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Antimicrobial Activity of Crude Extract and Carotenoid Pigments from Flowers

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A B S T R A C T

All human pharmaceuticals now in use are originally derived from natural sources. According to studies conducted by the World Health Organization (WHO), about 80% of the world's population relies on traditional medicine**.** Several important drugs such as Taxol, camptothecin, morphine and quinine have been isolated from plant sources. The first two are widely used as anticancer drugs, while the remaining are analgesic and antimalarial agents, respectively. 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). Thus natural sources make a very significant contribution to the health care system. The present study is aimed at studying the Antimicrobial activity of Crude Extract and Carotenoid pigments of selected Flowers of Medical Significance.

Keywords: Pharmaceuticals, WHO, Flowers, Antimicrobial activity and Health Care System.

A R T I C L E I N F O

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1. Introduction

Peltophorum pterocarpum (DC.) K. Heyne is a common deciduous tree grown in tropical countries. Different parts of this tree are used to treat diseases like stomatitis, insomnia, skin troubles, constipation, and ringworm. Its bark is used as medicine for dysentery, as eye lotion, embrocating for pains and sores. The traditional healers use the leaves in the form of decoction for treating skin disorders. A Research study conducted by extracting carotenoid pigments using 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* **(Jean Tony Amalya and Judia Harriet Sumathy, V, 2015).**

Hibiscus rosasinensis L. on the other hand 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 (**Nengguo Tao *et. al.*, 2010)**. 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 **(Sharma S *et. al.*, 2004)**. 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 y an adaptative response towards it without producing any cytotoxic effect **(Gauthaman K.K *et. al.*,2006) (Figure 1).**

Figure 1: Dried Flowers samples

Carotenoids

Carotenoids are important in human health. Carotene plays an essential role as sources of vitamin A. The most active role is protection against serious disorders such as cancer, heart diseases and degenerative eye diseases. It is an antioxidant and acts as regulators of the immune system. Carotenoids are a class of hydrocarbon (carotene) and their oxygenated derivatives (xanthophyls). In mammals, such as humans and monkeys, the most important metabolic products of carotenoids are the retinoids, including vitamin A and retinal. It was demonstrated that the formation of vitamin A from β-carotene could occur either by central or by eccentric cleavage of β carotene. α-carotene, β-carotene and β-cryptoxanthin can be converted to retinal or vitamin A in the intestine and liver by the enzyme 15-151 β-carotenoid dioxygenase **(Joanna Fiedor and Kvetoslva Burda, 2014)**. Such in vivo formation of retinal appears to be homeastatically controlled, such that conversion to retinol is limited in persons having adequate vitamin A. Age-related muscular degeneration (ARMD) associated with ageing can lead to a total blindness in healthy people. **(D. E. Okwu, 2008).** Carotenoids are known to suppress the growths of tumors in in vitro (test tube) and in vivo (animal) studies **(S. Sonia, K, *et. al.*, 2007)**. 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 **(A. Bendich, 1989).** Carotenoids besides the anticancerous effect, showed a strong antioxidant character, which plays an important role in the prevention and treatment of cardiovascular, ophthalmological, dermatological diseases and prevents the oxidative damages that are specific to ageing phenomena and also prevents the immunological disorders **(P.M.Dey and J.B.Haarborne, 1997).** Due to carotenoids great sensitivity to light, heat, oxygen, acids, their isolation from different raw materials must be accomplished choosing the optimal work conditions to gum up their degradation **(Delia -Gabriela Dumbravă *et. al.*, 2010).**

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 their applications in various fields of medical sciences.

2. Materials and Methods

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. After 48 hours, the extracts were filtered. The filtrates were used for further phytochemical analysis which includes Test for Carbohydrates, Proteins, Glycosides, Tannins, Alkaloids, Flavonoids, Terpenoids, Saponins, Resins, Quinones, Cardiac Glycosides, Coumarins, Ssteroids, Phytosteroids, Phenols, Anthraquinones and Phlobotannins. 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 antioxidant studies using Reducing

Power assay and Phosphomolybdenum methods and the Antimicrobial study were carried out.

Antimicrobial Activity of the Extracts

The antimicrobial contents present in the flower extracts and the carotenoid extracts 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 Petri plate, Gel puncturing machine and Plant extracts are the materials required.

Preparation of Media

1. Nutrient Broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (Hi Media) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by auto claving at 15lbs 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 37^{o}C. These bacterial cultures were used for seeding the petriplates.

2. Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (Hi Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15minutes. The autoclaved medium was mixed well and poured onto 100 mm petriplates (25-30ml/plate) while still molten. Petri plates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacteria strain. Wells were made in each of these plates using sterile cork borer. About 100 µl and 75 µl of 100mg/ml concentrations of flower solvent extracts and carotenoid 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.

3. Results and Discussions

Isolation of Carotenoid Pigments by Column Chromatography:

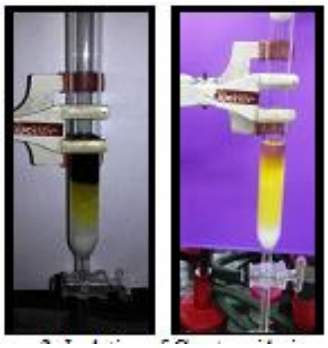

Figure 2: Isolation of Carotenoid pigment

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 2)**.The carotenoid pigments eluted with hexane was collected and stored in vials at -20ºC.

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).**

Antimicrobial Activity of the Extracts

The antimicrobial activity of the Ethanol, Ethyl acetate and Chloroform crude extracts of the samples includes flowers (Copper pod, Yellow bell, Hibiscus and Red jungle flame) and their respective isolated carotenoid pigments from each sample were studied against organisms namely *Staphylococcus aureus* and *Escherichia coli*.

The concentration of the each extracts used were 100µg/ml and they were studied using different µl change 100µl and 75µl of each sample extracts.Over all the extracts of sample of three different solvent showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* **(Table 2).** Particularly the Ethanolic crude extract and the Ethyl acetate crude extract of all the flowers extract showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli*. But the chloroform crude extracts of flowers (except Copper pod) showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* **(Figure 3 – 5) .**The Carotenoid pigment extracted from the flowers showed maximum antimicrobial activity against *Staphylococcus aureus* only. The activity was determined by measuring the zone of inhibition in mm.

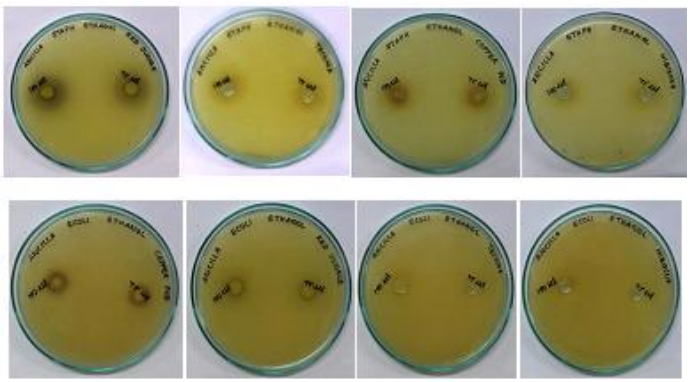

Figure 3: Antimicrobial Activity of Ethanol Crude Extract against organism

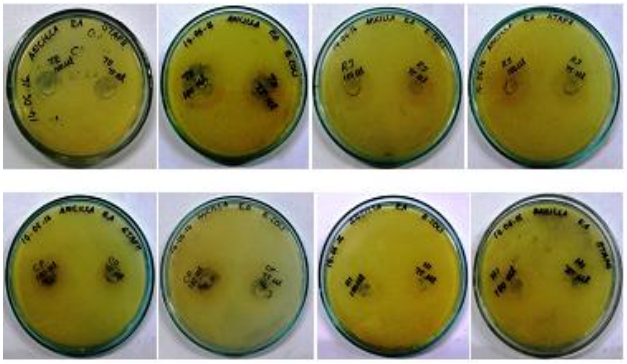

Figure 4: Antimicrobial Activity of Ethyl acetate Crude Extract against organism

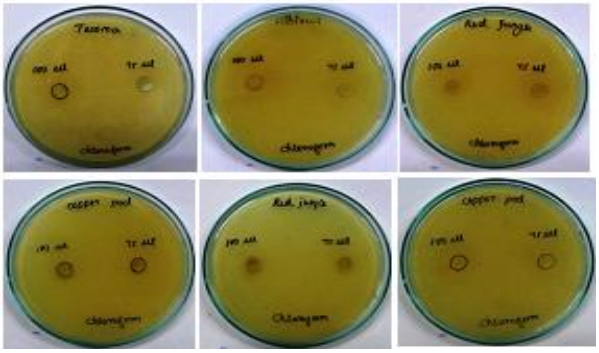

Figure 5: Antimicrobial Activity of Chloroform Crude Extract against organism

Table 1: Rf 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

Table 2 : Zone of Inhibition In mm

Sample	Ethanol				Ethyl acetate				Chloroform				Carotene sample			
	STAPH		E.COLI		STAPH		E.COLI		STAPH		E.COLI		STAPH		E.COLI	
	100 µl	75 µl	100 µl	75 µl	100 µl	75 µl	100 µl	75 µl	100 µl	75 µl	100 µl	75 µl	100 µl	75 µl	100 µl	75 µl
Copper POD	20	15	15	10	10	5	20	15	-	-	-	-	10	25	-	-
Yellow Bell	20	15	15	10	25	15	25	15	10	-	10	-	10	20	-	-
Red jungle	20	15	10	10	10	10	20	10	10	10	10	10	-	15	-	-
Hibiscus	15	10	10	10	10	10	5	-	10	10	15	10	10	15	-	-

4. Conclusion

Thus the present study reveals the Flowers, Copper pod and Yellow Bell to be the best and is highly recommended for its use as an effective antimicrobial compound. The antimicrobial activity of the crude extracts could be attributed to the presence of metabolic toxins or broad spectrum antibiotic compounds. Furthermore the carotenoid pigments can also be used as a natural alternative to chemical in preservation of food.

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