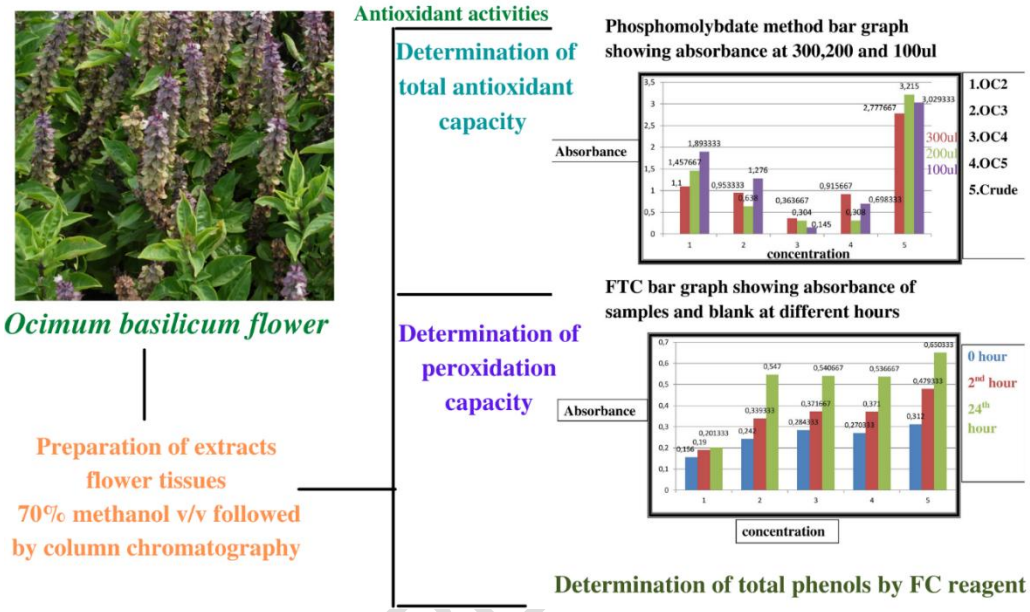
Original Research Article
Investigation of anti-oxidant potential in *Ocimum basilicum* flower.

.

GRAPICAL ABSTRACT



ABSTRACT

Aims: The present work is particularly focused on anti-oxidant properties of flower of *Ocimum basilicum* plant.
Study design: Study is basically designed on Column chromatography of extracts.
Place and Duration of Study: Sample collection and all experimental work was done in Chemistry Department Government College University, Lahore.
Methodology: The flower of *Ocimum basilicum* were collected, dried and grinded. It was soaked in methanol-water (70:30) in dark bottle for a week. Followed by a scheme (column chromatography). After TLC of extracts, three activities were done. Phosphomolybdate, Ferric thiocyanate FTC, and FC reagent for determination of antioxidant capacity, peroxidation, determination of total phenols respectively.
Results: The sample OC2 and crude have maximum absorbance at the concentration of 100ul, 200ul and 300ul. The results show that crude has maximum anti-oxidant capacity. The phenolic contents are in the increasing order of fraction OC2, OC5, and crude. The maximum phenolic contents are present in crude. Blank has the maximum ability for peroxidation for ferric thiocyanate complex by giving red colour.
Conclusion: Overall its concluded that *Ocimum basilicum* flower has antioxidant capacity as good as a standard anti-oxidant. It is recommended for use in food/medicine as natural herbal product.

Keywords: [Ocimum basilicum;flower,antioxidant capacity,FTC,FC*]*

1. INTRODUCTION

Ocimum basilicum belongs to family Lamiaceae. This annual herb having more than 150 species of the genus *Ocimum,* grows in several regions of world. The origin of *O. basilicum* commonly known as basil is from India, Afghanistan, Pakistan, Northern India and Iran, now this is cultivated worldwide [1]. Basil is most popular herb due to its number of advantages as a result this plant is often referred to as the "king of the herbs." There are three regions of world where *Ocimum* showed diversity i.e tropical South America including Brazil, tropical and subtropical regions of Africa and the other is tropical Asia [2].The region where *Ocimum* showed maximum diversity is the Africa [3].

Plants-based foods have got interest due to the presence of natural antioxidants such as polyphenols, flavonoids, vitamin C and vitamin E .All these anti-oxidants have reduced risk of cardiovascular, chronic diseases and certain types of cancer [4].

Ocimum basilicum plant as a whole is anti-malarial. In sweet basil leaves, high levels of an enzyme phenylalanine ammonia-lyase has been detected the enzyme has been purified and characterized. [5] Leaf extracts and essential oils have shown hypoglycemic activity along with [6] anti-inflammatory and anti-oxidant effects [7]. The flowering tops are carminative, galactogogue, stomachic and antispasmodic [8] however, basil seeds are used in constipation and piles. [9]

Reported research work is novel by considering flower of *Ocimum basilicum* in particular for the determination of anti-oxidant properties. Previous studies were restricted to leaves, stem and other aerial parts of this plant only.

2. MATERIAL AND METHODS

EXPERIMENTAL DETAILS

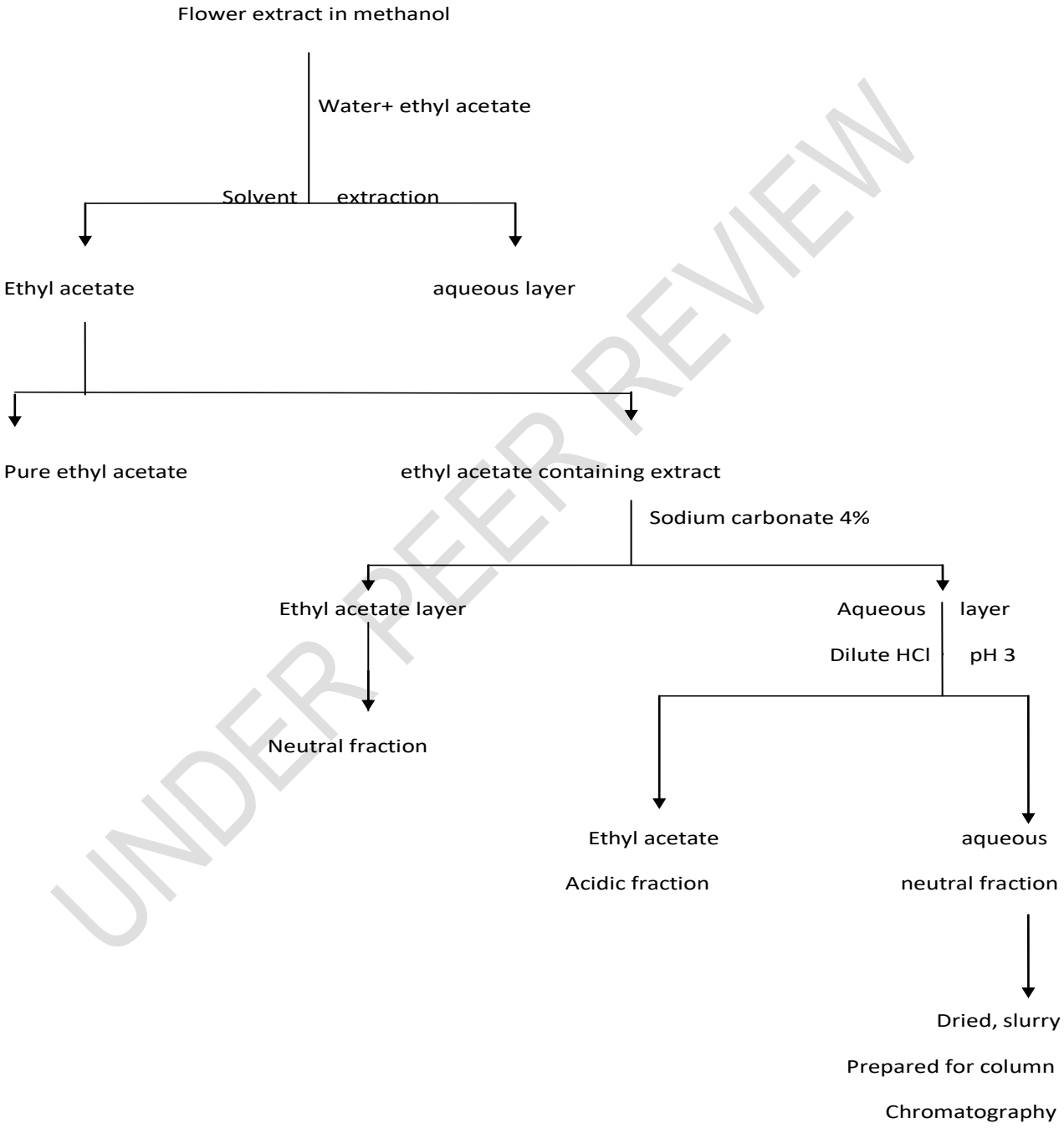
IR spectrum was recorded using KBr disk with a Perkin-Elmer 735B spectrometer. UV spectra were recorded on UV/VIS spectrophotometer 2300 (Shimadzu, Kyoto,Japan). Heidolph, Laborota 400, rotary evaporator was used to evaporate solvents from samples. Silica gel 60 (0.063-0.200 mm) for column chromatography and TLC silica gel 60 F254 aluminum sheets (20 x 20 cm) from Merck. Solvents of analytical grade were purchased from Panreac (Spain). All other chemicals and reagents of analytical grade were from Merck (Germany). Standard deviation (±SD) of repeated measurements was calculated using Microsoft Excel 2007.

METHODOLOGY

The flower of *Ocimum basilicum* was collected, dried and grinded.It was soaked in methanol-water (70:30) in dark bottle for a week.

To the dried flower extract of *O.basilicum* which was prepared in methanol different solvents were added to check its solubility. The order of solvents was Hexane 100%, hexane: ethyl acetate (1:2), ethyl acetate 100%, ethyl acetate: methanol (1:2). It was found flower extract was soluble in ethyl acetate. So, it was dissolved in ethyl acetate and water. For removal of aqueous contents the solvent extraction was done. The organic layer(OL) and aqueous layers(AQ) were completely separated. The organic layer(OL) was further processed. Ethyl acetate was evaporated from the extract(EE) and 4% sodium carbonate was added. This further gave two layers organic and aqueous, organic layer (ethyl acetate) was placed as neutral fraction(NF) and dil HCl was added to the aqueous layer of sodium carbonate and pH was maintained at 3. Again two layers obtained solvent extraction was done again and finally two layers obtained one was aqueous fraction(AF) and other as acidic fraction(AF) of ethyl acetate. (**Scheme 1**)

TLC was done for both the fractions. The spot in neutral fraction was more clearly compared to acidic fraction. Mixture of hexane and ethyl acetate was found best for neutral fraction (NF). The neutral fraction was made clearer by adding ethyl acetate, separating and filtering. Slurry of neutral fraction was prepared and column chromatography was done. (**Table 1**)



Scheme 1 Extraction and processing of flower extract of *Ocimum basilicum*

Table No.1 Different fractions of *Ocimum basilicum* by Column Chromatography

SR.NO	Solvent system		Fraction code	Volume ml
1	n- Hexane	100%	OC-1	500ml
2	Ethyl acetate:n-hexane	5%	OC-2	500ml
3	Ethyl acetate:n-hexane	20%	OC-3	500ml
4	Ethyl acetate:n-hexane	50%	0C-4	500ml
5	Ethyl acetate:n-hexane	80%	OC-5	500ml
6	Chloroform	100%	OC-6	500ml
7	Chloroform :methanol	1%	0C-7	500ml
8	Chloroform:methanol	5%	0C-8	500ml
9	Chloroform:methanol	10%	0C-9	500ml
10	Chloroform:methanol	20%	0C-10	500ml
11	Methanol	100%	OC-11	500ml

TLC was done for different fractions of column.TLC for fraction 1,3,4,5 was run in pure n-hexane. TLC for fraction 2,6,7,8,9,10,11 was run in 1:1 ethyl acetate –n-hexane. Spots were observed and TLC cards were sprayed with cerric sulphate reagent. Fractions showing same spots were mixed together. The fractions 5%,20%,50% and 80% (OC 2, OC 3, OC 4, OC 5) were separated and their TLC was again run in 2% ethyl acetate:n-hexane(2ml:8ml). These fractions were selected for carrying out anti-oxidant activities.

ANTIOXIDANT ACTIVITIES

PHOSPHOMOLYBDATE METHOD

To the 300,200 and 100ul of sample including fractions from the column and crude extract 2ml of phospho ammonium molybdate reagent was added and incubated at $95°C$ for 60 minutes.After cooling absorbance was read at 695nm against blank(phosphomolybdate reagent)[10].

DETERMINATION OF TOTAL PHENOLS BY FC REAGENT

Standard Gallic acid: To the 100ul of the standard 50ul FC reagent and 100ul of 10%Na_2CO_3 was added and volume was raised up to 2.5ml with methanol. It was kept for 30 minutes and absorbance was read at 765 nm. Different dilutions were done by taking 1ml from standard and making volume up to 2.5ml by adding methanol. Gallic acid calibration curve was drawn. The equation of the curve was y=2322.1x

Samples preparation: To different fraction and crude extract of volume 100ul, FC reagent 50ul, Na_2CO_3 100ul was added volume was raised up to 2.5 ml with methanol. Stayed for 30 minutes and reading for absorbance was taken at 765 nm. [11]

FERRIC THIOCYANATE FTC METHOD

To the 1mg of fractions and extract ethanol was added.1000ul was taken and mixed with 1000ul of 2.52% linolenic acid prepared in absolute ethanol.1000ul of 0.05M phosphate buffer (pH 7) and 1000ul of distilled water was added.(Phosphate buffer was prepared by dissolving0.05M disodium hydrogen phosphate di-hydrate in distilled water and pH was maintained by adding sodium hydroxide solution.)The mixture solution with screw cap was placed in dark oven at 40°C for 10 minutes.
After 10 minutes 100ul of solution was taken and 100ul of 30 % ammonium thiocyanate with 100ul of 0.02 M ferrous sulphate in 3.5%HCl was added and absorbance was read at 532nm. Gallic acid, ascorbic acid was used as standards. [12]

Table No 2a Anti-oxidant activity by phosphomolybdate method using 300ul sample

S.No	Samples code	Average absorbance at 300ul	(+-)Standard deviation
1	OC 2	1.1	0.08544
2	OC3	0.953333	0.066583
3	OC4	0.363667	0.072947
4	OC5	0.915667	0.010504
5	Crude fraction	2.777667	0.018475

Table No.2b Anti-oxidant activity by phosphomolybdate method using 200ul sample

S.No	Samples code	Average absorbance at 200ul	(+-)Standard deviation
1	OC 2	1.457667	0.448487
2	OC 3	0.638	0.008
3	OC 4	0.304	0.076236
4	OC 5	0.308	0.020664
5	Crude fraction	3.215	0

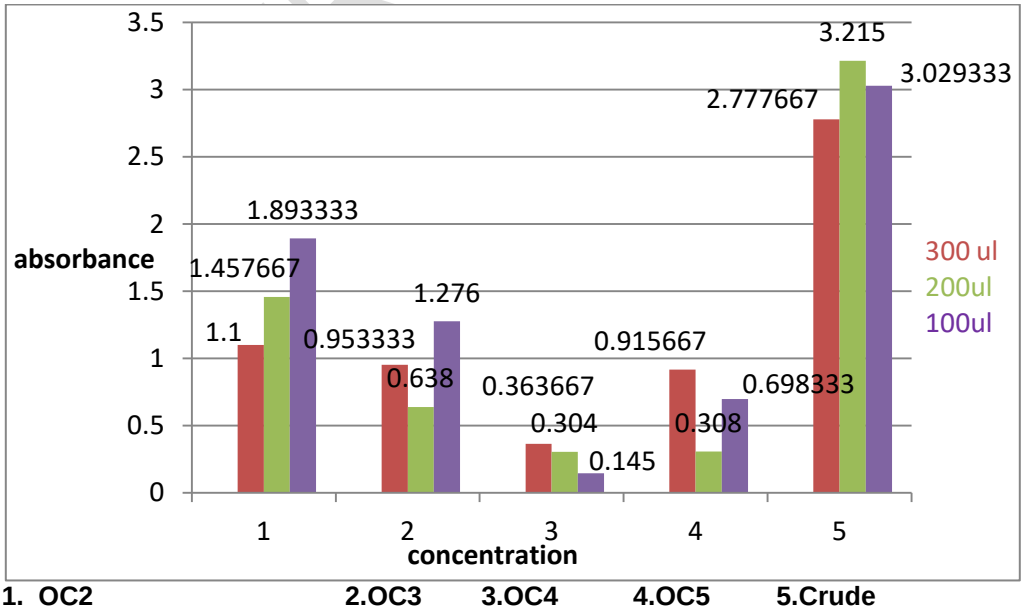
Table No.2c Anti-oxidant activity by phosphomolybdate method using 100ul sample

S.No	Samples code	Average absorbance at 100ul	(+-)Standard deviation
1	OC 2	1.893333	0.00611
2	OC 3	1.276	0.003
3	OC 4	0.145	0.091263
4	OC 5	0.698333	0.022053
5	Crude fraction	3.029333	0.0

3. RESULTS AND DISCUSSION

The phosphomolybdenum method is used to describe the antioxidant capacity. The method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound (the flower extract) and the formation of a green phosphate/Mo (V) complex with a maximum absorption at 695 nm. [13]
The results of phosphomolybdate activity at different concentrations are same. (**Table 2 a,b,c**) The sample OC2 and crude have maximum absorbance at the concentration of 100ul, 200ul and 300ul. However, crude has the much higher absorbance compared to OC2 at all three concentrations. The results conclude that crude has maximum anti-oxidant capacity. (**Fig 1**)

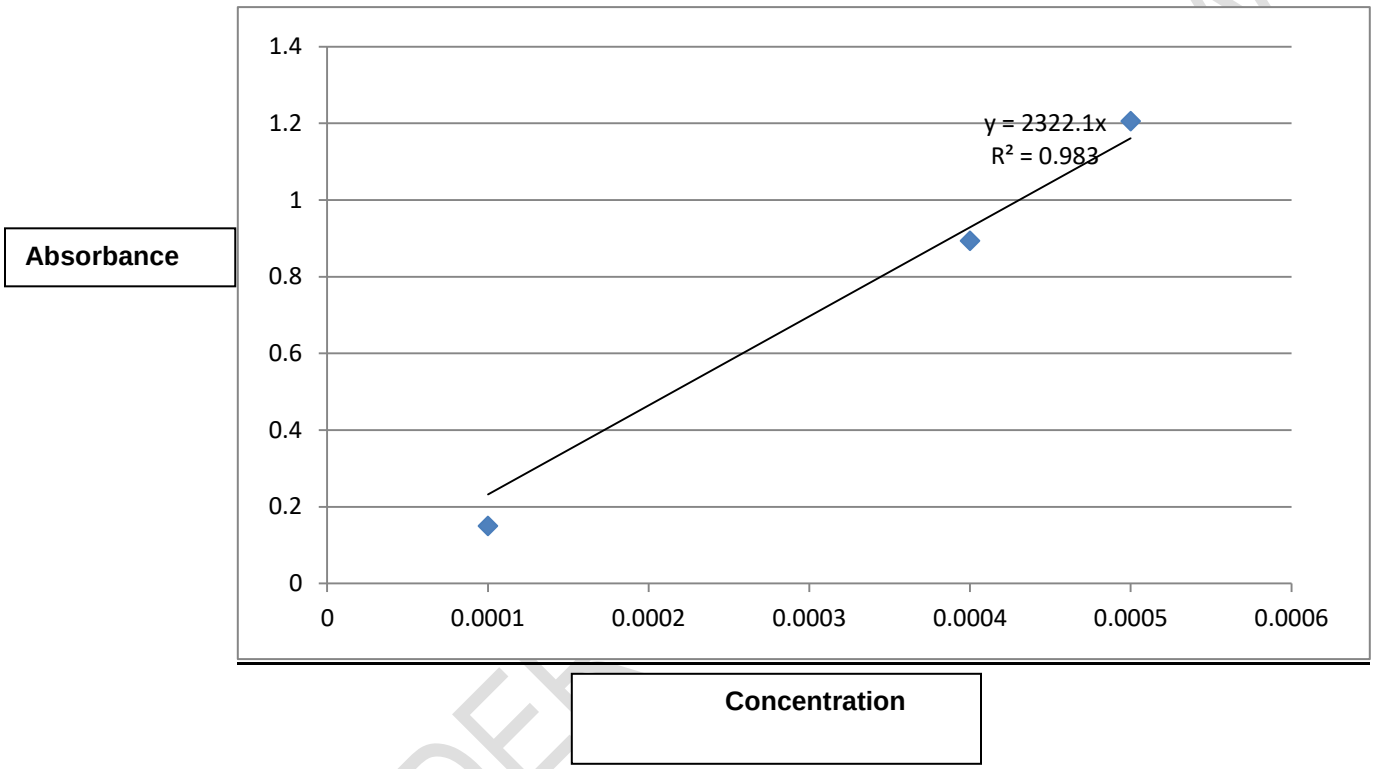
Fig 1 Phosphomolybdate method bar graph showing absorbance at 300,200 and 100ul



1. OC2 2.OC3 3.OC4 4.OC5 5.Crude

FC method has been used for the determination of polyphenols in flower extracts. The Folin-Ciocalteu reagent (FC) is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric determination of phenolic and polyphenolic compounds .The total phenolic contents from different fractions were found by plotting standard calibration curve of gallic acid by the equation y=2322.1x.(**Fig 2**)

Fig 2 Determination of total phenols by standard calibration curve Gallic acid:



The phenolic contents are in the increasing order of fraction OC2, OC5, and crude. The maximum phenolic contents are present in crude.(**Table 3**)

Table No.3 Total phenols mg/g equivalent of Gallic acid

S.No	Samples code	Total phenols mg/g equivalent of Gallic acid	(+-)Standard deviation
1	OC 2	4.49*e-7	0.068418
2	OC 3	3.04*e-7	0.143962
3	OC 4	4.35*e-7	0.067471
4	OC 5	6.70*e-7	0.069573
5	Crude fraction	8.87*e-8	0.01

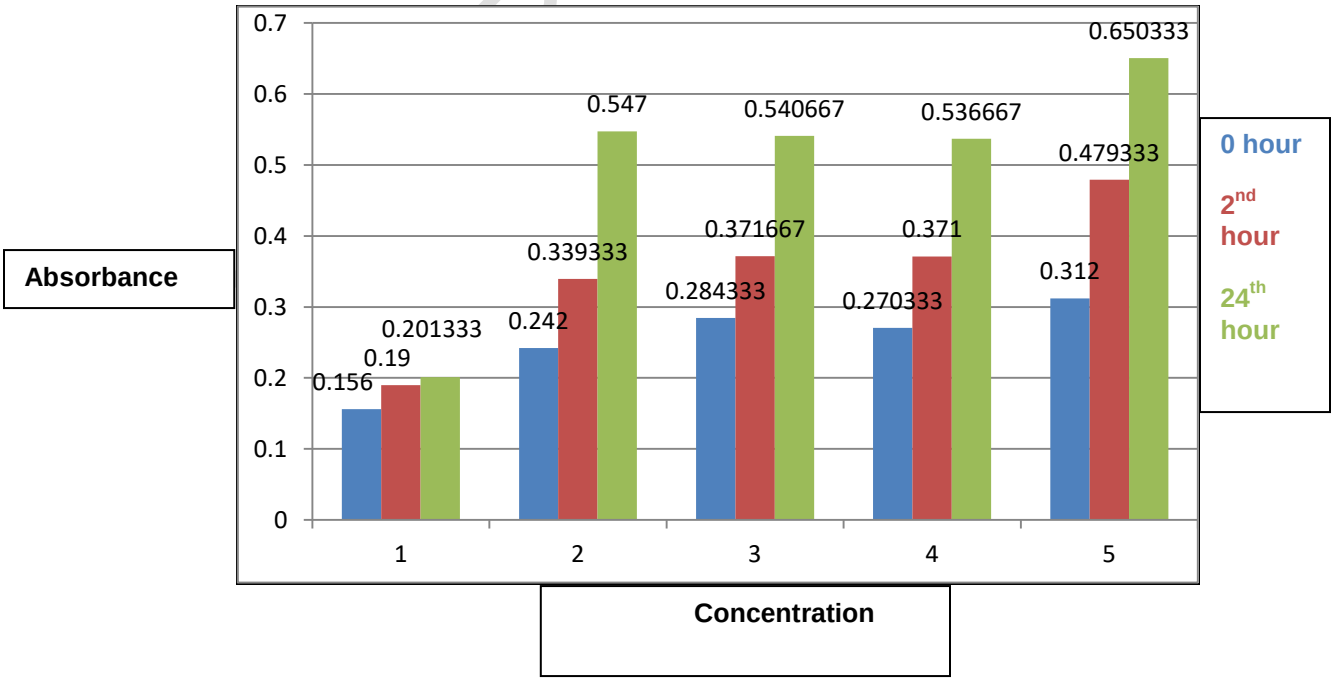
In FTC the cell membrane is permeable to free radicals that react rapidly with unsaturated fatty acids like linoleic acid that are embedded in the membrane. The free radical reacts with the unsaturated fatty acid, it is called lipid peroxidation. In this particular assay of FTC linoleic acid is used as the peroxide source. The peroxides generated react with ferrous (2+) chloride to form ferric (3+) ions. A ferric ion formed by an oxidant form a thiocyanate complex which is measured by a spectrophotometer at the wavelength of 500 nm. [14]

Fig 3 shows FTC ferric thiocyanate activity.Different samples shows different absorbance at different intervals of time.Three hours have been selected i.e zero hour,second hour and twenty fourth hour.It has been found with increase in interval of time there is increase in absorbance.Hence maximum absorbance has been shown by the samples at twenty fourth hour.(**Table 4**)

Table No.4 FTC method showing absorbance of samples and blank at different hours

S. No	Samples code	absorbance 0 hour	absorbance 2nd hour	absorbance 24th hour	(+-) Standard deviation 0 hr	2nd hr	24th hr
1	OC 2	0.156	0.19	0.201333	0.001	0.001	0.000577
2	OC 3	0.242	0.339333	0.547	0.001	0.004041	0.002646
3	OC 4	0.284333	0.371667	0.540667	0.00404	0.013429	0.015044
4	OC 5	0.270333	0.371	0.536667	0.00251	0.001	0.008083
5	Blank	0.312	0.479333	0.650333	0.001	0.007638	0.012014

Fig 3 FTC bar graph showing absorbance of samples and blank at different hours



Among all the samples blank shows maximum absorbance.So,blank has been selected for calculating percentage inhibition.Percentage inhibition calculated is 69% .So,it is concluded that blank has the maximum ability to form ferric thiocyanate complex by giving red colour.

4. CONCLUSION

The results of activities suggest that the flower extract possess oxidants compounds, phenols,polyphenols and show peroxidation.So,its concluded *Ocimum basilicum* flower show as good results as leaf extract[15] like an standard anti-oxidant itself.On basis of antioxidant potential flower extract is recommended for use in food for inhibition of diseases like obesity and metabolic disorder.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

[1]Telci I, Bayram E, Yilmaz G, Avci B Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.*). Biochem. Syst.Ecol.,* (**2006**) 34: 489-497.

[2] Khosla,M.K.Study on inter relationship ,phylogeny and evolutionary tendencies in genus ocimum.*Journal of plant anatomy and morphology*(**1995**) 6:93-106.

[3] Hedge,I.C .A global survey of the biogeography of the Labiatae.*Advances in Labiatae science,The Royal Botanical Garden* ,New London. (**1992**)7-17 in R.M Harley and T.Reynods,eds.

[4] Choi, Y., Jeong, H. S., & Lee, J. Antioxidant activity of methanolic extracts from some grains consumed in Korea*. Food Chemistry,* (**2007**)103: 130_138.

[5]Z. Hao, D.J. Charles, Y. Liangli, J.E. Simon, Purification and characterization of a phenylalanine ammonialyase from *Ocimum basilicum*, *Phytochemistry*(**1996**)43:735–739.

[6]J.C. Aguiyi, C.I. Obi, S.S. Gang, A.C. Igweh Hypoglycaemic activity of *Ocimum gratissimum* in rats *Fitoterapia,* 71 (**2000**):444–446

[7]M. Sharma, K. Kishore, S.K. Gupta, S. Joshi, D.S. Arya Cardioprotective potential of *Ocimum sanctum* in isoproterenol induced myocardial infarction in rats *Molecular and Cellular Biochemistry,* 225 (**2001**): 75–83

[8] Sajjadi, S. E. Analysis of the essential oils of two cultivated basils (Ocimum basilicum L.) from Iran*. Daru*, (**2006)** 14(3), 128_130.

[9] Husain A., O.P. Virmani, A. Sharma, A. Kumara and L.N. Mirsa,. Major essential oil – Bearing plants in India. *Central institute of medicinal and aromatic plants.* Lucknow,India **1988.**

[10] Huda-Faujan.N,Noriham. A, Norrakiah.AS,Babji.AS,Antioxidant activity of plants methanolic extracts containing phenolic compounds. *African Journal of Biotechnology,* (**2009**) Vol. 8 (3):484-489.

[11] Shahwar .D,Raza .MA,Mirza ASM,Abbasi .MA,Ahmad UV,. Comparative Study on the Antioxidant and Antimicrobial Activities of Stem-Bark Extract of Litchi chinensis and its Organic Fractions. *Journal- Chemical Society of Pakistan* (**2010)**32(3):357-362.

[12]Osawa.T,Namiki.M,A Novel Type of Antioxidant Isolated from Leaf Wax of Eucalyptus leaves. *Agricultural and Biological Chemistry*, (**1981**) Vol 45, (3):735–739.

[13] Prieto P, Pineda M and Aguilar M ; Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. *Analytical Biochemistry* (**1999**)269: 337-341.

[14] Cai, Y.; Luo, Q.; Sun, M.; Corke, H. Antioxidant activity and phenolic compounds of 12 traditional Chinese medicinal plants associated with anticancer*. Life Sci*. **2004**, 74, 2157–2

[15]Patil D.D, Mhaske D.K,Wadhawa G.C,) Antibacterial and Antioxidant study of Ocimum basilicum Labiatae (sweet basil) .*Journal of Advanced Pharmacy Education & Research***(2011***)* 2: 104-112.