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Research Article

A STUDY ON THE ANTICANCER ACTIVITY OF CRUDE EXTRACT AND CAROTENOID PIGMENTS FROM FLOWERS

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ABSTRACT

Medicinal plants have been identified and used throughout human history. Plants make many chemical compounds that are for biological functions. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. The use of plants as medicines pre-dates written human history. **Ethnobotany**, the study of traditional human uses of plants, is recognized as an effective way to discover future medicines. In 2001, researchers identified 122 compounds used in modern medicine which were derived from traditional plant sources; 80% of these have had a traditional use identical or related to the current use of the active elements of the plant**.** Some of the pharmaceuticals currently available to physicians are derived from plants that have a long history of use as herbal remedies, including aspirin, digoxin, quinine, and opium. Some compounds from plants that have been particularly important for human medicine include: morphine from the Opium Poppy (*Papaver somniferum* L.), aspirin from the White Willow Tree (*Salix alba* L.), and the anticoagulant coumadin from spoiled sweet clover (*Melilotus officinalis* L.Pall) Tropical plants such as the Madagascar, or Rosy, Periwinkle (*Catharanthus roseus* L.G.Don) have yielded vinblastine (which has revolutionized the treatment of Hodgkin's lymphoma, turning a disease that was once uniformly fatal into one that can now be totally cured in many patients) and vincristine (which has done the same for acute childhood leukemia). The present study is aimed at studying the Anticancer Activity of Crude Extract and Carotenoid Pigments of certain selected Flowers of Medicinal Importance.

Keywords: Medicinal Plants, Ethnobotany, Pharmaceuticals, Flowers and Anticancer Activity.

INTRODUCTION

Plants have been used in traditional medicine since prehistoric period and play a significant role to heal human diseases and disorders. According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno pharmacological information is generally considered as an effective approach in the discovery of new antioxidant and ant-infective agents from higher plants. Many medicinal plants contain large amounts of antioxidants other than vitamin C and carotenoids (**Figure 1**). In recent years, in order to discover novel antioxidant and antimicrobial drugs, screening of plants has been accelerated[1].

Figure 1: Dried Flowers samples

Plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. Plants are rich sources of ecologically developed secondary metabolites, which are potential remedies for different ailments. *Ixora coccinea* L. is traditionally used for its hepatoprotective, chemoprotective, antimicrobial, anti-oxidant, anti-nociceptive, anti–mitotic and anti-inflammatory activities. Decoctions of roots are used for nausea, hiccups and anorexia whereas powdered roots are used for sores and chronic ulcers. In indo – china, root decoctions are used to clarify the urine, poultice fresh leaves and stems for sprains, eczema, boils and contusions[2].

Peltophorum pterocarpum (DC.) K.Heyne is a very common deciduous tree grown in tropical countries. Different parts of this tree are used to treat many diseases like stomatitis, insomnia, skin troubles, constipation, and ringworm. Its bark is used as medicine for dysentery, as eye lotion, embrocation for pains and sores. The traditional healers use the leaves in the form of decoction for treating skin disorders. The carotenoid pigments extracted by Column Chromatography showed antibacterial activity against *Staphylococcus aureus, Enterobacter sp. Streptococcus sp.and Escherichia coli* whereas the crude leaf and flower extracts showed antibacterial activity against *Staphylococcus aureus, Enterobacter sp., Streptococcus sp., Salmonella paratyphi and Escherichia coli*[3].

Hibiscus rosasinensis L. is widely cultivated in the tropics as an ornamental plant. It has been reported that the hypoglycemic activity of this extract is not mediated through insulin release and this increases the potential use of this species for human health purposes. Moreover, there is very important evidence of the anticancer action of hibiscus extract against the tumor promotion stage of cancer development, in mouse skin with ultraviolet radiation[4].On the other hand, the ancient Indian medicinal literature reported that the flowers of hibiscus have beneficial effects in heart diseases, mainly in myocardial ischemic disease, due to its enhancement of the myocardial endogenous antioxidants by an adaptative response and without producing any cytotoxic[5].

Tecoma stans (Bignoniaceae) known as yellow elder is an erect shrub or small tree. The plant has been used for a variety of purposes in herbal medicine, treating diabetes and digestive problems. Extracts from *Tecoma stans* leaves have been found to inhibit the growth of the yeast infection. *T. stans* have active phytochemicals which are able to inhibit plant and animal pathogenic bacteria and fungi. The ethanol and methanol extract fractions showed significantly antimicrobial activity against all Gram-positive and Gram negative bacteria and different fungi tested. Strong antioxidant properties were confirmed in the ethanol and methanol extract fractions. These activities may be due to strong occurrence of polyphenolic compounds such as flavonoids, tannins, alkaloids, steroids, phenols and Saponins. The antioxidant activity was comparable with standard ascorbic acid and BHT. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an antimicrobial and antioxidant agent from *T. stans* plant. This medicinal plant by *in vitro* results appear as interesting and promising and may be effective as potential sources of novel antimicrobial and antioxidant drugs[6].

CAROTENOIDS

Carotenoids are an abundant group of naturally occurring pigments. They occur ubiquitously in all organism of conducting photosynthesis (**Figure 2**). They are found in photosynthetic membranes of phototropic bacteria and cyanobacteria. More than 600 different carotenoids from natural sources have been isolated and characterized [7]. Carotenoids consist of 40 carbon atoms (Tetraterpenes) with conjugated double bonds. They consist of 8 isoprenoid units joined in such a manner that the rearrangement of isoprenoid units is reversed at the centre of the molecule so that the two central methyl groups are in a 1, 6 position and the remaining non terminal methyl groups are in a 1,5 position relationship[8].

Carotenoid hydrocarbons are called carotenes and their derivatives containing oxygen are called xanthophylls. Because of the extensive double bond system in the carotenoid molecule, a carotenoid can exist in a large number of geometric isomers (cis/trans isomers). Most Carotenoids are, in fact, found to be in the all-trans form, but cis isomers do exist[9]. The most obvious structural feature of a carotenoid molecule is the chromophore of conjugates double bonds which, in carotenoids of plant tissues, varies from 3 in the colourless phytoene to 13 in canthaxanthin, which is red. This double bond system also renders them susceptible to isomerization and oxidative degradation[10].

Carotenoids are known to suppress the growths of tumors in in vitro (test tube) and in vivo (animal) studies[11]. The various carotenoids such as lycopene, β-carotene, α-carotene, lutein and canthaxanthin can decrease malignant transformation of cells. There have been positive reports on dietary carotenoids improving fertility or reproduction capacity in a number of animals[12].

STRUCTURES OF TYPICAL CAROTENOIDS

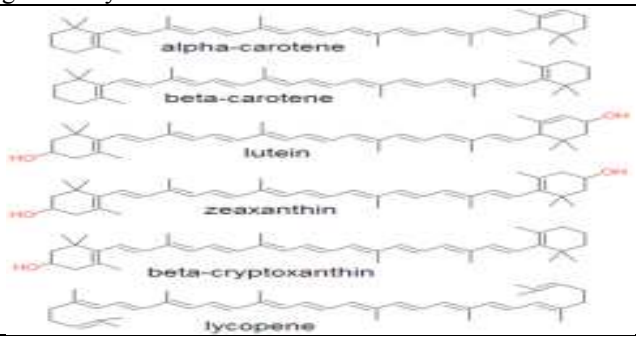

Figure 2: Structure of Carotenoids

The greater the number of conjugated double bonds, the higher the λmax values. Thus, the most unsaturated acyclic carotenoid lycopene, with 11 conjugated double bonds, is red and absorbs at the longest wavelengths (λmax at 444, 470, and 502 nm). At least 7 conjugated double bonds are needed for a carotenoid to have perceptible color. Thus, Beta- carotene is light yellow[13].

CAROTENOIDS AND THEIR BIOLOGICAL ROLE

Carotenoids play a very important role in the human health. They are known to be very efficient physical and chemical quenchers of singlet oxygen (O2), as well as potent scavengers of other reactive oxygen species (ROS), thus acting as very important natural antioxidants. This is of special significance, because the uncontrolled generation and concomitant increase of ROS level in the body results in "oxidative stress", an essential contributor to the pathogenic processes of many diseases**.** Some carotenoids, such as lycopene, zeaxanthin, lutein, capsanthin, and canthaxanthin are not converted into vitamin A in the body. But again, they are powerful cancer fighters, prevalent in fruits and vegetables. There is abundant evidence that lycopene in particular helps reduce the risk for prostate cancer [14]. The present study is aimed at isolating carotenoid pigments from various **Flowers** such as Copper pod, Yellow bell, Hibiscus and Red jungle flame which are rich in beta carotene and to evaluate and compare their Anticancer activity.

MATERIALS AND METHODOLOGY

SAMPLES USED IN THE PRESENT STUDY ARE AS FOLLOWS

Yellow bell (*Tecoma stans* (L.) Juss.ex Kunth.)
Red jungle flame (*Ixora Coccinea* L.)
Copper pod (*Peltophorum pterocarpum* (DC.) K.Heyne.)
Hibiscus (*Hibiscus rosasinensis* L.)

PREPARATION OF EXTRACTS

The Flowers were collected and dried in shade for few weeks. The dried samples were ground into powder. 5gm of the dried sample powder was weighed and immersed in 50 ml of the solvents – Ethanol, Ethyl acetate and Chloroform for 48 hours. The carotenoid pigments were isolated using Column Chromatography and was quantified using the formula

Total carotenoid content (µg/g) = A x V (ml) x 10^4 / $A^{1\%}$cm x W (g)

Where A is the absorbance of the carotenoid pigment at 450 nm, V is the total extract volume, $A^{1\%}$cm is the absorption coefficient of β carotene in hexane (2600), W is the sample weight. The samples were further subjected to Thin Layer Chromatography. The Anticancer activity was assessed using MTT Assay Method.

ANTICANCER ACTIVITY OF THE EXTRACTS - MTT ASSAY

This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (eg. isopropanol) and the released, solubilized formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

Cancer cell lines (MCF7)**,** 96 well plate**,** Dulbecco's Modified Eagle Medium, Foetal Bovine Serum, Antibiotics**,** MTT Reagent, Dimethylsulphoxide were the materials required. Cancer cell lines were purchased from Cancer Institute, Chennai. The cells were grown in a 96 well plate in Dulbecco's Modified Eagle Medium, supplemented with 10% Foetal Bovine Serum and antibiotics (Penicillin-G). About 200µl of the cell suspension was seeded in each well and incubated at 37°C for 48 hours with 5% CO_2 for the formation of confluent monolayer. The monolayer of cells in the plate was exposed to various concentrations of the Flower extracts and their carotenoid extracts and incubated for 24hours. The cytotoxicity was measured using MTT (5mg/ml). After incubation at 37°C in a CO_2 incubator for four hours, the medium was discarded and 200µl of DMSO was added to dissolve the formazon crystals. The absorbance was read in a micro plate reader at 570nm.

Cyto toxicity was calculated by the following formula:

Viability % = (Test OD/Control OD) x 100
Cell toxicity % = 100 – Viability %

RESULTS AND DISCUSSIONS

ISOLATION OF CAROTENOID PIGMENTS BY COLUMN CHROMATOGRAPHY

Carotenoid pigments were effectively separated from the sample extracts separately in a silica gel column with 100% hexane. The yellow colour band which gets separated when eluted with 100% hexane is identified to be carotenoid pigments **(Figure 3)**.The carotenoid pigments eluted with hexane was collected and stored in vials at -20ºC.

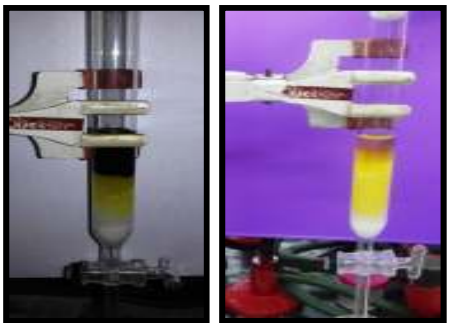

Figure 3: Isolation of Carotenoid pigment

QUANTIFICATION OF CAROTENOIDS

The total carotenoid content quantified are as follows

Total carotenoid content in copper pod = 0.232x10x10^4 / 2600x10 = **0.89 µg/g.**

Total carotenoid content in yellow bell = 0.258x10x10^4 / 2600x10 = **0.99 µg/g.**

Total carotenoid content in hibiscus = 0.237x10x10^4 / 2600x10 = **0.91 µg/g.**

Total carotenoid content in red jungle flame = 0.242x10x10^4 / 2600x10 = **0.93 µg/g.**

THIN LAYER CHROMATOGRAPHY

The crude extracts and the purified carotenoid pigments and the standard were subjected to thin layer chromatography. The standard used was beta carotene. The mobile phase used was hexane and acetone in the ratio 6:4. The respective Rf values

for the Flowers (Copper pod, Yellow bells, Hibiscus and Red jungle flame) were calculated **(Table 1).**

Table 1 : R_f Values of Crude Extract and Carotenoid				
Sample	**Ethanol Crude**	**Ethyl Acetate Crude**	**Chloroform Crude**	**Carotenoide Pigment**
Copper Pod	0.91	0.95	0.94	0.94
Yellow Bell	0.91	0.95	0.94	0.94
Hibiscus	0.97	0.97	0.95	0.94
Red Jungle Flame	0.97	0.95	0.95	0.94

ANTICANCER ACTIVITY OF THE EXTRACTS - MTT ASSAY

The cytotoxicity of the crude extracts of Ethanol, Ethyl acetate, Chloroform and the purified carotenoid pigments of each sample was analysed against human breast cancer cell lines, MCF 7 using MTT assay. It is an colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2 -yl) 2,5-diphenyltetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase in the live cells. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. Cells were treated with 100µg and 150µg of the crude extract and carotenoid extracts **(Figures 4 - 6).**

Figure 4: Cells before treatment of the extracts

Figure 5: Cells after adding the extracts

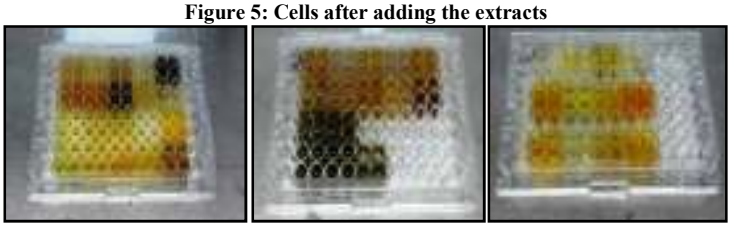

Figure 6: Cells after adding MTT Reagent

The Ethyl acetate crude extracts of Copper pod and Red jungle flame and the Chloroform crude extracts of Yellow bell and Hibiscus showed increased cytotoxicity when compared to other two solvents. The crude extract and their respective isolated carotenoid pigment showed higher cytotoxicity. Over all **Copper pod** and **Yellow bell** gave the best results in anticancer activity among the flowers **(Table 2 and Figures 7 - 8).**

Table 2: Anticancer activity of Flowers									
Sample	**Conc.**	**Ethanol**		**Ethyl Acetate**		**Chloroform**		**Carotenoid**	
		Cell Viability %	**Cell Toxicity %**	**Cell Viability %**	**Cell Toxicity %**	**Cell Viability %**	**Cell Toxicity %**	**Cell Viability %**	**Cell Toxicity %**
Copper Pod	100	67.65	32.35	56.22	43.78	68.06	31.94	54.89	45.11
	150	56.42	43.58	45.1	54.9	62.04	37.96	44.89	55.11
Tecoma	100	65.71	34.29	68.97	31.03	60.91	39.09	63.67	36.33
	150	58.06	41.94	67.34	32.66	52.55	47.45	61.22	38.78
Hibiscus	100	72.65	27.35	71.12	28.88	66.02	33.98	64.89	35.11
	150	70.3	29.7	56.63	43.37	62.85	37.15	62.04	37.96
Red Jungle Flame	100	67.34	32.66	52.34	47.66	69.38	30.62	65.51	34.49
	150	62.24	37.76	52.04	47.96	66.53	33.47	51.22	48.78

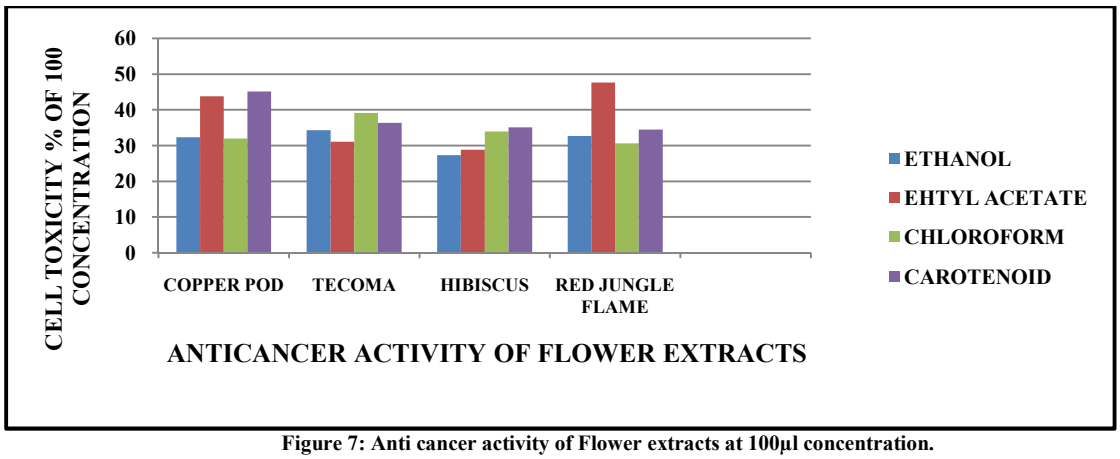

Figure 7: Anti cancer activity of Flower extracts at 100µl concentration.

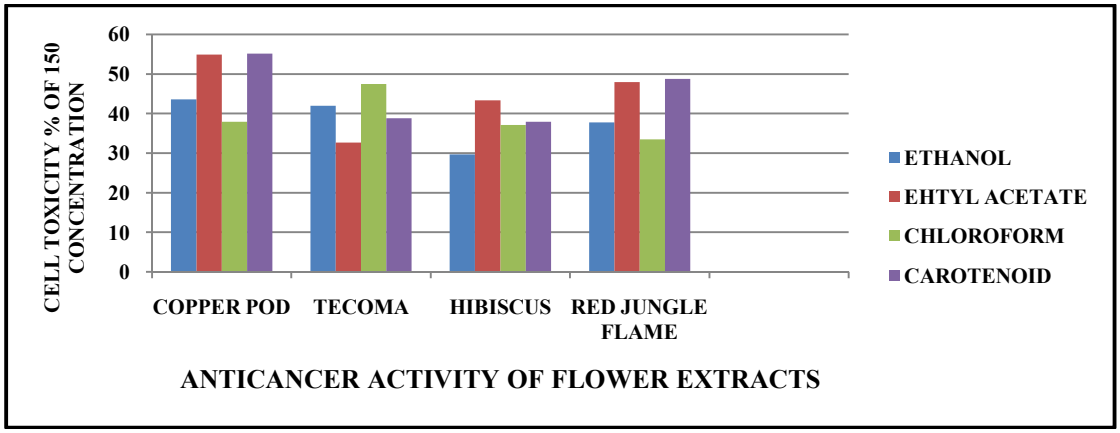

Figure 8: Anti cancer activity of Flower extracts at 150µl concentration.

CONCLUSION

The results of MTT assay on the human breast cancer cell lines, MCF 7 showed dose dependent increase in cytotoxicity of the extracts on the cancer cells. As the concentration of the extracts increases, the cytotoxicity to the cells increases, suggesting the anticancer activity of the extracts. However, the cytotoxicity percentage was maximum in the isolated carotenoid pigment extracts than the crude extracts of all three solvents. Thus the present study reveals that the **Flowers Copper pod and Yellow Bell** to be the best in Anticancer activity and is highly recommended for consumption for prevention of dreadful diseases and for a healthy living in the long run.

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