anti-cancer agents*, in Ethnopharmacology, [Eds. Elaine Elisabetsky, Nina L. Etkin], in Encyclopedia of Life Support Systems (Developed under the Auspices of the UNESCO, Eolss Publishers, Oxford, UK.

Dashora, N., Sodde, V., Prabhu, K. S. and Lobo, R. 2011. *In vitro* cytotoxic activity of Dendrophthoe falcata on human breast adenocarcinoma cells-MCF-7. Int. J. Cancer Res., 7: 47-54.

Diantini, A., Subarnas, A., Lestari, K., Halimah, E., Susilawati, Y., Supriyatna, Julaeha, E., Achmad, T. H., Suradji, E. W., Yamazaki, C., Kobayashi, K., Koyama, H. and Abdulah, R. 2012. *Kaempferol-3-O-rhamnoside isolated from the leaves of Schima wallichii Korth. inhibits MCF-7 breast cancer cell proliferation through activation of the caspase cascade pathway*. Oncology Letters 3: 1069-1072.

Ferlay J, Shin, H.R., Bray, F., Forman, D., Mathers, C. and Parkin, D. M: GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide. International Agency for Research on Cancer, 2010. Available from: http://globocan.iarc.fr. Accessed August 27, 2011.

Koshimizu, K., Murakami, A., Hayashi, H., Ohigashi, H., Subarnas, A., Gurmaya, K.J. and Ali, A. 1998. *Biological activities of edible and medicinal plants from Indonesia and Malaysia*. The Tokyo International Forum on Conservation and Sustainable Use of Tropical Bioresources. Tokyo. Japan. 203-208.

Newman, D. J, Cragg, G. M. and Snader, K. M. 2003. *Natural products as sources of new drugs over the period 1981-2002*. J. Nat. Prod., 66(7): 1022-1037

Newman, D. J. and Cragg, G. M. 2007. *Natural products as sources of new drugs over the last 25 years*. J Nat Prod 70(3): 461-77.

Sakarkar, D. M. and Deshmukh, V. N. 2011. Ethnopharmacological review of traditional medicinal plants for anticancer activity. Int. J. Pharm. Tech. Res., 3(1): 298-308.

Ren, W., Qiao, Z., Wang, H., Zhu, L. and Zhang, L. 2003. *Flavonoids: Promising anticancer agents*, Medicinal Research Reviews, 23(4): 519–534.