International Journal of Current Trends in Pharmaceutical Research

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Research Article Open Access

Antimicrobial and Anticancer Activity of the Leaf, Flower and Carotenoid Extracts of *Peltophorum Petrocarpum*

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A B S T R A C T

Nature is our greatest medicine cabinet. It has provided mankind with numerous cures even for deadly diseases. Still there are so many cures that lie untapped in earth's ecosystem and many researches are being done in order to find the cures for many illnesses. *Peltophorum pterocarpum* is a very common deciduous tree grown in tropical countries. Flowering occurs from March-May, although sporadic flowering may occur throughout the year (particularly in young trees), and a second flush of flowers may occur in September-November. Under natural conditions, *P. pterocarpum* is a lowland species, rarely occurring above an altitude of 100 m. It frequently grows along beaches and in mangrove forests, especially the inner margins of mangroves. The species prefers open forest conditions. *Peltophorum pterocarpum* will grow in tropical climates with a dry season of 1-3 months. The tree prefers light to medium free draining alkaline soils although it tolerates clay soils. Different parts of this tree are used to treat many diseases like stomatitis, insomnia, skin troubles, constipation, 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 present study is aimed at analyzing the Anti - microbial and Anti – cancer activity of the leaf, flower and carotenoid extracts of *Peltophorum petrocarpum.*

Keywords: Nature, Peltophorum pterocarpum, Tradional Healers, Anti - microbial and Anti–cancer activity.

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Article History: Received 21 October 2014, **Accepted** 24 December 2014, **Available Online** 15 January 2015

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Manuscript ID: IJCTPR2362

PAPER-QR CODE

Citation: Jean Tony Amalya J and V. Judia Harriet Sumathy. Antimicrobial and Anticancer Activity of the Leaf, Flower and Carotenoid Extracts of *Peltophorum Petrocarpum*. *Int. J. Curnt. Tren. Pharm, Res.*, 2015, 3(1): 748-753.

1. Introduction

Peltophorum pterocarpum is a very common deciduous tree grown in tropical countries and known by a variety of names such as Yellow Poinciana, Golden Flame, Copper pod, Rusty shield bearer and Yellow flamboyant (**Figure 1**).

General Uses of *Peltophorum Pterocarpum*

The following are the general usage of *P. pterocarpum.* Fodder: It is suitable for use as a fodder. Apiculture: In India, it is a source of pollen for the dammer bee (*Trigona iridipennis*). Fuel: The tree is used as fuelwood. Timber: The sapwood is greyish-white, turning grey-brown on aging. The heartwood is light reddish-brown or black, moderately hard, moderately heavy, and somewhat lustrous, with a straight to interlocking grain (Bundit Yuangsoi *et.al.,* 2008). The wood is used locally for light construction purposes, cabinet making, sawn or hewn building timbers, woodware, woodcarving and marquetry (www.worldagroforestry.org). Tannin or dyestuff: The bark of *P. pterocarpum* has been an important component of the dark or black 'soga' dye in Java, used for batik work. It is also used for tanning leather, and preserving and dyeing fishing nets. In Indonesia, the bark is used for fermenting palm wine. (Orwa C *et. al.,* 2009)

Figure 1: Tree, flowers and leaves of *Peltophorum pterocarpum*

Different parts of this tree are used to treat many diseases like stomatitis, insomnia, skin troubles, constipation, ringworm. Its flower extract is known to be a good sleep inducer and used in insomnia treatment. Its bark is used as medicine for dysentery, as eye lotion, embrocation for pains and sores (**Gyorgyi Horvath *et.al.*, 2010)**. The traditional healers use the leaves in the form of decoction for treating skin disorders. Stem infusion of *Peltophorum pterocarpum* is used in dysentery, for gargles, tooth powder and muscular pain. Flowers are used as an astringent to cure or relieve intestinal disorders after pain at childbirth, sprains, bruises and swelling or as a lotion for eye troubles, muscular pains and sores. It is also assumed by **Rao K.N., 2007** that the flowers of *Peltophorum pterocarpum* contain a rich content of carotenoids. There are so many chemical constituents isolated from different parts of the tree and these chemical constituents are known to exhibit several biological activities such as antimicrobial activity, antioxidant activity, cytotoxic activity, antiglycemic activity, aldose reductase inhibition activity, cardiotonic activity, choline esterase inhibitory activity, etc. **(Shyamal K. Jash *et. al.*, 2014).**

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 (O_2), 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 **(Joanna Fiedor and Kvetoslava Burda, 2014).**

These colorful plant pigments are some of which the body can turn into vitamin A, an essential vitamin needed for good eye health. These precursors to vitamin A are sometimes called provitamin A (**Karunai Raj *et.al.*, 2012)**. Bright-orange beta-carotene is the most important carotenoid for adequate vitamin A intake because it yields more vitamin A than alpha- or gamma-carotene. Some carotenoids, such as lycopene, do not convert to vitamin A at all. Lycopene, the orange-red pigment found in tomatoes and watermelon, is still of value, however, because it's an antioxidant even more potent than beta-carotene. The other carotenoids are also valuable antioxidants. **(www.encyclopedia.com).** Most other carotenoids, such as alpha- and gamma-carotene, plus cryptoxanthin and beta-zeacarotene have less vitamin A activity than beta-carotene, but offer ample cancer prevention. Some carotenoids, such

Leaf and Flower Carotenoids

Carotenoids accumulate in the photosynthetic tissues of all higher plants. Both carotenes and xanthophylls are found in leaves with the same four major carotenoids, β- carotene, lutein, violaxanthin and neoxanthin. Minor components include α-carotene, β-cryptoxanthin, zeaxanthin, antheraxanthin and lutein 5,6 epoxide. During leaf senescence the chloroplast disintegrates and esterification of the xanthophylls occurs. The carotenoids of flower petals can be divided into three main groups: a) highly oxygenated carotenoids such as auroxanthin and flavoxanthin b) carotenes, sometimes in high concentrations, ex: β-carotene in Narcissus and c) species specific, ex: Crocetin from Crocus. Flower carotenoids are frequently esterified (**Jain S C, Pancholi B and Jain R, 2011)**.

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 **Nengguo Tao *et.al.*, 2010)**.

2. Materials and Methods

Isolation of Carotenoid Pigments from *Peltophorum Pterocarpum* by Column Chromatography

Column chromatography involves the separation of compounds based on the differences in partitioning between mobile and stationary phases. The stationary phase is placed in a support through which the mobile phase is passed. The stationary phase serves as an adsorbent. Several types of interactions can aid in developing the desired separation **(Pavia D.L., *et. al.*, 1999).** Plant material (leaves and flowers of *P. pterocarpum*), mortar and pestle, acetone, centrifuge tubes, hexane, distilled water, burette, cotton, silica gel are the materials required. About 10g of leaves are weighed, washed of impurities and dried in air.

The leaves are ground in a mortar and pestle by adding acetone little by little. The leaves are ground until a fine paste is obtained. Now the mixture along with acetone is transferred to the centrifuge tube. The mortar and pestle is rinsed with acetone and again the mixture is transferred to the centrifuge tube. 5 ml of hexane is added to the centrifuge tube and capped and the mixture is shaken thoroughly. Then 5 ml of distilled water is added and shaken thoroughly with occasional venting. The mixture is centrifuged to break the emulsion. The pigment layer is the top hexane layer, which should be dark green. Most of the acetone will dissolve in the water. The pigment layer is carefully pipetted out into a clean test tube. The dark green hexane solution of the leaf pigments in the test tube may contain traces of water that must be removed before separating the components through column chromatography.

To dry the solution 0.5g of anhydrous sodium sulphate is added to the hexane solution. Sodium sulphate is allowed to contact all parts of the hexane solution. After standing for 5 minutes, the liquid is transferred to another clean test tube. This extract is used for column chromatography. A dry and clean burette is clamped onto a burette stand and a very small plug of cotton is pushed to the bottom of the burette. 15g of silica gel is taken and mixed well with 100% hexane in a slurry like consistency and the mixture is poured into the burette carefully. The silica gel must be kept wet with solvent all the time. Now the set up is allowed to stand for an hour without disturbance so that the silica gel gets packed well. After an hour, the solvent is drained till it reaches a little above the silica gel. Then the flower/leaves extract is poured gently into the burette. As the extract drains into the silica gel, the pigments begin to separate into a yellow colour carotene band and a green colour chlorophyll band. The pigment of interest here is the yellow carotenes. So, the yellow carotene band is eluted with 100% hexane and the eluant is collected separately.

The carotenes are light sensitive and so care must be taken to protect the pigments from light. The fractions collected are evaporated and the thick concentrated carotene fractions are used for TLC. The total carotenoid content is calculated and the absorbance of the extracted carotenoids was measured at 450nm. The extracted carotenoids were quantified by the following 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. **(Alcides Oliveira R.G., *et. al.*, 2010).**

Antimicrobial Activity of the Extracts

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters. Muller Hinton Agar Medium, 24 hour bacterial cultures, Sterile Petriplates, Gel puncturing machine and Plant extracts were the materials required.

Preparation of Reagents

1. Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L): One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes. The broth was cooled to room temperature after sterilizing and then the bacterial cultures were inoculated in them. The cultures were incubated for 24 hours in a shaker at 37ºC. These bacterial cultures were used for seeding the petriplates.

Procedure

Petriplates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial cultures. Wells were made in each of these plates using sterile cork borer. About 100 µl of different concentrations of plant solvent extracts were added into the wells and allowed to diffuse.The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

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-diphenyl tetrazolium 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, solubilised 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, 96 well plate, Dulbecco's Modified Eagle Medium, Foetal Bovine Serum, Antibiotics, MTT Reagent and Dimethyl sulphoxide were the materials required.

Procedure

Human Breast Cancer cell lines MCF 7 were purchased from Biozone Laboratories, 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^{o}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 carotenoid extracts and incubated for 24hours. The cytotoxicity was measured using MTT (5mg/ml). After incubation at 37^{o}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 microplate reader at 570nm.

Cytotoxicity was calculated by the following formula

Viability % = (Test OD/Control OD) x 100

Cell toxicity % = 100 – Viability %

3. Results and Discussion

Isolation of Carotenoid pigments by Column Chromatography

Figure 2: Isolation of pigments by Column Chromatography

Carotenoid pigments were effectively separated from the leaves and flower extracts separately in a silica gel column with 100 % hexane. The yellow band that separates when eluted with 100% hexane is identified to be the carotenoid pigments. Thus the carotenoid pigments with hexane was collected and stored in glass vials at -20^{o}C (**Figure 2).**

Quantification of Carotenoids

The absorbance of the extracted carotenoids was measured at 450nm. The extracted Carotenoids were quantified by the following 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.

Thus,

Total Carotenoids content in flowers

= 0.145x10x10^4/2600x10

= 0.56µg/g

Antimicrobial Activity of the Extracts

The antibacterial activity of the ethanol extracts of leaf, flower, leaf carotenoid and flower carotenoid extracts were studied against eight organisms namely, *Staphylococcus aureus, Enterobacter sp., Streptococcus sp., Salmonella paratyphi, Escherichia coli, Pseudomonas sp., Bacillus sp. and Proteus sp.* The concentrations of the four extracts used were 100µg/ml.

The extracts showed antibacterial activity against *Staphylococcus aureus, Enterobacter sp., Streptococcus sp., Salmonella paratyphi and Escherichia coli.* They did not show any activity against *Pseudomonas sp., Bacillus sp. and Proteus sp*. The activity was determined by measuring the zone of inhibition in mm.

Table 1: Antimicrobial activity of the extracts against Bacteria

S.No	Bacteria	Zone of Inhibition in mm			
		Leaf extract	**Flower extract**	**Leaf Carotenoid Extract**	**Flower Carotenoid Extract**
1	*Staphylococcus aureus*	2	2	2	3
2	*Enterobacter sp.*	3	2	3	2
3	*Streptococcus sp.*	4	2	2	3
4	*Salmonella paratyphi*	2	3	-	-
5	*Escherichia coli*	3	-	-	2
6	*Pseudomonas sp.*	-	-	-	-
7	*Bacillus sp.*	-	-	-	-
8	*Proteus sp.*	-	-	-	-

The ethanolic flower extract showed activity against *Staphylococcus aureus* and *Sterptococcus* sp. Similar results were shown by **Sukumaran S et al (2011**) in their work for the methanolic extracts of the flowers of *Peltophorum pterocarpum*. The ethanolic leaf extracts showed antibacterial activity against *Staphylococcus aureus, Enterobacter sp., Streptococcus sp., Salmonella paratyphi and Escherichia coli* **(Table 1 & Figure 3)**.

Figure 3: Antibacterial activity of the leaf, flower and carotenoid extracts

The carotenoids extracted from the leaves and flowers showed antibacterial activity against *Staphylococcus aureus, Enterobacter sp., Streptococcus sp.* The flower carotenoid extracts showed antibacterial activity against *Escherichia coli* too. Similar results were reported by **Nengguo Tao *et. al.*, 2010** on analyzing the antibacterial activity of the carotenoid extracts obtained from the peel of *Citrus grandis* Osbeck. The antibacterial activity of the leaf and flower extracts could be attributed to the presence of some metabolic toxins or broad spectrum antibiotic compounds. Thus, the leaf and flower extracts could be used as effective antimicrobial compounds. The carotenoid extracts may be used as natural alternatives for chemicals in food preservation.

Anticancer Activity

Table 2: Cell viability and Cell toxicity of the extracts

S.No	Concentration (µg/ml)	Leaf Extract		Leaf Carotenoid Extract		Flower extract		Flower Carotenoid Extract	
		Cell Viability (%)	**Cell Toxicity (%)**	**Cell Viability (%)**	**Cell Toxicity (%)**	**Cell Viability (%)**	**Cell Toxicity (%)**	**Cell Viability (%)**	**Cell Toxicity (%)**
1.	25	89.69	10.31	75.15	24.85	82.88	17.12	75.67	24.33
2.	50	88.86	11.14	69.17	30.83	78.86	21.14	72.26	27.74
3.	100	82.16	17.84	57.11	42.89	68.65	31.35	67.73	32.27

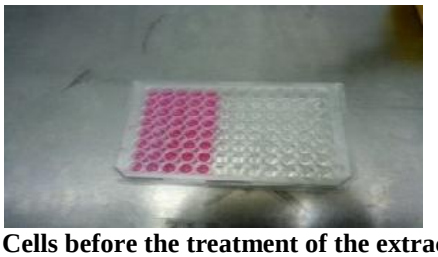

Figure 4: Cells before the treatment of the extracts

Figure 5: Cells after the treatment with the extracts and MTT Reagent

The cytotoxicity of the carotenoids and the crude extracts on human breast cancer cell lines, MCF 7 was analysed. MTT assay as described is a technique of analyzing the cytotoxicity of substances based on the conversion of the yellow MTT reagent into purple insoluble formazon crystals, by the enzymatic action of mitochondrial dehydrogenase in live cells. Cells were treated with 25µg, 50µg and 100µg of the carotenoid extracts and the crude extracts. The results of the assay on the cancer cell lines showed dose dependent increase in cytotoxicity of the extracts on these cells, suggesting the anticancer activity of the extracts. However, the cytotoxicity percentage was higher in the carotenoids extracts than the crude extracts **(Table 2 & Figures 4 - 5)**.

4. Conclusion

Carotenoids and some of their metabolites are suggested to play a protective role in a number of ROS-mediated disorders, such as, *i.e.*, cardiovascular diseases, several types of cancer or neurological, as well as photosensitive or eye-related disorders. Carotenoids are also suggested to participate in: (i) the stimulation of the immune system; (ii) the modulation of intracellular signaling pathways (gap junction communication); (iii) the regulation of the cell cycle and apoptosis; (iv) the modulation of growth factors; (v) cell differentiation; and (vi) the modulation of various types of receptors or adhesion molecules and many other physiologically significant processes. Thus carotenoids are pigments that are found to have multi-medicinal value. 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.* The anticancer activity studied using MTT assay showed higher anticancer activity than the crude leaf and flower extracts.

5. References

1. Alcides Oliveira R G, Lucia De Carvalho M J, Marilia Nutti R, Jose De Carvalho L V and Wania Goncalves Fukuda. "Assessment and degradation study of total carotenoid and Beta carotene in bitter yellow cassava varieties" *African Journal of Food Science*, **2010**, **4(4)**, pp. 148 – 155.
2. Bundit Yuangsoi, Orapint Jintasataporn, Nontavit Areechon and Prathak Tabthipwon**.** "Validated TLC-densitometric analysis for the determination of carotenoids in fancy carp (*Cyprinus carpio*) serum and the application for pharmacokinetic parameter assessment" *Songklanakarin Journal of Science and Technology*, **2008,** 30(6), 693-700.
3. Gyorgyi Horvath, Peter Molnar, Agnes Farkas, Laszlo Gy. Szabo, Erika Turcsi and Jozsef Deli **(2010)** "Separation and Identification of Carotenoids in flowers of *Chelidonium majus L.* and Inflorescences of *Solidago Canadensis L."Chromatographia* .
4. Jain S C, Pancholi B and Jain R**.** "*Peltophorum pterocarpum* (DC.) Baker ex. K. Heyne Flowers: Antimicrobial and Antioxidant Efficacies" *Research Journal of Medicinal Plant,* **2011,** 5: 274-280.
5. Joanna Fiedor and Kvetoslava Burda**.** "Potential Role of Carotenoids as Antioxidants in Human Health and Disease" *Nutrients*, **2014,** 6: 466-488.
6. Karunai Raj M, Balachandran C, Duraipandiyan V, Agastian P, Ignachimuthu S and Vijayakumar A **(2012)** "Isolation of terrestribisamide from *Peltophorum pterocarpum* (DC) Baker ex K.Heyne and its antimicrobial, antioxidant and cytotoxic activities" *Medicinal Chemistry Research*.
7. Nengguo Tao, Yumei Gao, Yuejin Liu and Fei Ge. "Carotenoids from the peel of Shatian Pummelo (Citrus grandis Osbeck) and its antimicrobial activity" *American-Eurasian J. Agric &Environ. Sci.,* **2010,** 7(1):110-115.
8. Orwa C, Mutua A, Kindt R, Jamnadass R and Simons A **(2009)**. Agroforestree Database: a tree reference and selection guide version 4.0.
9. Pavia, D L, Lampman, G M, Kriz, G S, and Engel R G **(1999)** "Isolation of Chlorophyll and Carotenoid pigments from Spinach" *Introduction to Organic Laboratory Techniques: A Microscale Approach third edition,* Saunders College Publishing: New York.
10. Rao K N, **2007** Trees and Tree Tales – Some Common trees of Chennai, 30-31.
11. Shyamal K. Jash, Raj K. Singh, Sasadhar Majhi, Atasi Sarkar and Dilip Gorai, "*Peltophorum pterocarpum*: Chemical and Pharmacological aspects" *International Journal of Pharmaceutical Sciences and Research*, **2014**, 5(1): 26-36.
12. Sukumaran S, Kiruba S, Mahesh M, Nisha S R, Miller Paul Z, Ben C P and Jeeva S**.** "Phytyochemical constituents and antibacterial efficacy of the flowers of Peltophorum pterocarpum (DC.) Baker ex Heyne" *Asian Pacific Journal of Tropical Medicine,* **2011**, 735-738.