INTRODUCTION

Among various forms of cancer, melanoma is a malignant neoplasm of melanocytes, most frequently arising from the skin. Melanoma has increased to the level of a serious public health problem during the past 20 years. Melanoma tumor is accounted for 2·6% of the global cancer incidence and 1·1% of cancer-related deaths. Even if these data rank melanoma eighth or ninth in incidence, its doubling rate every 10–20 years is more worrying. The curative surgical treatment of melanoma remains a significant clinical challenge and trials of postsurgical adjuvant therapy have proved largely unsuccessful with the majority inducing severe side effects at therapeutically effective doses.

In recent perspectives, cancer is considered to be a complex disease which requires a combinational therapy including the use of Natural compounds. The pharmacological efficacy of plant based products has created a revolutionary interest and awareness among the scientific community. Development of compounds with pharmacological efficacies from Natural Products has currently become an important area of research. Plants are the sources of half of the pharmaceuticals in our modern medicinal cabinet. Several biologically active compounds in a plant work together to produce greater effect then single chemical because specific combination allows inclusion of Natural Products that can work at different aspect and stages like short term energy, long term endurance, or weight control. Multidrug approach, broad spectrum medicine and combination therapy are just more or the same. Chemical partnership in the plant extract is the reason to believe that Natural compounds might inhibit cancer growth when used in combination. The synergistic or antagonistic effect of various component of plant material may enhance the therapeutic effect simultaneously reducing the side effects which may not occur when one or more isolated chemical components are used. Working with plant extracts, disease may not gain resistance because they are really a cocktail of active biochemical rather than single compounds. The proven efficacies of herbalism have created a revolutionary new interest and awareness among the scientific community. Plants are the sources of half of the pharmaceuticals in our modern medicine cabinet. Primary metabolites are often concentrated in plant organs. The secondary metabolites serve as a defense system against various infestations. Thus, unlike compounds synthesized in the laboratory, secondary compounds from plants are virtually guaranteed to have biological activity. Plants are known to produce a wide range of secondary metabolites such as alkaloids, terpenoids, polyacetylenes, flavonoids, quinines, phenyl propionates, amino acids and recently recognised naturally occurring plant derived cancer preventive isothiocyanates have been proved for useful medicinal properties.

Advancements made in the application of modern physico chemical methods of isolation, purification and various spectroscopic techniques have strengthened the research base in the fields of Phytochemistry. Discoveries of physiological and pharmacological functions [Active bio Principle] of medicinal plants, has initiated extensive research in this field to utilize the medicinal properties of the plant in alternative human sufferings. Such green magic bullets hopefully would delay resistance for cancer growth, while other may yield bioactive principles which can become our therapeutic armamentarium.
OBJECTIVES

Natural Products Research Laboratory, Dayalbagh Educational Institute has explored significant antimelanoma activity of the taramira (*Eruca sativa*) seed oil. This important finding has motivated us to exploit the antimelanoma property of the plant *Eruca sativa* seed oil and characterisation of its bioactive principle for the sustainable management of rapidly scattered melanoma disease and to develop safe, non-resistant, poor-man friendly and target oriented herbal drug.

The work will be carried out with the following specific objectives:

- Characterization of glucosinolate derived cancer preventive isothiocyanates (possible bioactive principles) in taramira oil.

- *In vitro* assessment of antimelanoma bioefficacy of possible bioactive principle ITCs (single and combinational) against reference drug (Doxorubicin).

- *In vivo* assessment of antimelanoma bioefficacy of ITCs (single and combinational) against reference drug (Doxorubicin) and its comparison with taramira seed oil.
PLANT SELECTED FOR STUDY

**Eruca sativa** (Mill). *(Taramira, Rocket)*

Taramira comprises a number of species of the *Brassicaceae* (*Cruciferae*) family belonging to the *Eruca* (Miller) and *Diplotaxis* (DC.) genera. *Eruca sativa* (Mill.) or *Eruca vesicaria* (L.) has its origin in the Mediterranean region (Zeven et al., 1982) but is widely distributed all over the World (Warwick, 1994). It is mostly harvested wild or cultivated as an edible vegetable with distinct spicy flavor of young leaves. Taramira seeds are also used for the production of oil, known as “Jamba oil” (Padulosi, 1995) and appreciated for pungent taste sprouts. Plant grow up to 80 cm, leaves are more or less sessile, characterized by pungent taste, which are used for flavoring *salads* (Bianco, 1995). Seeds are brown in colour.

**Pharmacological properties**

Taramira is considered as medicinal plant with many reported properties. It is used in traditional pharmacopoeia for many different purposes: it is antiphlogistic, astringent, depurative, diuretic, digestive, emollient, tonic, stimulant, laxative, stomachic, anti-inflammatory for colitis and rubefacient (Arietti, 1965; Uphof, 1968; Balme, 1978 and Perry, 1978). Tender leaves are reported to have stimulant, stomachic, diuretic and antisorbutic activity (Bhandari and Chandel, 1996). Taramira oil is used to get rid off lice and dandruff. It is sometimes used as antibiotic to treat infection of the respiratory and urinary tracts (Mennicke et al., 1988). Aqueous extracts of the plant tissues have shown herbicidal potential (Bialy et al., 1990).
CHEMICAL COMPOSITION

Taramira plant contains 67 volatile components, representing 96.52% of the oil. Taramira oil contains both saturated and unsaturated fatty acids. Some important saturated and unsaturated fatty acids are: Arachidic acid, Behenic acid, Erucic acid, Lignoceric acid, Linoleic acid (Omega-6), Linolenic acid (omega-3), Oleic acid and Stearic acid (Ali and Mckay, 1982; Muuse et al., 1992; Miyazawa et al., 2002.) The plant Eruca sativa has been reported to contain Glucoerucin (Kjaer and Gmelin, 1955).

![Erucic acid](image)

Erucic acid

![Glucoerucin](image)

Glucoerucin

![Sinigrin](image)

Sinigrin

Like other cruciferous vegetables, Eruca sativa contains a range of health promoting phytochemicals including Carotenoids, Vitamin C, Fibers, Flavonoids, Isoflavonoids, Polyphenols and Glucosinolates (GLs) (Steinmetz et al., 1991). The Phytochemicals like GLs have recently garnered great interest for their potential role in the maintenance of human health. In particular, significant cancer risks reduction with increasing Brassicaceae consumption (Jeffery et al., 2001 and Poppel et al., 1999). It has been speculated that the isothiocyanates (ITCs), obtained from myrosinase hydrolysis of GLs (By chewing, cutting or processing the vegetable), are in great part responsible for the protective effects of Brassica vegetable (Zhang et al., 1992 and Fahey et al., 1997). Glucosinolate-containing cruciferous vegetables such as cabbage, broccoli and Brussels sprouts are correlated with reduced incidence of cancer (Graham et al., 1978).
There are evidences that ITCs are Anticarcinogenic (Mossoba et al., 1989 and Fimognaria et al., 2004, Dong Xiao et al., 2008, Khoobchandani et al., 2011 and Moon et al., 2011). Tumors were inhibited in mice and rats that were given ITCs both before and after administering known carcinogens (Fenwick et al., 1983 and Wattenberg, 1981). Short-term chemical mechanisms appear to be involved in the suppression such as high toxicity toward cancer cells (Nastruzzi et al., 1996). Thus, if ITC is consumed in normal dietary levels, it may be more beneficial than being harmful (Zigang Dong ACS, 2003, Piercey, M. June, 2009).

**ISOTHIOCYANATES: AN OVERVIEW**

Glucosinolates are sulfur-containing molecules produced from aminoacids by the secondary metabolites. Glucosinolates are not biologically active, but are the precursor for the formation of a variety of potential allelochemicals; most important of these are Isothiocyanates (ITCs). They occur predominantly in various families: Tovariaceae, Resedaceae, Capparaceae, Moringaceae and Brassicaceae. (Fenwick et al., 1983). Species belonging to these families are widely consumed or cooked as salad vegetables (cabbage, Brussels, sprouts, cauliflower, radish, water cress) or condiments (horseradish, mustard caper) cruciferous forages (kale, rape, turnip) and oilseed meals (rape, turnip rape) are used as foodstuffs for animals. (Fenwick et al., 1982). Glucosinolates on enzymatic degradation by myrosinase enzyme (thioglucoside glucohydrolase, in presence of water release isothiocyanates (ITCs) organic cyanides and ionic thiocyanates (SCN⁻). Degradation also occurs thermally or by acid hydrolysis (Kjaer, 1976; Mac leod et al., 1981). Myrosinases are fairly specific towards glucosinolates (Durham and Poulton, 1990). These enzymes cleave the sulfur-glucose bond.
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CHEMOPREVENTIVE ROLE OF ISOTHIOCYANATES

Many synthetic ITCs exert versatile chemopreventive effects against tumors induced by chemical carcinogens in various animal organs, suggesting that common mechanisms underlie their chemopreventive properties (Zhang et al., 1994; Hecht et al., 1995). Cruciferous vegetable-derived ITCs are highly effective in prevention of chemically induced cancers in animals (Hecht et al., 2000; Talalay et al., 2001). A number of studies have documented the chemopreventive properties of Isothiocyanates (Zhang et al., 1992; Fahey et al., 2002). Many isothiocyanates, both natural and synthetic, display anticarcinogenic activity because they reduce activation of carcinogens and increase their detoxification. Various studies show that they exhibit anti-tumor activity by affecting multiple pathways including apoptosis (Thornalley et al., 2000; Thornalley et al., 2002), MAPK signaling, oxidative stress (Thornalley et al., 2001) and cell cycle progression.

The chemopreventive potential of the isothiocyanates has been attributed to several different actions (Hecht, 2000; Zhang et al., 2006). The isothiocyanates are strong inhibitors of phase I enzymes, particularly the cytochrome P450 enzymes (Goosen et al., 2000; Nakajima et al., 2001). Another important activity of the isothiocyanates is induction of phase II detoxification enzymes including sulfotransferases, NAD(P)H quinone oxidoreductases, and N-acetyltransferases (Xu et al., 2005). Phase II enzymes catalyse the conjunction of carcinogens with endogenous ligands, resulting in the formation of hydrophilic conjugates, which are often less toxic and more easily excreted in the urine or bile (Holtzclaw et al. 2004). The isothiocyanates activate phase II enzymes and consequently reduce carcinogen titre within the body (Rose et al., 2000).

The chemopreventive effects of the isothiocyanates were traditionally attributed to the enhancement of carcinogen detoxification by phase II induction and the blocking of carcinogen activation by phase I inhibition (Hecht, 1999). Both of these actions explain the ability of the isothiocyanates to prevent tumourigenesis when administered prior to carcinogen exposure (Morse et al., 1991; Jiao et al., 1997; Kassie et al., 2002).

MATERIAL AND METHODS

Plant species: Eruca sativa Mill. Thell

In vitro: Cell line [B16F10 (Melanoma)]

In vivo: Animal model C57BL/6

METHODOLOGY

Extraction of seed oil

E. sativa seed oil would be obtained by standard hot water extraction from ground seeds (100 gm) for 3-5 hours. Aqueous fraction would be separated and dried over anhydrous Na2SO4. Seed oil would be sealed under nitrogen and kept refrigerated (+4°C) in the dark.
Characterization of Isothiocyanates

GC-MS screening of the seed oil for the identification of glucosinolates and their corresponding breakdown products (isothiocyanates) would be carried out.

In-vitro studies:

Appropriate cell lines would be obtained from National Cell centre of Science, Pune, and grown in MEM / RPMI 1640 medium supplemented with 1% fetal calf serum (FCS), antibiotic at 37°C, 5% CO₂ in an incubator for 24 hours.

MTT Assay:

The cytotoxic effect of Natural compounds against human cancer cell lines/Animal Cell lines would be determined by a colorimetric assay, quantitated by the ability of living cells to reduce the yellow dye MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium] bromide to a blue formazan product (Pandey et al., 2006).

Cells would be seeded in 96-well microplates and incubated for 24h, at 37°C, 5% CO₂ humidified. After 24h, the extract dissolved in media would be added in each well and incubated for 3 days (72 hr). Doxorubicin would be used as positive control. First column used as negative control containing no drug no extract.

(a) Evaluation of Cells Survival:

Cell survival would be evaluated by MTT dye which is reduced by the living cells. Absorbance would be determined by ELISA plate reader at 540 nm.

(b) Determination of IC₅₀

The inhibition rate (%) = \[1-(\text{Absorbance} \, 540 \, \text{of sample}/\text{Absorbance} \, 540 \, \text{of control})\].

Trypan Blue Exclusion Assay

Cells will be seeded in tissue culture petri plates and adhere for 24 hr in CO₂ incubator at 37°C. The medium was replaced with incomplete MEM medium for 24 hr in CO₂ incubator at 37°C. Trypan blue dye (0.1 ml, 0.4% in water) was mixed with cell suspension, 15 min prior to completion of incubation period. At the end of incubation period, the petri plates were carefully taken out and sodium dodecyl sulfate (1.0%) was added. Viability was expressed as a percentage of control number of cells excluding Trypan blue dye (Frieauff et al., 2001).

Fine Needle Aspiration Cytology (FNAC)

Fine needle aspiration cytology (FNAC) entails using a narrow gauge (25-22G) needle to collect a sample of a lesion for microscopic examination. It allows a minimally invasive, rapid diagnosis of tissue. Aspiration cytology offers a relatively cheap, quick, and accurate tool for the diagnosis and follows up of cancer (Roskell et al., 2004).
**In vivo studies**

C57BL/6 mice of 6 weeks of age would be kept in groups five per cage and fed with control diet and water. The animals would be acclimatized for 1 week before use and maintained throughout at standard condition as follows: 24 hr, 22 ± 2°C temperature, 50% humidity and 12 hr light / dark cycle. Mice would be divided into two main groups normal and cancerous. Cancerous groups would be subdivided into various subgroups: control untreated cancerous animals, animals treated with various plant extracts and animals treated with reference drug (Doxorubicin).

**Induction of melanoma cells in mice**

At the 4 to 6 weeks of age, 1 x 10^5 viable cells in DMEM medium supplemented with 10% FCS would be induce in the mice.

**Administration of test samples**

Animals would be treated with the test samples at the first day, after induction of cell line. Another group of animals would be treated with test samples when the tumor becomes 0.5x 0.5cm (ex-vivo) or sub-centimetre in vivo.

**Measurement of tumor volume**

The volume of the tumor would be recorded by using calipers method and calculated by using the formulae: 

\[ \text{Tumor volume} = \frac{4}{3} \times \pi \times \left( \frac{1}{2} \times \text{smaller diameter} \right)^2 \times \left( \frac{1}{2} \times \text{larger diameter} \right) \]  

(Gupta et al., 2007)

**Histological investigation**

Sample of tumor would be fixed in 10% Formalin and processed for histology. Tissues embedded in paraffin wax would be sectioned and examined for the architect of the tissues for histological changes if any and blood vessels count.

**Chromosomal and micronucleus assay**

Cytogenetic damage in the bone marrow cells would be studied by chromosomal aberration assay in terms of chromatid break, chromosomal break and asymmetrical exchanges using method (Savage, 1993) Damage induced by the test substance to the chromosomes would be studied by micronucleus method (Schimid, 1975).
REFERENCES


Olsen, O. and Sørensen, H., Glucosinolates and Amines in Reseda Media. Phytochemistry, 19, 1783-1787 (1980).


