International Journal of Current Trends in Pharmaceutical Research

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Research Article **Open Access**

A Study on the Antioxidant, Antimicrobial, Anticancer Activity and Phytochemical Analysis of Crude Extract and Carotenoid Pigments from Fruits, Vegetables and Flowers

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A B S T R A C T

Traditional medicine has a long history of serving people all over the world. India is without doubt a herbal hub. Medicinal plants that are native to India and their use in various traditional systems of medicine are indeed awe-inspiring. The Ethnobotany of ubiquitous plants provides a rich resource for natural drug research and development. The World Health Organization (WHO) defines traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness". 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. The present study is aimed at studying the Antioxidant, Antimicrobial and Anticancer activity of Crude extract and Carotenoid pigments of selected Fruits, Vegetables and Flowers and identify their pivotal role in combating dreadful diseases in the field of Medical Science.

Keywords: Traditional Medicine, Ethnobotany, Fruits, Vegetables and Flowers

A R T I C L E I N F O

CONTENTS

Article History: Received 19 September 2016, **Accepted** 27 October 2016, **Available Online** 15 November 2016

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Manuscript ID: IJCTPR3159

PAPER-QR CODE

Citation: V. Judia Harriet Sumathy. A Study on the Antioxidant, Antimicrobial, Anticancer Activity and Phytochemical Analysis of Crude Extract and Carotenoid Pigments from Fruits, Vegetables and Flowers. *Int. J. Curnt. Tren. Pharm, Res.*, 2016, 4(6): 322-337.

1. Introduction

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 **(www.nature.com)**. Nature has been providing medicines to treat our diseases and relieve our suffering for many thousands of years **(Fabricant DS and Farnsworth NR, 2001).** Despite great advances in rational drug design, in which new medicines are synthesized based on knowledge of specific molecular targets, most prescribed medicines used in industrialized countries today still are derived from, or patterned after, natural compounds from plants, animals, and microbes. This is particularly true for drugs that treat infections and cancers **(www.chgeharvard.org).** Medicinal plants have been identified and used throughout human history. Plants make many chemical compounds that are for biological functions **(Tapsell LC, *et al.* 2006).** At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (**Lai PK and Roy J 2004)**. The use of plants as medicines pre-dates written human history. 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 **(Swain, *et. al.*, 1968).** Indeed, nearly half of all human pharmaceuticals now in use were originally derived from natural sources. Nature may still be the best place to hunt for lifesaving compounds **(www.scientificamerican.com).** According to studies conducted by the World Health Organization (WHO), about 80% of the world's population relies on traditional medicine **(WHO News, 2002).** About 121 drugs prescribed in USA today come from natural sources, 90 of which comes either directly or indirectly from plant sources. Forty-seven percent of the anticancer drugs in the market come from natural products or natural product mimics **(Benowitz, S, 1996).**

Fruits and Their Health Benefits

Figure 1: Dried Fruit samples

Fruits have been recognized as good sources of vitamins and minerals, and for their role in preventing vitamin C and vitamin A deficiencies **(J. Zhao, 2007)**. Fruits are important sources of many nutrients, including potassium, fiber, vitamin C and folate (folic acid) **(Morton, 1987; Gunaseelan, 2004; Bori *et. al.*, 2007)**. People who eat fruit as part of an overall healthy diet generally have a reduced risk of chronic diseases **(Singh and Bhat, 2003)**. United States Department of Agriculture (USDA's) My Plate organization encourages making half our plate with fruits and vegetables for healthy eating. They insist on incorporating fruits rich in vitamin C which contain phytochemicals for added health benefits (**Figure 1**).

The nutrients in fruits are vital for health and maintenance of our body. The potassium in fruit reduces the risk of heart disease and stroke. Potassium may also reduce the risk of developing kidney stones and also helps to decrease bone loss as one ages. Folate (folic acid) helps the body in the formation of red blood cells **(Mokbel, *et. al.*, 2005)**. Women of childbearing age who become pregnant and those in the first trimester of pregnancy need adequate folate **(Amit and Shailandra, 2006)**. Folate helps prevent neural tube birth defects, such as spina bifida. Eating a diet rich in fruit may reduce the risk for stroke, other cardiovascular diseases and type-2 diabetes and protects against certain cancers **(Omojasola and Jilani, 2009)**. Fruits help to maintain optimum health due to the health promoting phytochemicals and phytonutrients they contain – many of which are still being identified. Phytonutrients are vital in both; health promotion and disease prevention One to 2-1/2 cups of fruits are recommended each day for a healthy living **(www.healthyeating.org).** The dietetic and therapeutic properties of all citrus fruits are similar due to their phytonutrient contents **(D. E. Okwi and I. N. Emenike, 2006).**

Vegetables and their Health Benefits

Vegetables can be eaten either raw or cooked and play an important role in human nutrition, being mostly low in fat and carbohydrates, but high in vitamins, minerals and fiber **(Velíšek, 1999)**. Particularly important are the antioxidant vitamins A, C and E. When vegetables are included in the diet, there is found to be a reduction in the incidence of cancer, stroke, cardiovascular disease and other chronic ailments **(Jeszka, 1997)**. Research has shown that compared with individuals who eat less than three servings of fruits and vegetables each day, those that eat more than five servings have an approximately twenty percent lower risk of developing coronary heart disease or stroke (**Figure 2**). Vegetables contain a great variety of phytochemicals (bioactive non-nutrient plant compounds), some of which have been claimed to have antioxidant, antibacterial, antifungal, antiviral and anti carcinogenic properties **(www.encyclopedia.com).**

Figure 2: Dried Vegetable samples

Natural antioxidants such as vitamin C, tocopherols, flavonoids and other phenolic compounds are known to be present in certain plants **(Pakade V, *et. al.*, 2013).** Recent studies have shown the importance of vegetables in a healthy diet in preventing degenerative diseases caused by oxidative stress **(P.R. Onkar, 2013)**. Vitamins and phytochemicals, such as ascorbic acid, carotenoids,

polyphenols, and fiber have been regarded as the bioactive substances responsible for these effects and as spinach shows all these qualities it is highly recommended to add a daily intake of it **(Ana P. Tiveron *et. al*., 2012).**

Flowers and Their Health Benefits

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 **(Sharma S *et. al*., 2004)**. 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 **(Gauthaman K.K *et. al*., 2006)**. Many medicinal plants contain large amounts of antioxidants other than vitamin C and carotenoids (**Figure 3**). In recent years, in order to discover novel antioxidant and antimicrobial drugs, screening of plants has been accelerated **(Rajamurugan R, *et. al*., 2013).**

Figure 3: Dried Flower samples

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value. Plants are used in modern medicine where they occupy a very significant place as raw material for important drugs. 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 **(Elumalai, 2012).**

Carotenoids

Carotenoids are an abundant group of naturally occurring pigments. They occur ubiquitously in all organisms which conduct photosynthesis**.** They are found in photosynthetic membranes of phototropic bacteria and cyanobacteria. More than 600 different carotenoids from natural sources have been isolated and characterized **(www.upb.pitt.edu)**. 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).** 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, infact found to be in the all-trans form, but cis isomers do exist **(www.nature.com)**. 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 **(P.M.Dey and J.B.Haarborne, 1997).**

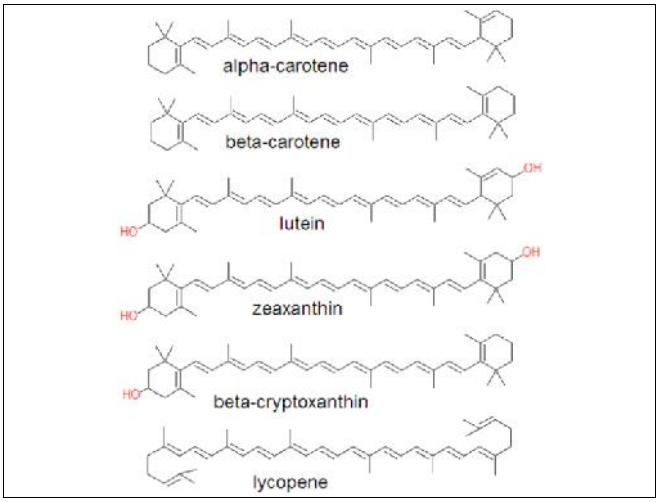


Figure 4: Structure of Carotenoids

The greater the number of conjugated double bonds, the higher the λmax values (**Figure 4**). 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)**.

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

2. Materials and Methods

Samples Used in the Present Study are as Follows

Orange (*Citrus reticulate* Blanco)
Lemon (*Citrus limon* (L.)Brum.f.)
Pineapple (*Ananas comosus* (L.)Merr.)
Banana (*Musa acuminate* Colla.)
Green spinach (*Sauropus androgynus* (L.)Merr.)
Beetroot (*Beta vulgaris* L.)
Red spinach (*Amaranthus dubius* Mart.ex Thell.)
Carrot (*Daucus carota* L.)
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 Fruits, Vegetables and 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 & 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 and FTIR studies. The antioxidant studies using Reducing Power assay and Phosphomolybdenum methods, the Antimicrobial studies and the anticancer activity using MTT Assay methodology were carried out.

3. Results and discussions

Qualitative Phytochemical Analysis

The following results were obtained for the phytochemical analysis of various Fruits **(Orange, Lemon, Pineapple and Banana),** Vegetables **(Carrot, Beetroot, Red Spinach and Green spinach)** and Flowers **(Copper pod, Yellow Bells, Red Jungle Flame and Hibiscus)** extract. Preliminary phytochemical screening of the Fruits (Orange, Lemon, Pineapple and Banana) extracts showed the presence of Carbohydrate, Glycosides, Tannins, Alkaloids, Flavonoids, Terpenoids, Saponin, resins, Quinones, Cardiac glycosides, Coumarins, Steroids and Phenols **(Table 1).** Preliminary phytochemical screening of the Vegetables (Carrot, Red spinach, Green spinach and Beet root) extracts showed the presence of Carbohydrate, Glycosides, Alkaloids, Flavonoids, Terpenoids, Saponin, Resins, Quinones, Cardiac glycosides, Coumarins and Steroids (**Table 2).**

Preliminary phytochemical screening of the flowers (Copper pod, Yellow bell, Hibiscus and Red jungle flame) extracts showed the presence of Carbohydrate, Glycosides, Tannins, Alkaloids, Flavonoids, Terpenoids, Saponin, Resins, Quinones, Cardiac glycosides, Steroids and Phenols (**Table 3).**

Over all, the samples Orange, Lemon, Pineapple, Carrot, Red spinach, Green spinach, Copper pod, Yellow bell, Hibiscus and Red jungle flame showed the presence of maximum phytochemicals in the Ethanolic extract. Banana and Beet root samples showed the presence of maximum phytochemicals in the Ethyl acetate extract and all the fruits, vegetables and flowers samples showed the minimum phytochemicals in Chloroform extract compared to the Ethanolic and Ethyl acetate extracts. Phytochemicals plays an important role in a plant's metabolic activities. Based on the phytochemicals present, the plant exhibited various activities such as antimicrobial, antioxidant and anticancer activities.

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

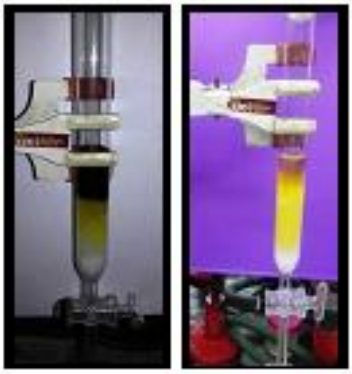


Figure 5: Isolation of Carotenoid pigment

Quantification of Carotenoids

The extracted carotenoids were quantified and the following results were obtained.

Total carotenoid content in orange = 0.245x10x10^4 / **2600x10 = 0.94 µg/g.**

Total carotenoid content in lemon = 0.220x10x10^4 / **2600x10 = 0.84 µg/g.**

Total carotenoid content in pineapple = 0.251x10x10^4 / **2600x10 = 0.96 µg/g.**

Total carotenoid content in banana = 0.254x10x10^4 / **2600x10 = 0.97 µg/g.**

Total carotenoid content in carrot = 0.252x10x10^4 / **2600x10 = 0.96 µg/g.**

Total carotenoid content in red spinach = $0.231x10x10^4$ / **2600x10 = 0.88 µg/g.**
Total carotenoid content in green spinach = $0.252x10x10^4$ / **2600x10 = 0.96 µg/g.**
Total carotenoid content in beet root = $0.145x10x10^4$ / **2600x10 = 0.56 µg/g.**
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 are subjected to thin layer chromatography **(Figure 6 - 8).** 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 Fruits, Vegetables and Flowers were calculated **(Table 4).**

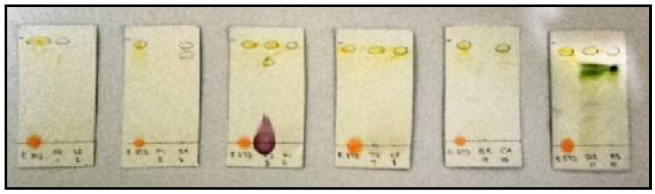


Figure 6: Thin Layer Chromatography of Ethanol Crude Samples

Figure 7: Thin Layer Chromatography of Ethyl acetate Crude Samples

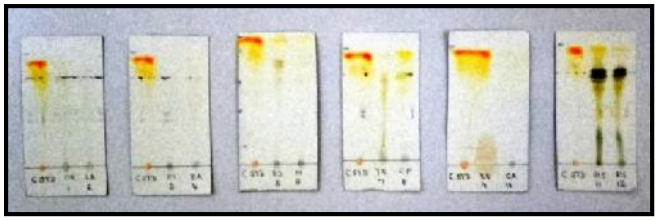
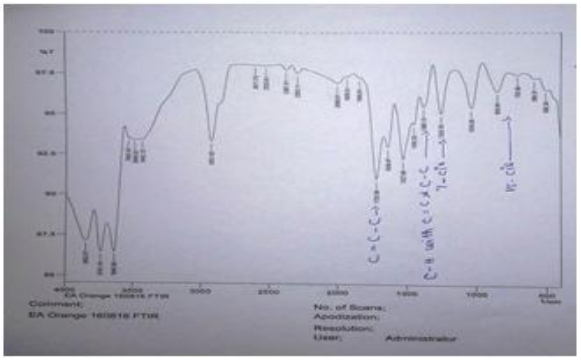


Figure 8: Thin Layer Chromatography of Chloroform Crude Samples

Fourier Transform Infrared Spectroscopy

The FTIR spectrum of β-carotene spectrum was recorded in the range of 4000-500 cm^{-1} respectively. In the spectrum of β-carotene the infrared band at between 1250 cm^{-1} and 740cm^{-1} are characteristic of 7-cis configuration and the infrared band at 780 cm^{-1} is characteristic of 15-cis configuration isomers being present. All isomers specifically cis-isomer of β-carotene gave two characteristic coupled C==C—C stretchings at 1720 cm^{-1} and 1680 cm^{-1} in the infrared spectrum. Thus in the FTIR analysis of samples of Orange, Banana, Carrot, Green spinach, Copper pod and Yellow bell the presence of beta carotene was confirmed **(Figures 9-14).** It is recommended to use freshly isolated Beta carotene as biological important source in the synthesis of vitamin A, the deficiency of which leads to affect visions and as it is been reported earlier that structure of beta carotene gets degraded at storage time. **(J. Marshell, 1998).**



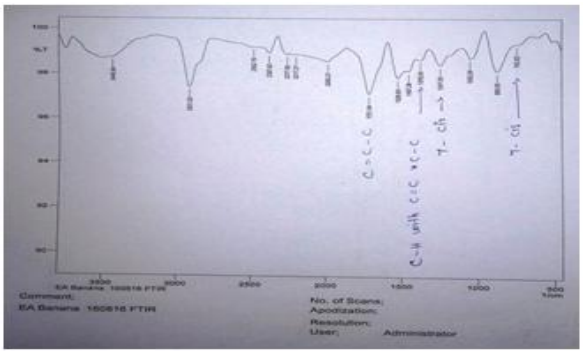
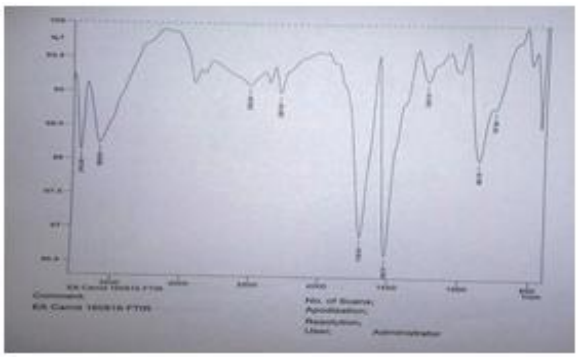


Figure 9 -10: FTIR result of Fruits



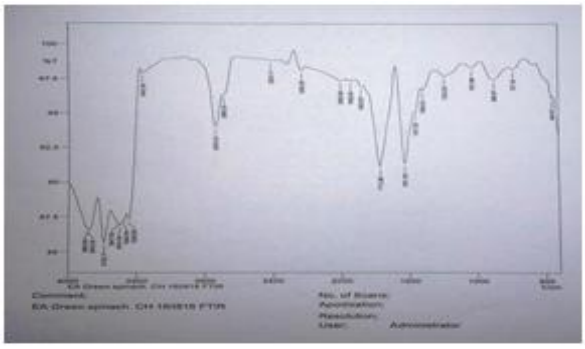
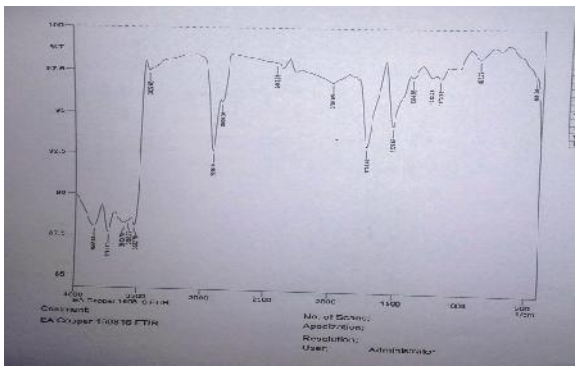


Figure 11-12: FTIR result of Vegetables



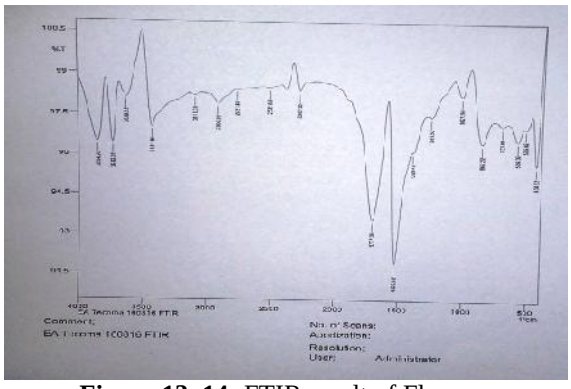


Figure 13–14: FTIR result of Flowers

Antioxidant Activity of the Extracts

Reducing Power Assay:

The reducing power assay was used to test the reducing capability of the extracts **(Figures 15 - 16).**

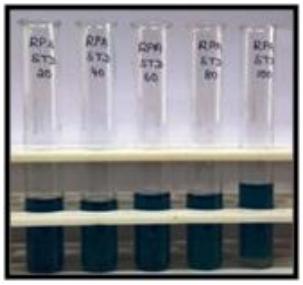


Figure 15: Standard test of Reducing Power assay.

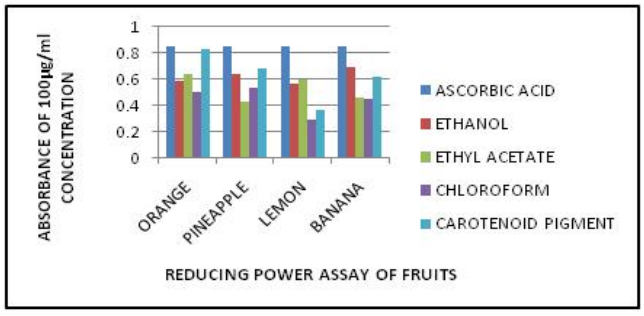


Figure 16: Reducing power activity of Fruits extracts

The Ethyl acetate crude extracts of (Carrot and Green spinach) and the Chloroform crude extracts of Beet root and Red spinach showed increased activity when compared to other two solvents. But their respective isolated carotenoid pigment showed higher activity than the crude. Over all **Carrot** and **Green spinach** gave the best results in Reducing Power assay among the Vegetables **(Table 6 and Figure 17).**

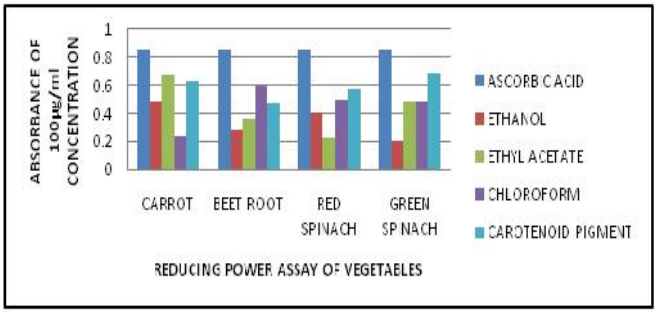


Figure 17: Reducing power activity of Vegetable extracts

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 7).**

Over all **Copper pod** and **Yellow bell** gave the best results in Reducing Power assay among the Flowers **(Figure 18).**

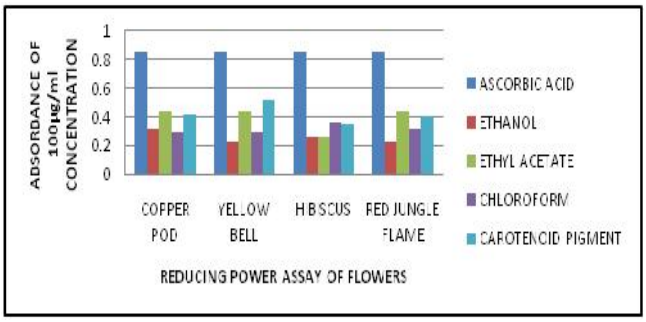


Figure 18: Reducing power activity of Flower extracts

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 19).**

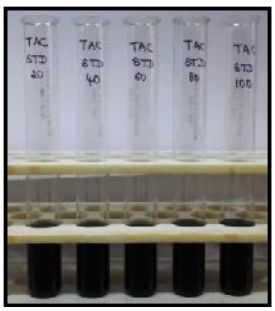


Figure 19: Standard test of Total antioxidant activity

The Ethanol crude extracts of (Orange, Pine apple and Lemon) and the Chloroform crude extract of Banana showed increased activity when compared to other two

solvents. But their respective isolated carotenoid pigment showed higher activity than the crude**.** Over all **Orange** and **Banana** gave the best results in Total antioxidant activity among the Fruits **(Table 8 and Figure 20).**

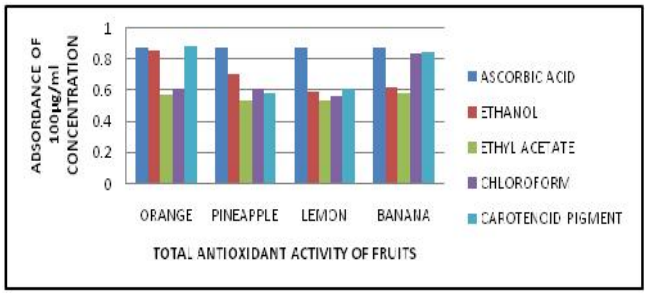


Figure 20: Total Antioxidant capacity by phosphomolybdenum method of Fruits extract.

The Ethanol crude extracts of Carrot, the Ethyl acetate crude extract of red spinach and the Chloroform crude extract of Beet root and Green spinach showed increased activity when compared to other two solvents. But their respective isolated carotenoid pigment showed higher activity than the crude. Over all **Carrot** and **Green Spinach** gave the best results in Total antioxidant activity among the Vegetables **(Table 9 and Figure 21).**

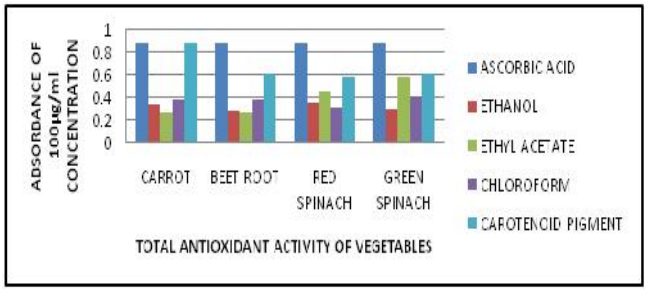


Figure 21: Total Antioxidant capacity by phosphomolybdenum method of Vegetables extract.

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 10).** Over all **Copper pod** and **Yellow bell** gave the best results in Total antioxidant activity among the Flowers **(Figure 22).**

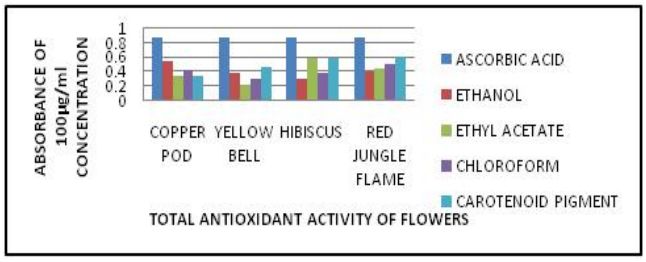


Figure 22: Total Antioxidant capacity by phosphomolybdenum method of Flowers extract.

Antimicrobial Activity of the Extracts

The antimicrobial activity of the Ethanol, Ethyl acetate and Chloroform crude extracts of the samples includes fruits (Orange, Lemon, Pineapple and Banana) vegetables (Carrot, Red spinach, Green spinach and Beet root) and 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*. Over all the extracts of sample of three different solvent showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* **(Table 11).**

Particularly the Ethanolic crude extract of all fruits and vegetables showed activity against *Staphylococcus aureus* and not against *Escherichia coli* **(Figure 23).** The flowers extract showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli*. The Ethyl acetate crude extract of all fruits (except lemon) vegetables and flowers showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* **(Figure 24).** The chloroform crude extracts of fruits (Pine apple and Banana) all vegetables and flowers (except Copper pod) showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli* **(Figure 25).** The Carotenoid pigment extracted from the fruits, vegetables and flowers showed maximum antimicrobial activity against *Staphylococcus aureus* only.

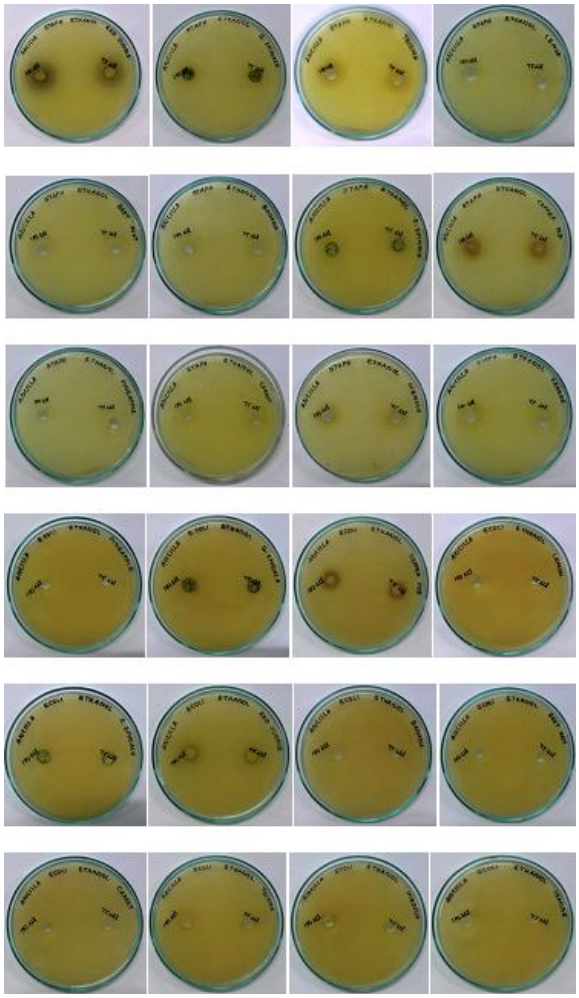


Figure 23: Antimicrobial Activity of Ethanol Crude Extract against organism

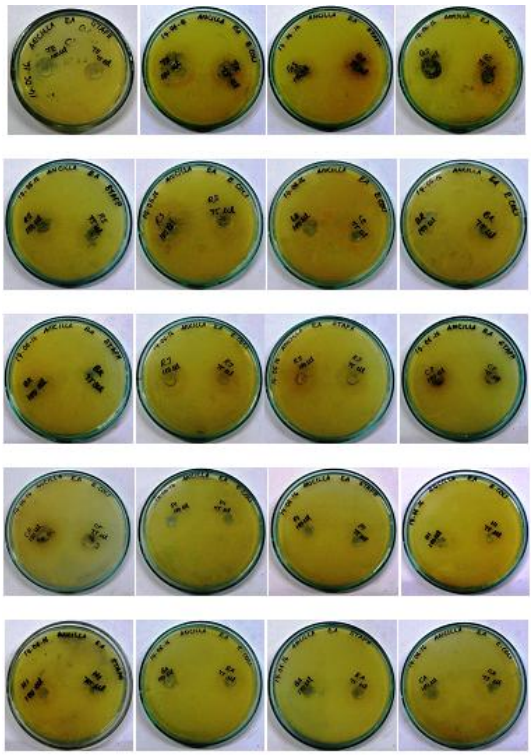


Figure 24: Antimicrobial Activity of Ethyl acetate Crude Extract against organism

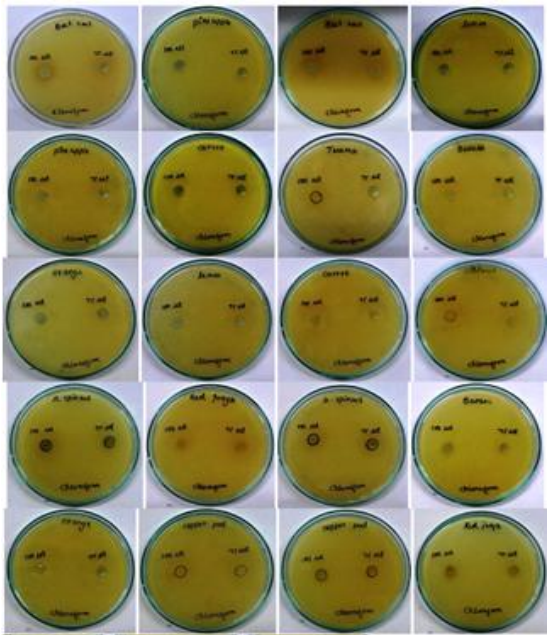


Figure 25: Antimicrobial Activity of Chloroform Crude Extract against organism

The antimicrobial activity of the crude extracts is attributed to the presence of some metabolic toxins or broad spectrum antibiotic compounds. Thus these extracts could be used as effective antimicrobial compounds and the carotenoid pigment can also be used as a natural alternative to chemical in preservation of food.

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. **(Figures 26 - 28).**

Figure 26: Cells before treatment of the extracts

Figure 27: Cells after adding the extracts

Figure 28: Cells after adding MTT Reagent

The Ethyl acetate crude extracts of Orange, Pineapple, Banana and Lemon showed increased cytotoxicity when compared to other two solvents. The crude and their respective isolated carotenoid pigment showed higher cytotoxicity than the ethyl acetate crude. Over all **Orange** and **Banana** gave the best results in anticancer activity among the fruits **(Table 12 and Figure 29 - 30).**

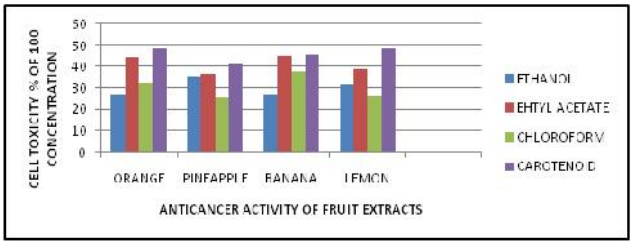


Figure 29: Anti cancer activity of Fruit extracts at 100µl concentration.

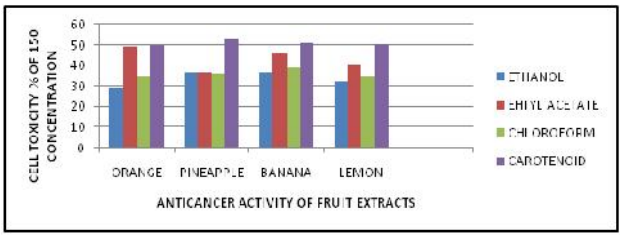


Figure 30: Anti cancer activity of Fruit extracts at 150 µl concentration.

The Ethyl acetate crude extracts of Carrot, Red spinach and Green spinach and the Ethanol crude extracts of Beet root

showed increased cytotoxicity when compared to other two solvents. The crude extract and their respective isolated carotenoid pigment showed higher cytotoxicity. Over all **Carrot** and **Green spinach** gave the best results in anticancer activity among the vegetables **(Table 13 and Figure 31 - 32).**

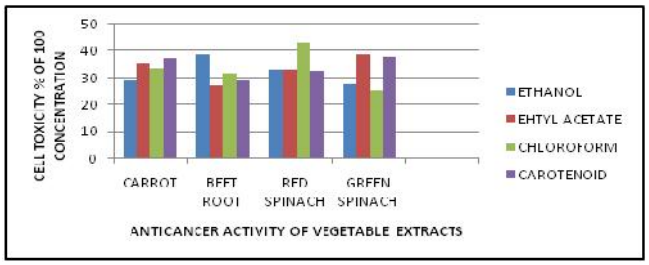


Figure 31: Anti cancer activity of Vegetable extracts at 100µl concentration

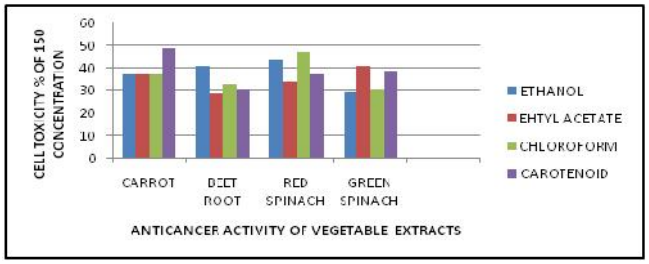


Figure 32: Anti cancer activity of Vegetable extracts at 150µl concentration.

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 14 and Figure 33 - 34).**

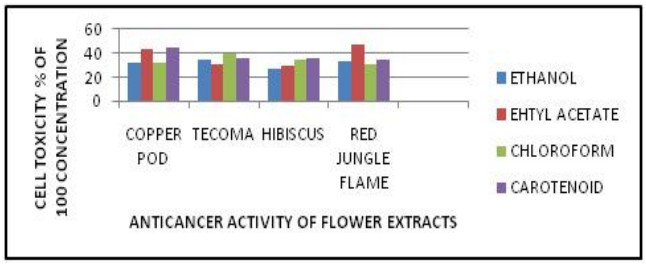


Figure 33: Anti cancer activity of Flower extracts at 100µl concentration.

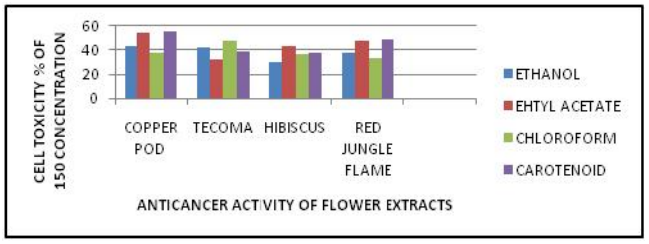


Figure 34: Anti cancer activity of Flower extracts at 150µl concentration.

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.

4. Conclusion

The solvent (ethanol, ethyl acetate and chloroform) crude extracts of various fruits (Orange, Lemon, Pineapple and Banana), Vegetables (Carrot, Beetroot, Red Spinach and Green spinach) and Flowers (Copper pod, Yellow Bells, Red Jungle Flame and Hibiscus) were subjected to phytochemical analysis.

Over all, the samples Orange, Lemon, Pineapple, Carrot, Red spinach, Green spinach, Copper pod, Yellow bell, Hibiscus and Red jungle flame showed the presence of maximum phytochemicals in the Ethanolic extract. Banana and Beet root samples showed the presence of maximum phytochemicals in the Ethyl acetate extract and all the fruits, vegetables and flowers samples showed the minimum phytochemicals in Chloroform extract compared to the Ethanolic and Ethyl acetate extracts. The carotenoids were extracted from the fruits (Orange, Lemon, Pineapple and Banana), Vegetables (Carrot, Beetroot, Red Spinach and Green spinach) and Flowers (Copper pod, Yellow Bells, Red Jungle Flame and Hibiscus) by column chromatography and subjected to thin layer chromatography. The pigments were further analysed by Fourier transform infrared spectroscopy to find the carotenoid pigment.it was found that beta carotene is the extracted carotenoid pigment present in the samples. The crude extract and the carotenoid extracts were then analysed for their antioxidant, antimicrobial and anti cancer 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 Orange, Banana, Carrot, green spinach, Copper pod and Yellow bell showed highest activity.

The Ethanolic crude extract of all fruits and vegetables showed activity against *Staphylococcus aureus* and not against *Escherichia coli.* The flowers extract showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli*. The Ethyl acetate crude extract of all fruits (except lemon) vegetables and flowers showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli*. The chloroform crude extracts of fruits (Pine apple and Banana) all vegetables and flowers (except Copper pod) showed antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli*. Thus the Carotenoid pigment extracted from the fruits, vegetables and flowers showed maximum antimicrobial activity against *Staphylococcus aureus* only. 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 the Fruits, Orange and Banana, Vegetables, Carrot and Green Spinach and Flowers, Copper pod and Yellow Bell to be the best in all the three activities and is highly recommended for consumption for prevention of dreadful diseases and for a healthy living in the long run.

Table 1: Phytochemicals present in Fruit extracts

Test	Orange			Lemon			Pineapple			Banana		
	E	EA	C	E	EA	C	E	EA	C	E	EA	C
Carbohydrate	+	+	-	+	-	-	+	+	-	-	+	-
Carbohydrate	-	-	+							-	+	+
Protein	-	-	-	-	-	-	-	-	-	-	-	-
Protein	-	-	-							-	-	-
Glycoside	+	+	+	+	-	-	+	+	+	+	+	+
Tannins	+	-	-	-	-	-	-	+	-	-	-	-
Alkaloides	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloides	+	+	+	+	+	-	+	-	-	-	-	-
Flavonoides	+	+	+	+	-	-	+	+	+	-	+	-
Terpenoides	+	+	+	-	-	-	+	+	+	-	+	+
Saponins	+	-	-	-	-	-	+	+	+	-	-	-
Resins	+	-	-	-	-	-	+	-	-	-	+	-
Quinones	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	-	-	+	+	-
Coumarines	+	+	+	+	+	+	+	+	+	+	+	-
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Phytosteroids	-	-	-	-	-	-	-	-	-	-	-	-
Antraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-
Phenols	+	-	-	+	-	-	-	-	-	-	-	-

Table 2: Phytochemicals present in Vegetable extracts.

Test	Carrot			Red spinach			Green spinach			Beet root		
	E	EA	C	E	EA	C	E	EA	C	E	EA	C
Carbohydrate	+	+	+	-	-	+	+	-	-	-	+	+
Carbohydrate	-	-	-	-	-	-	-	-	-	-	-	-
Protein	-	-	-	-	-	-	-	-	-	-	-	-
Protein	-	-	-	-	-	-	-	-	-	-	-	-
Glycoside	+	+	-	+	-	-	+	+	+	+	+	-
Tannins	-	-	-	+	+	+	+	+	+	-	-	-
Alkaloides	+	+	+	-	+	+	-	+	+	+	+	+
Alkaloides	+	+	+	-	+	-	+	+	-	-	-	+
Flavonoides	+	+	+	-	+	+	-	-	+	+	-	-
Terpenoides	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	-	-	+	+	-	-	-	-	-	-	-
Resins	+	-	+	+	-	-	-	-	-	-	-	+
Quinones	+	+	+	+	-	-	-	-	-	+	+	+
Cardiac glycosides	+	+	-	+	+	+	+	+	+	+	+	+
Coumarines	+	+	+	-	-	-	-	-	-	+	+	-
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	-	-	-	+	-	+	+	-	-	-	+	+
Phytosteroids	-	-	-	-	-	-	-	-	-	-	-	-
Antraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-	-	+	+

Table 3: Phytochemicals present in Flower extracts.

TEST	Copper pod			Yellow bell			Hibiscus			Red jungle flame		
	E	EA	C	E	EA	C	E	EA	C	E	EA	C
Carbohydrate	+	+	-	+	+	-	+	-	-	+	-	+
Carbohydrate				+	+	+	-	-	-			
Protein	-	-	-	-	-	-	-	-	-	-	-	-
Protein	-	-	-	-	-	-	-	-	-	-	-	-
Glycoside	+	+	-	+	+	+	+	+	-	+	+	+
Tannins	+	-	-	-	-	-	+	-	-	+	-	-
Alkaloides	+	+	+	+	+	+	-	+	+	-	+	+
Alkaloides	-	-	+	+	+	+	-	-	+	-	+	-
Flavonoides	+	+	-	+	-	+	-	+	+	-	+	+
Terpenoides	+	+	+	+	+	+	+	+	-	+	+	+
Saponins	+	-	-	-	-	+	-	-	-	+	-	-
Resins	+	-	-	+	-	-	+	-	-	+	-	-
Quinones	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	-	+	+	+	+	+	-	+	+	+
Coumarines	-	+	+	-	+	+	-	+	+	-	+	-
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	-	-	+	-	-	+	-	-	+	-	-
Phytosteroids	-	-	-	-	-	-	-	-	-	-	-	-
Antraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-	+	-	-

Table 4: Rf values of samples

Rf Values of Crude Extract and Carotenoid				
Sample	**Ethanol crude**	**Ethyl acetate crude**	**Chloroform crude**	**Carotenoide pigment**
Orange	0.95	0.94	0.94	0.94
Lemon	0.92	0.92	0.92	0.92
Pineapple	0.92	0.92	0.92	0.92
Banana	0.94	0.91	0.95	0.94
Carrot	0.94	0.91	0.94	0.94
Red spinach	0.95	0.95	0.94	0.94
Green spinach	0.95	0.95	0.94	0.94
Beet root	0.92	0.91	0.94	0.92
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 5 : Reducing Power Activity of Fruit Extracts

Sample	Conc (µg/ml)	Standard Ascorbic Acid OD	Ethanol	Ethyl acetate	Chloroform	Carotenoid pigment
Orange	20	0.17	0.5	0.48	0.35	0.53
	40	0.45	0.53	0.55	0.41	0.6
	60	0.63	0.55	0.58	0.44	0.63
	80	0.80	0.57	0.62	0.45	0.65
	100	0.85	0.59	0.64	0.5	0.83
Pineapple	20	0.17	0.54	0.3	0.42	0.5
	40	0.45	0.57	0.33	0.44	0.55
	60	0.63	0.61	0.36	0.48	0.58
	80	0.80	0.63	0.39	0.51	0.63
	100	0.85	0.64	0.43	0.53	0.68

Lemon	20	0.17	0.45	0.47	0.16	0.23
	40	0.45	0.47	0.53	0.2	0.24
	60	0.63	0.5	0.57	0.25	0.29
	80	0.80	0.51	0.58	0.27	0.32
	100	0.85	0.56	0.6	0.29	0.36
Banana	20	0.17	0.54	0.42	0.35	0.24
	40	0.45	0.58	0.43	0.37	0.36
	60	0.63	0.61	0.44	0.41	0.45
	80	0.80	0.63	0.45	0.43	0.54
	100	0.85	0.69	0.46	0.45	0.62

Table 6 : Reducing Power Activity of Vegetable Extracts

Sample	Conc (µg/ml)	Standard Ascorbic Acid OD	Ethanol	Ethyl acetate	Chloroform	Carotenoid pigment
Carrot	20	0.17	0.4	0.56	0.16	0.25
	40	0.45	0.42	0.59	0.18	0.32
	60	0.63	0.44	0.61	0.2	0.52
	80	0.80	0.46	0.63	0.22	0.57
	100	0.85	0.48	0.67	0.24	0.63
Beet root	20	0.17	0.2	0.23	0.51	0.24
	40	0.45	0.22	0.26	0.53	0.29
	60	0.63	0.24	0.29	0.55	0.32
	80	0.80	0.26	0.33	0.57	0.36
	100	0.85	0.28	0.36	0.59	0.47
Red spinach	20	0.17	0.28	0.14	0.42	0.42
	40	0.45	0.33	0.16	0.44	0.44
	60	0.63	0.36	0.18	0.46	0.45
	80	0.80	0.38	0.2	0.48	0.48
	100	0.85	0.4	0.22	0.5	0.57
Green spinach	20	0.17	0.12	0.4	0.38	0.29
	40	0.45	0.14	0.42	0.42	0.32
	60	0.63	0.16	0.44	0.44	0.36
	80	0.80	0.18	0.46	0.45	0.47
	100	0.85	0.2	0.48	0.48	0.69

Table 7 : 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 Jungle Flame	20	0.17	0.12	0.38	0.25	0.25
	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

Table 8: Total Antioxidant Activity of Fruit Extracts

Sample	CONC (µg/ml)	Standard Ascorbic Acid OD	Ethanol	Ethyl acetate	Chloroform	Carotenoid pigment
Orange	20	0.16	0.63	0.47	0.47	0.65
	40	0.42	0.66	0.49	0.49	0.7
	60	0.55	0.69	0.52	0.54	0.75
	80	0.74	0.74	0.54	0.58	0.79
	100	0.88	0.86	0.57	0.61	0.89
Pineapple	20	0.16	0.57	0.43	0.51	0.47
	40	0.42	0.59	0.46	0.53	0.51
	60	0.55	0.63	0.49	0.56	0.53
	80	0.74	0.65	0.51	0.59	0.56
	100	0.88	0.68	0.53	0.61	0.58
Lemon	20	0.16	0.55	0.43	0.46	0.52
	40	0.42	0.56	0.49	0.48	0.55
	60	0.55	0.57	0.5	0.5	0.57
	80	0.74	0.58	0.51	0.52	0.59
	100	0.88	0.59	0.53	0.56	0.61
Banana	20	0.16	0.51	0.4	0.65	0.55
	40	0.42	0.53	0.44	0.67	0.67
	60	0.55	0.56	0.48	0.69	0.69
	80	0.74	0.59	0.52	0.71	0.74
	100	0.88	0.62	0.58	0.84	0.85

Table 9 : Total Antioxidant Activity of Vegetable Extracts

Sample	Conc (µg/ml)	Standard Ascorbic Acid OD	Ethanol	Ethyl acetate	Chloroform	Carotenoid pigment
Carrot	20	0.16	0.22	0.11	0.26	0.67
	40	0.42	0.27	0.21	0.29	0.69
	60	0.55	0.3	0.23	0.32	0.72
	80	0.74	0.32	0.26	0.36	0.78
	100	0.88	0.35	0.27	0.39	0.88
Beet root	20	0.16	0.2	0.11	0.23	0.53
	40	0.42	0.23	0.15	0.27	0.55
	60	0.55	0.25	0.19	0.3	0.57
	80	0.74	0.27	0.23	0.36	0.6
	100	0.88	0.29	0.27	0.39	0.61
Red spinach	20	0.16	0.21	0.25	0.15	0.49
	40	0.42	0.25	0.31	0.19	0.52
	60	0.55	0.28	0.36	0.22	0.54
	80	0.74	0.32	0.39	0.26	0.57
	100	0.88	0.36	0.45	0.31	0.58
Green Spinach	20	0.16	0.18	0.41	0.3	0.54
	40	0.42	0.2	0.47	0.32	0.56
	60	0.55	0.26	0.49	0.36	0.57

	80	0.74	0.3	0.54	0.39	0.59
	100	0.88	0.3	0.59	0.42	0.61

Table 10: 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

Table 11 : 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
Orange	10	10	-	-	10	10	10	10	-	-	15	10	15	10	-	-
Lemon	15	10	-	-	-	-	5	-	-	-	10	10	10	15	-	-
Pineapple	15	10	-	-	5	-	5	-	10	-	10	-	10	25	-	-
Banan	10	10	-	-	15	5	20	10	10	-	10	10	15	10	-	-
Carrot	10	10	-	-	15	-	10	5	10	10	-	-	10	15	-	-
Beet root	10	10	-	-	10	-	5	-	20	15	15	10	10	15	-	-
Red spinach	20	12	-	-	15	-	25	15	10	10	10	5	10	15	-	-
Green spinach	15	10	-	-	10	5	15	10	10	10	15	10	5	5	-	-
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	-	-

Table 12 : Anticancer Activity Of Fruits

Sample	Conc	Ethanol		Ehtyl acetate		Chloroform		Carotenoid	
		Cell Viability %	Cell Toxicity %	Cell Viability %	Cell Toxicity %	Cell Viability %	Cell Toxicity %	Cell Viability %	Cell Toxicity %
Orange	100	73.36	26.64	55.4	44.6	67.44	32.56	51.22	48.78
	150	70.61	29.39	51.02	48.98	65.61	34.39	50.3	49.7
Pineapple	100	64.79	35.21	63.36	36.64	74.08	25.92	59.08	40.92
	150	63.46	36.54	63.16	36.84	63.77	36.23	47.14	52.86

Banana	100	73.06	26.94	54.69	45.31	62.04	37.96	54.38	45.62
	150	63.26	36.74	54.08	45.92	61.02	38.98	48.97	51.03
Lemon	100	68.36	31.64	61.02	38.98	73.67	26.33	51.22	48.78
	150	67.85	32.15	60	40	64.89	35.11	49.79	50.21

Table 13 : Anticancer Activity of Vegetables

Sample	Conc	Ethanol		Ethyl acetate		Chloroform		Carotenoid	
		CELL VIABILITY %	CELL TOXICITY %	CELL VIABILITY %	CELL TOXICITY %	CELL VIABILITY %	CELL TOXICITY %	CELL VIABILITY %	CELL TOXICITY %
Carrot	100	71.22	28.78	64.79	35.21	66.83	33.17	62.75	37.25
	150	62.85	37.15	62.44	37.56	62.85	37.15	51.12	48.88
Beet root	100	61.12	38.88	72.75	27.25	68.46	31.54	71.22	28.78
	150	59.28	40.72	71.22	28.78	67.14	32.86	69.28	30.72
Red spinach	100	67.34	32.66	56.93	43.07	67.04	32.96	67.65	32.35
	150	56.22	43.78	52.55	47.45	66.32	33.68	62.44	37.56
Green spinach	100	72.44	27.56	61.42	38.58	75.1	24.9	62.24	37.76
	150	70.4	29.6	59.18	40.82	69.38	30.62	61.32	38.68

Table 14 : Anticancer Activity of Flowers

Sample	Conc	Ethanol		Ehtyl 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

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