WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 11, 1173-1183. Research Article ISSN 2277– 7105

# ANTIOXIDANT ACTIVITY OF CRUDE EXTRACT AND CAROTENOID PIGMENTS FROM FLOWERS

**Ancilla Senoretta B. and *Dr. V. Judia Harriet Sumathy**

Postgraduate & Research Department of Biotechnology Women's Christian College, Chennai – 600 006.

Article Received on
12 Sept. 2016,

Revised on 02 Oct. 2016,
Accepted on 22 Oct. 2016

DOI: 10.20959/wjpr201611-7294

***Corresponding Author**
**Dr. V. Judia Harriet Sumathy**
Postgraduate & Research Department of Biotechnology Women's Christian College, Chennai – 600 006.

## ABSTRACT

Plants in any form has always remained to be a major source of traditional way of managing health problems and different economic issues. Importance of the plants basically originates due to the presence of specific biological active classes of organic compounds. On the planet earth, according to one report, more than 258,650 species of higher plants and round about similar number of lower plants exist but not more than 10% of higher plants species are being used for their medicinal properties. Over 50% of all modern clinical drugs are obtained from natural sources. So in this way natural products have played a vital role in drug development programs of pharmaceutical industries. Further, due to the long list of side effects of purely synthesized drugs or medicines more than 80% world's population still emphasize to use traditional and old medicinal system such as Homeopathy, Unani, Ayurveda, Sidha and Naturopathy. The extract of *H. rosa-sinensis* has already been tested for the treatment of oxidative stress diseases including diabetes and tumor. Hence the present study is aimed at studying the Antioxidant property of few Flowers of Medicinal Importance.

**KEYWORDS:** Plants, Planet Earth, Traditional Medicine, Flowers and Antioxidant Property.

## INTRODUCTION

Natural products have been the basis of treatment of human diseases for a long period of time. Modern medicine or allopathy has gradually developed over the years due to the scientific and observational efforts of scientists- however the basis for its development

remains in the roots of traditional medicine and therapies. Herbal medicinal preparations and their proprietary products are also being used more and more widely throughout the world.

*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 and adaptive response towards it without producing any cytotoxic effect **(Gauthaman K.K *et. al.*, 2006) (Figure 1).**


**Figure 1: Dried Flowers samples**

Ancient Indian medicinal literature reported that the flowers of *H. rosasinensis* 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 effects **(Gauthaman *et. al.*, 2006)**. Recently, **Nade *et. al.* (2011)** suggested that *Hibiscus rosa* has a protective role against age and scopolamine-induced amnesia, indicating its utility in management of cognitive disorders. Moreover, there is very important evidence of the anticancer action of *H. rosa sinensis* extract against the tumor promotion stage of cancer development, in mouse skin with ultraviolet radiation **(Sharma *et.al.*, 2004)**. The crude extract of aerial parts of *H. rosa sinensis* and its subsequent fractions, clearly showed the presence of two components that have cholinomimetic and calcium antagonist activities. So, the possible pharmacological rationale use of the plant for constipation and diarrhea was suggested **(Gilani *et. al.*, 2005)**.

*Ixora coccinea* (Rubiaceae), a small to medium sized hardy shrub cultivated for ornamental purpose were also used in traditional Indian medicine. Antimicrobial activity of *I.coccinea* leaves and flower extracts have been reported. Anti-inflammatory and antimitotic activities from leaf extracts have been reported. They have also been reported to have anti-inflammatory activity comparable to indomethacin. Flowers were also reported to possess cytotoxic and antitumour activity in mice injected with Dalton's lymphoma ascetic (DLA) cells. Flowers extracts were reported to contain triterpenoid, ursolic acid. The flowers afforded two new cycloartenol esters, lupeol fatty ester, lupeol, oleanolic acid and sitosterol. Flowers showed protective effects against cyclophosphamide and cisplastin induced systemic toxicity. Wound healing properties of alcoholic extract of flower was also reported and it was shown to significantly increase the enzymatic profiles of Wistar rats (**Nagaraj *et. al*, 2011).**

*Tecoma stans* have active phytochemicals which are able to inhibit plant and animal pathogenic bacteria and fungi. The ethanol and methanol extract fractions showed significant antimicrobial activity against all Gram-positive and Gramnegative 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 **(Govindappa M *et. al*, 2011).**

## CAROTENOIDS

Carotenoids are an abundant group of naturally occurring pigments. 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 **(Joanna Fiedor and Kvetoslva Burda, 2014 and D. E. Okwu, 2008).**

Carotenoids besides the anti-cancerous 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. Due to carotenoids great sensitivity to light, heat, oxygen and 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 ultraviolet and visible spectrum is the first diagnostic tool for the identification of carotenoids **(Figure 2).** The wavelength of maximum absorption (λ max) and the shape of the spectrum (spectral fine structure) are characteristic of the chromophore. Most carotenoids absorb maximally at three wavelengths, resulting in three peaks Spectra (**Seow-Mun Hue *et. al.*, 2011)**. 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 **(Delia B. Rodriguez-Amaya, 2001)**.

## STRUCTURES OF TYPICAL CAROTENOIDS

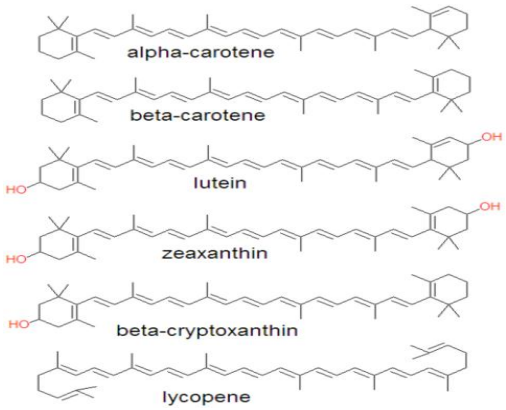


**Figure 2: Structure of Carotenoids**

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.

## 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. After 48 hours, the extracts were filtered. 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 methodology were carried out.

## 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.

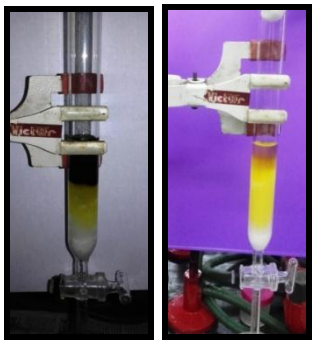

**Figure 3: Isolation of Carotenoid pigment**

## QUANTIFICATION OF CAROTENOIDS

The total carotenoid content quantified are as follows

Total carotenoid content in copper pod = $0.232 \times 10 \times 10^4 / 2600 \times 10$ = **0.89 µg/g.**

Total carotenoid content in yellow bell = $0.258 \times 10 \times 10^4 / 2600 \times 10$ = **0.99 µg/g.**

Total carotenoid content in hibiscus = $0.237 \times 10 \times 10^4 / 2600 \times 10$ = **0.91 µg/g.**

Total carotenoid content in red jungle flame = $0.242 \times 10 \times 10^4 / 2600 \times 10$ = **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 : 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 |

## ANTIOXIDANT ACTIVITY OF THE EXTRACTS

### 1. REDUCING POWER ASSAY

The reducing power assay was used to test the reducing capability of the extracts. Their ability to reduce the potassium ferricyanide (Fe3+) complex to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex is determined by measuring the absorbance at 700 nm of sample with standard **(Figure 4).**

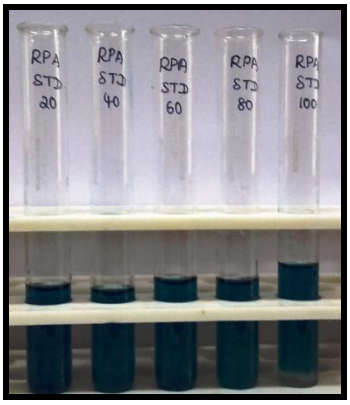


**Figure 4: Standard test of Reducing Power assay**

The concentration dependent activity was observed in all the crude extracts and carotenoid extracts. The reducing power of the extracts increased with increase in concentration. However, the isolated carotenoid pigments from their respective samples showed higher reducing activity compared to the crude solvent extracts.

The Ethyl acetate crude extracts of (Copper pod, Yellow bell and Red jungle flame) and the Chloroform crude extract of Hibiscus showed increased activity but their isolated carotenoid pigment showed higher activity than the crude **(Table 2).**

Over all **Copper pod** and **Yellow bell** gave the best results in Reducing Power assay among the Flowers **(Figure 5).**

**TABLE 2: REDUCING POWER ACTIVITY OF FLOWER EXTRACTS**

| SAMPLE | CONC (µg/ml) | STANDARD ASCORBIC ACID OD | ETHANOL | ETHYL ACETATE | CHLOROFORM | CAROTENOID PIGMENT |
|---|---|---|---|---|---|---|
| COPPER POD | 20 | 0.17 | 0.24 | 0.38 | 0.16 | 0.24 |
| | 40 | 0.45 | 0.26 | 0.39 | 0.2 | 0.26 |
| | 60 | 0.63 | 0.28 | 0.4 | 0.24 | 0.29 |
| | 80 | 0.80 | 0.3 | 0.42 | 0.26 | 0.35 |
| | 100 | 0.85 | 0.32 | 0.44 | 0.29 | 0.42 |
| YELLOW BELL | 20 | 0.17 | 0.12 | 0.26 | 0.16 | 0.25 |
| | 40 | 0.45 | 0.14 | 0.39 | 0.19 | 0.27 |
| | 60 | 0.63 | 0.16 | 0.4 | 0.22 | 0.34 |
| | 80 | 0.80 | 0.2 | 0.42 | 0.26 | 0.38 |
| | 100 | 0.85 | 0.22 | 0.44 | 0.29 | 0.52 |
| HIBICUS | 20 | 0.17 | 0.18 | 0.14 | 0.25 | 0.19 |
| | 40 | 0.45 | 0.2 | 0.18 | 0.29 | 0.22 |
| | 60 | 0.63 | 0.22 | 0.21 | 0.31 | 0.26 |
| | 80 | 0.80 | 0.24 | 0.23 | 0.33 | 0.3 |
| | 100 | 0.85 | 0.26 | 0.26 | 0.36 | 0.35 |
| RED | 20 | 0.17 | 0.12 | 0.38 | 0.25 | 0.25 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **JUNGLE FLAME** | 40 | 0.45 | 0.14 | 0.39 | 0.27 | 0.27 |
| | 60 | 0.63 | 0.16 | 0.4 | 0.27 | 0.34 |
| | 80 | 0.80 | 0.2 | 0.42 | 0.29 | 0.38 |
| | 100 | 0.85 | 0.22 | 0.44 | 0.32 | 0.4 |

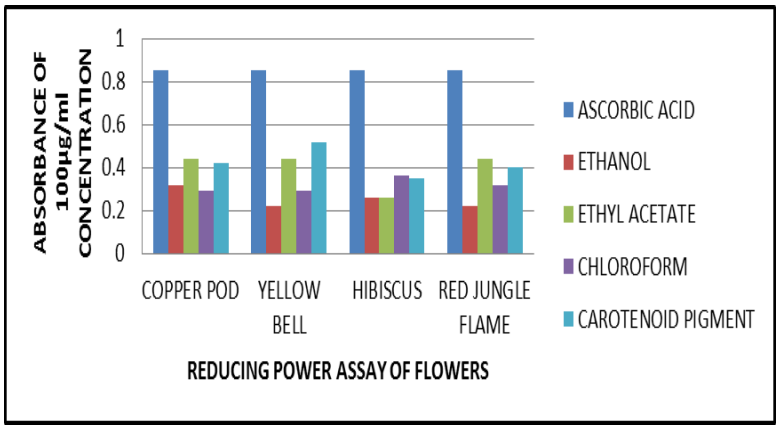


**Figure 5: Reducing Power activity of Flower extracts**

## 2. TOTAL ANTIOXIDANT ACTIVITY BY PHOSPHOMOLYBDENUM METHOD

The phosphomolybdenum assay was used to determine the antioxidant capacity of the extracts based on the reduction of Mo (VI) – Mo (V) by the antioxidants and subsequent formation of a green phosphate/Mo (V) complex by measuring the absorbance at 695 nm of the sample with standard **(Figure 6).**

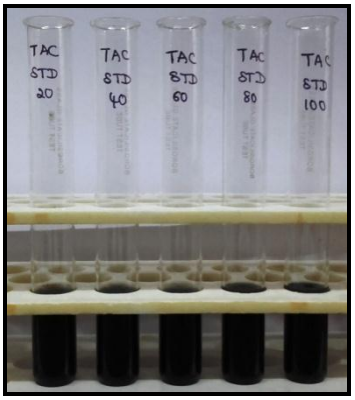


**Figure 6: Standard test of Total antioxidant activity**

The Ethanol extracts of (Copper pod and Yellow bell), the Ethyl acetate extract of Hibiscus, the Chloroform extract of Red jungle flame and their respective carotenoid pigment showed higher activity than the crude **(Table 3).**

Over all **Copper pod** and **Yellow bell** gave the best results in Total Antioxidant activity among the Flowers **(Figure 7).**

| TABLE 3 : TOTAL ANTIOXIDANT ACTIVITY OF FLOWER EXTRACTS | | | | | | |
|---|---|---|---|---|---|---|
| **SAMPLE** | **CONC (µg/ml)** | **STANDARD ASCORBIC ACID OD** | **ETHANOL** | **ETHYL ACETATE** | **CHLOROFORM** | **CAROTENOID PIGMENT** |
| **COPPER POD** | 20 | 0.16 | 0.39 | 0.2 | 0.34 | 0.15 |
| | 40 | 0.42 | 0.4 | 0.26 | 0.36 | 0.21 |
| | 60 | 0.55 | 0.42 | 0.29 | 0.38 | 0.29 |
| | 80 | 0.74 | 0.47 | 0.31 | 0.4 | 0.31 |
| | 100 | 0.88 | 0.50 | 0.35 | 0.42 | 0.49 |
| **YELLOW BELL** | 20 | 0.16 | 0.25 | 0.12 | 0.17 | 0.25 |
| | 40 | 0.42 | 0.3 | 0.16 | 0.19 | 0.39 |
| | 60 | 0.55 | 0.33 | 0.18 | 0.22 | 0.42 |
| | 80 | 0.74 | 0.36 | 0.21 | 0.26 | 0.45 |
| | 100 | 0.88 | 0.39 | 0.22 | 0.3 | 0.47 |
| **HIBICUS** | 20 | 0.16 | 0.15 | 0.42 | 0.25 | 0.5 |
| | 40 | 0.42 | 0.19 | 0.48 | 0.28 | 0.52 |
| | 60 | 0.55 | 0.22 | 0.52 | 0.31 | 0.57 |
| | 80 | 0.74 | 0.25 | 0.56 | 0.34 | 0.59 |
| | 100 | 0.88 | 0.31 | 0.59 | 0.38 | 0.6 |
| **RED JUNGLE FLAME** | 20 | 0.16 | 0.3 | 0.22 | 0.32 | 0.39 |
| | 40 | 0.42 | 0.33 | 0.28 | 0.36 | 0.42 |
| | 60 | 0.55 | 0.36 | 0.34 | 0.38 | 0.45 |
| | 80 | 0.74 | 0.39 | 0.39 | 0.46 | 0.47 |
| | 100 | 0.88 | 0.41 | 0.44 | 0.52 | 0.61 |

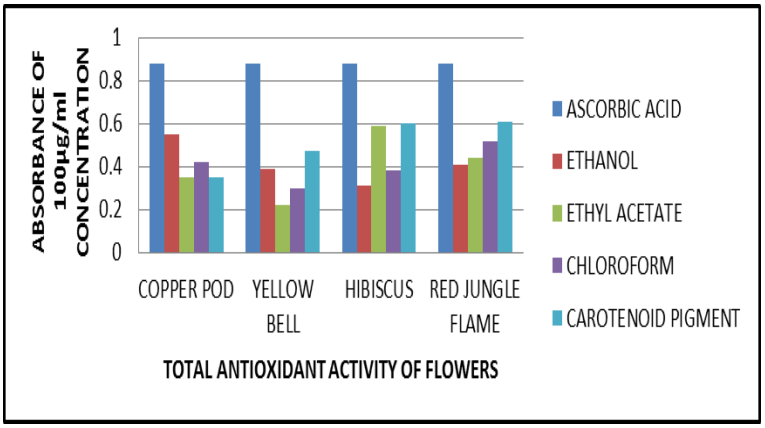


**Figure 7: Total Antioxidant capacity by phosphomolybdenum method of Flowers extract**

The Reducing power assay and Total antioxidant capacity of the extracts were increased with increase in concentration. However, the isolated carotenoid pigments from their respective samples showed higher reducing activity compared to the crude solvent extracts.

## CONCLUSION

The carotenoids were extracted from the Flowers **(Copper pod, Yellow Bells, Red Jungle Flame and Hibiscus)** by column chromatography and subjected to thin layer chromatography. The crude extract and the carotenoid extracts were then analysed for their antioxidant activity. The antioxidant activity was carried out using reducing power assay and phosphomolybdenum method. In both the methodologies done, the carotenoid pigments from the sample **Copper pod and Yellow bell** showed highest activity. Thus the present study reveals the Flowers, **Copper pod and Yellow Bell** to be the best in Antioxidant activities and is highly recommended for consumption for prevention of dreadful diseases and for a healthy living in the long run.

## BIBLIOGRAPHY

1. Delia B. Rodriguez-Amaya (2001) "A guide to Carotenoid Analysis in Foods".
2. Delia-Gabriela Dumbravă, Nicoleta-Gabriela Hădărugă, Daniel I. Hădărugă, Camelia Moldovan, Diana Rab (2010) "Determination by RP-HPLC of β-carotene concentration from orange (*Citrus sinensis* L.) fruits peel extracts" *Journal of Agro alimentary Processes and Technologies*, 16(2): 242-246.
3. Gauthaman K.K., Saleem M.T., Thanislas P.T., Prabhu V.V., Krishnamoorthy K.K., Devaraj N.S. and Somasundaram J.S (2006) "Cardio protective effect of the *Hibiscus rosasinensis* flowers in an oxidative stress model of myocardial ischemic reperfusion injury in rat" *BMC Complementary and Alternative Medicine*, 6: 32.
4. Gilani A.H., Bashir S., Janbaz K.H. and Shah A.J (2005) "Presence of cholinergic and calcium channel blocking activities explains the traditional use of *Hibiscus rosa sinensis* in constipation and diarrhea" *Ethnopharmacol*, 102: 94–289.
5. Govindappa M, Sadananda TS, Channabasava R, Jeevitha MK, Pooja KS and Vinay B. Raghavendra (2011) "Antimicrobial, Antioxidant Activity and Phytochemical Screening of *Tecoma stans* (L.) Juss. ex Kunth" *Journal of Phytology*, 3(3): 68-76.
6. Jean Tony Amalya J and V. Judia Harriet Sumathy (2015) "Antimicrobial and Anticancer Activity of the Leaf, Flower and Carotenoid Extracts of *Peltophorum Petrocarpum*." *International Journal of Current Trends in Pharmaceutical Research*, 3(1): 748-753.
7. Joanna Fiedor and Kvetoslava Burda (2014) "Potential Role of Carotenoids as Antioxidants in Human Health and Disease" *Nutrients*, 6: 466-488.

8. Nade V.S., Kanhere S.V., Kawale L. A. and Yadav A.V (2011) "Cognitive enhancing and antioxidant activity of ethyl acetate soluble fraction of the methanol extract of *Hibiscus rosa sinensis* in scopolamineinduced amnesia" *Indian Pharmacol,* 43(2): 137–142.
9. Nagaraj S, Baskar L, Papiya MM, Muthukumar K, Simon SJ, Ashok Kumar P (2011) "Evaluation of wound healing and antimicrobial potentials of Ixora coccinea root extract" *Asian Pacific Journal of Tropical Medicine*, 959-963.
10. Nengguo Tao, Yumei Gao, Yuejin Liu and Fei Ge (2010) "Carotenoids from the peel of Shatian Pummelo (Citrus grandis Osbeck) and its antimicrobial activity" *American-Eurasian J. Agric & Environ. Sci.,* 7(1): 110-115.
11. Okwu. D.E (2008) "Citrus fruits: a rich source of phytochemicals and their roles in human health" *International Journal of Chemistry and Science,* 6(2): 451-471.
12. Seow-Mun Hue, Amru Nasrulhaq and Chandran Somasundaram (2011) "Comparative Study on the Antioxidant Activity of Leaf Extract and Carotenoids Extract from *Ipomoea batatas* var. Oren (Sweet potato) Leaves" *World Academy of Science, Engineering and Technology*, 58.
13. Sharma S., Khan N. and Sultana S (2004) "Effect of Onosma echioides on DMBA/croton oil mediated carcinogenic response, hyperproliferation and oxidative damage in murine skin" *Europe Cancer Prevention,* 13: 40–53.