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Investigation of Antioxidant Potential in *Ocimum basilicum* Flower

Sadaf Khurshid Kayani [a*]

[a] *Department of Chemistry, Government College University, Lahore 54000 Pakistan.*

Authors' contributions

The sole author designed, analysed, interpreted and prepared the manuscript.

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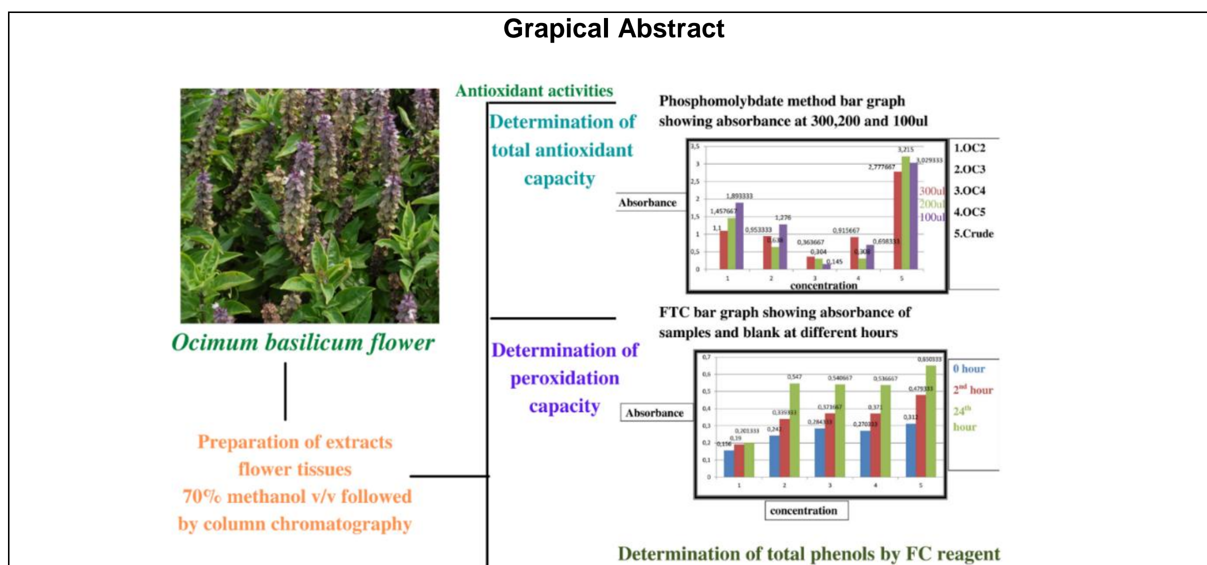
ABSTRACT



*Corresponding author: Email: sadafkyani04@gmail.com;

Aims: The present work is particularly focused on antioxidant 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. The study comprises duration of 6 months.
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 Folin-Ciocalteu (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 100µl, 200µl and 300µl. The results show that crude has maximum antioxidant capacity. The phenolic contents are in the increasing order of fraction OC2, OC5, and crude. The maximum phenolic contents are present in crude. Reference has the maximum ability for peroxidation for ferric thiocyanate complex by giving red colour.
Conclusion: Overall it is concluded that *Ocimum basilicum* flower has antioxidant capacity as good as a standard antioxidant. It is recommended in food/medicine as natural herbal product.

Keywords: Ocimum basilicum; flower; antioxidant capacity; ferric thiocyanate; folin-ciocalteu.

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 native to India, Afghanistan, Pakistan, Northern India and Iran, now this plant is cultivated worldwide [1].

Ocimum basilicum called sweet basil, is botanically described as a branched plant that grows between 0.3 and 1.3 m height. It has light green silky leaves which grow in opposite directions and contain many oily glands that store essentials oils. The flowers are colored, from white to purple and arranged in a terminal spike [2].

Ocimum species are not only characterized by an abundance of compounds such as phenolic acids, but also volatile oils [3].

Foods based on plants are considered, as they possess natural antioxidants like flavonoids polyphenols, vitamin C and vitamin E. The antioxidants have minimized risk of cardiovascular, chronic diseases and certain types of cancer [4].

It has been found that essential oils from *Ocimum basilicum* act as a strong antiviral agent against DNA viruses like herpes simplex viruses, adenoviruses and hepatitis B virus. Besides RNA viruses like coxsackievirus and enterovirus [5,6,7].

The complex composition of Ocimum species extract determines its strong anti-inflammatory, antibacterial and antiviral activity. The most powerful therapeutic species of Ociumum, are *Ocimum basilicum, Ociumum gratissiumum* and *Ocimum sanctum* [8].

Studies have shown that hydroalcoholic extract of *Ocimum basilicum* has antiosteoporotic effect, bone protection against osteoporosis induced by glucocorticoids [9].

Reported research work is novel by considering flower of *Ocimum basilicum.* Previous studies were restricted to leaves, stem and other aerial parts of this plant only.

The aim of this study is determination of antioxidant properties particularly, in flower of *Ocimum basilicum.*

2. MATERIALS AND METHODS

2.1 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.

2.2 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.

As organic solvents like methanol, ethanol, and hydro-alcoholic mixtures are mostly used for the extraction of flavonoid and phenolic compounds [10].

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 dilute HCl was added to the aqueous layer of sodium carbonate and pH was maintained at 3. Two layers were 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).

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.

2.3 Antioxidant Activities

2.3.1 Phosphomolybdate method

To the 300,200 and 100µl 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) [11].

2.3.2 Determination of total phenols by folin-ciocalteu (FC reagent)

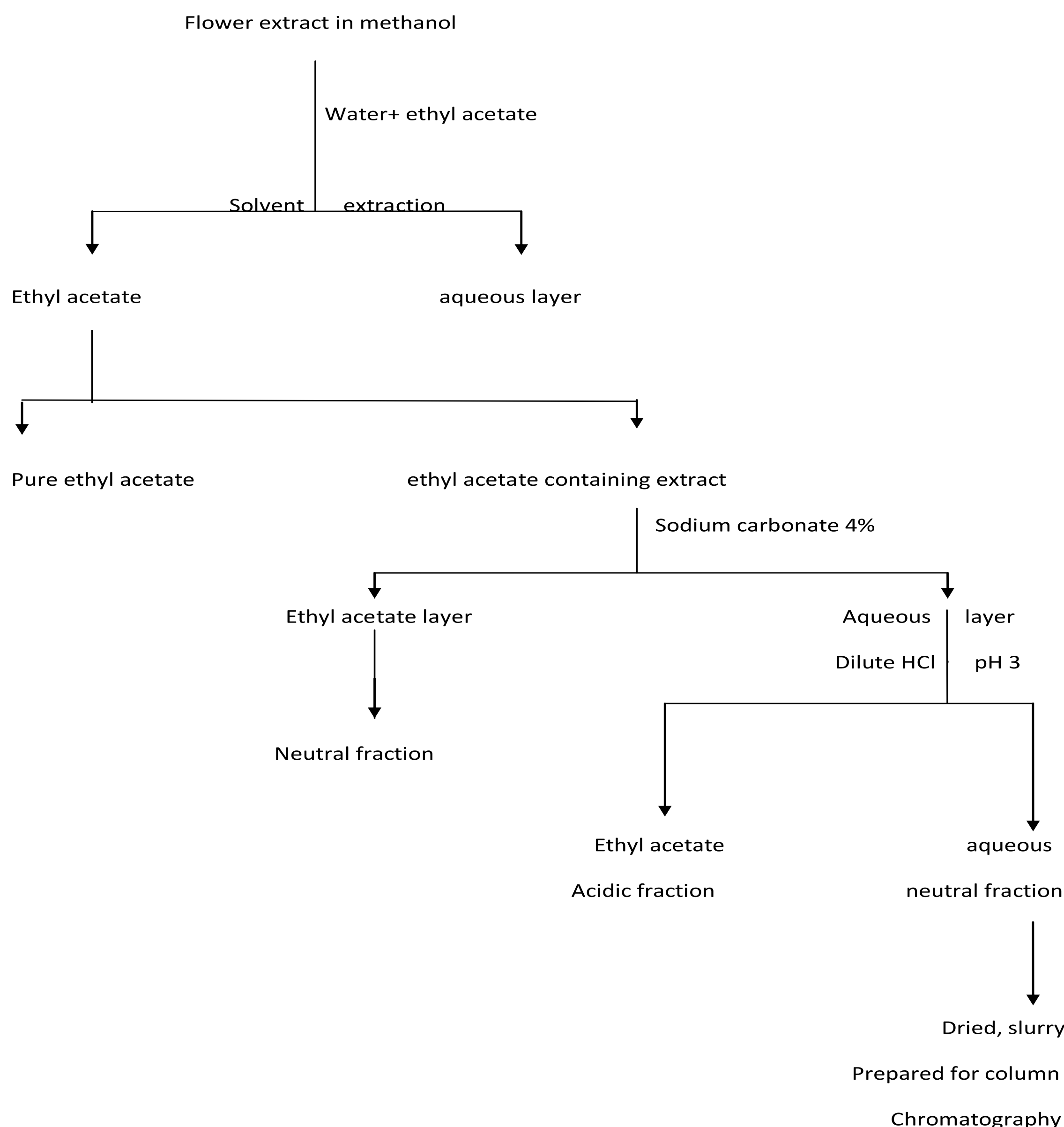
Standard Gallic acid: To the 100 µl of the standard 50 µl Folin-Ciocalteu (FC reagent) and 100 µl of 10%Na_2CO_3 was added and volume was raised up to 2.5ml with methanol. It was kept for 30 minutes and absorbance was read at 765 nm. Different dilutions were done by taking 1ml from standard and making 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 100µl, Folin-Ciocalteu (FC reagent) 50µl, Na_2CO_3 100µl 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 [12].

2.3.3 Ferric thiocyanate (FTC) method

To the 1mg of fractions and extract ethanol was added.1000µl was taken and mixed with 1000µl of 2.52% linolenic acid prepared in absolute ethanol.1000µl of 0.05M phosphate buffer (pH 7) and 1000µl 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℃ for 10 minutes.

The peroxide value was determined by using thiocyanate as a coloring agent. After 10 minutes 100µl of solution was taken and 100µl of 30 % ammonium thiocyanate with 100µl of 0.02 M ferrous sulphate in 3.5%HCl was added and absorbance was read at 532nm [13].



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

Table 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%	OC-4	500ml
5	Ethyl acetate:n-hexane	80%	OC-5	500ml
6	Chloroform	100%	OC-6	500ml
7	Chloroform :methanol	1%	OC-7	500ml
8	Chloroform:methanol	5%	OC-8	500ml
9	Chloroform:methanol	10%	OC-9	500ml
10	Chloroform:methanol	20%	OC-10	500ml
11	Methanol	100%	OC-11	500ml

Table 2. Antioxidant activity by phosphomolybdate method using 300µl sample

S.No	Samples code	Average absorbance at 300µl	(+-)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 3. Antioxidant activity by phosphomolybdate method using 200µl sample

S.No	Samples code	Average absorbance at 200µl	(+-)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 4. Antioxidant activity by phosphomolybdate method using 100µl sample

S.No	Samples code	Average absorbance at 100µl	(+-)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 for antioxidant capacity. In this method the reduction of Mo (VI) to Mo (V) by the antioxidant compound takes place. The formation of a green phosphate/Mo (V) complex is at the maximum absorption of 695 nm [14].

The results of phosphomolybdate activity at different concentrations are same (Tables 2, 3, 4). The sample OC2 and crude have maximum absorbance at the concentration of 100µl, 200µl and 300µl. However, crude has the much higher absorbance compared to OC2 at all three concentrations. The results lead to the conclusion that the raw material has maximum antioxidant capacity. (Fig. 1).

The Folin-Ciocalteu reagent (FC) is 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).

Chemically, phenols constitute the largest and most important class of antioxidants.

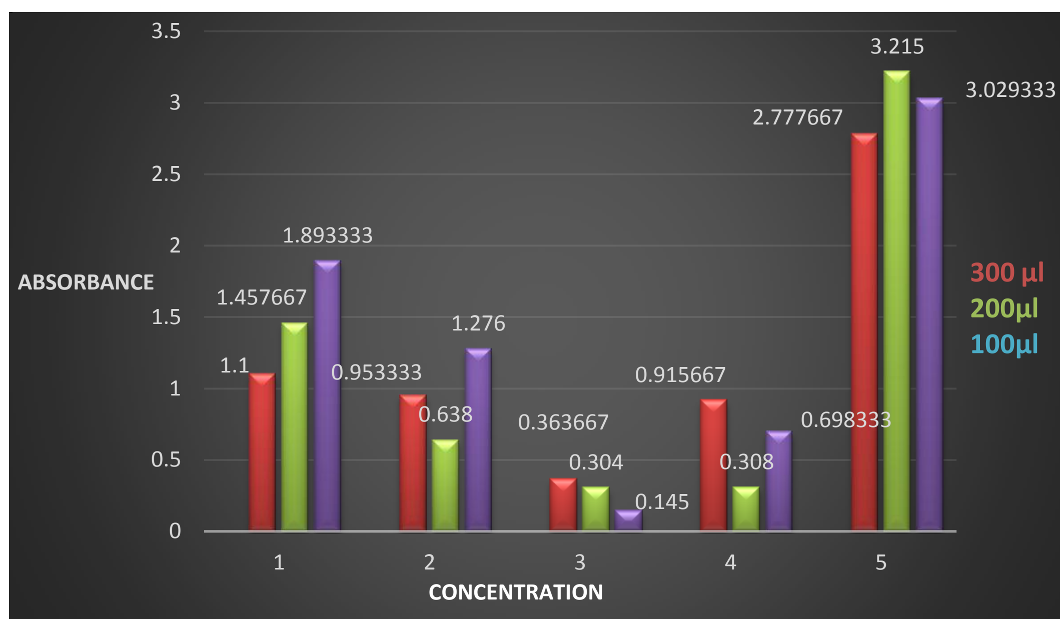
Phenolic contents are in the increasing order of fraction OC2, OC5, and crude.

The maximum phenolic contents are present in crude. (Table 5).

Thiocyanate method is used for the determination of peroxidation. The graph was plotted between absorbance and time for each fraction. Tocopherol was used as a reference.

Fig. 3 shows (FTC) ferric thiocyanate activity. Different samples shows different absorbance at different intervals of time - zero hour,second hour and twenty fourth hour.It has been found with increase in interval of time there is increase in absorbance. Therefore, the maximum absorbance has been shown by the samples at twenty fourth hour (Table 6).

Maximum peroxidation shown at twenty fourth hour is by the reference. Among samples peroxidation is in the increasing order of fraction OC3, OC5, and maximum by OC4.



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

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

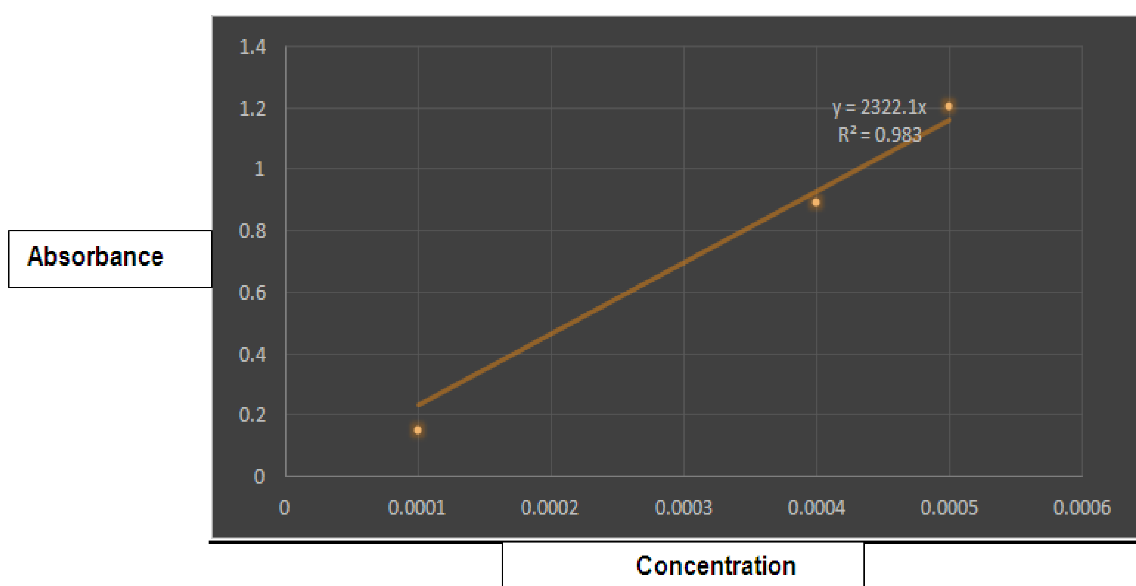


Fig 2. Determination of total phenols by standard calibration curve Gallic acid

Table 5. 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

Table 6. Ferric thiocyanate (FTC) method showing absorbance of samples and reference 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	Reference	0.312	0.479333	0.650333	0.001	0.007638	0.012014

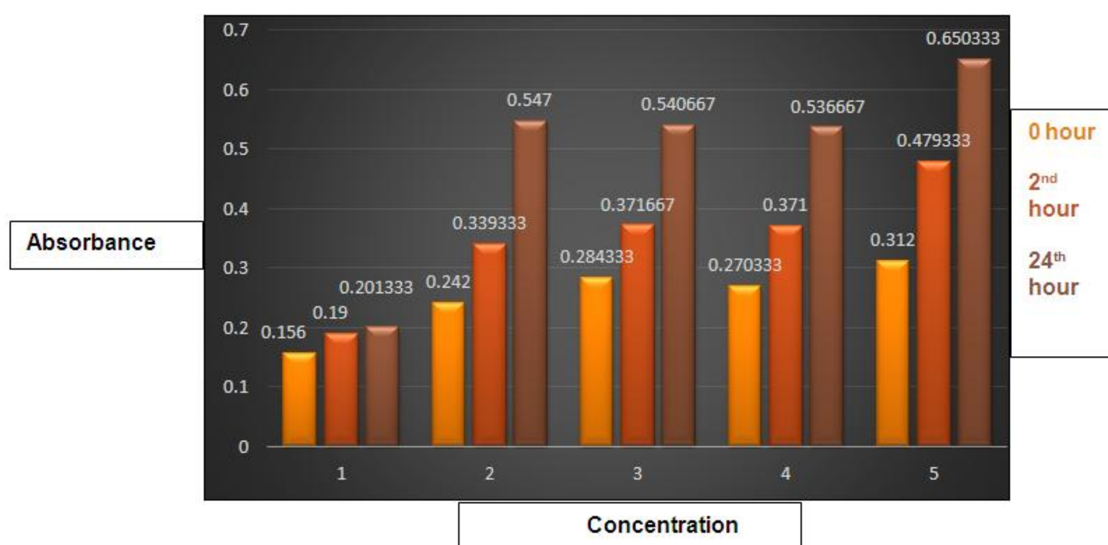


Fig. 3. Ferric thiocyanate (FTC) bar graph showing absorbance of samples and reference at different hours

The results of graphs showed that extract of flower have shown significant antioxidant activity and these results are comparable with the activity of tocopherol.

As reference has maximum peroxidation. So,reference has been selected for calculating percentage inhibition.Percentage inhibition calculated is 69% .

The results of all activities proof that high content of antioxidant compounds are present in flower of this plant. Flower extract can also be used in future like leaves extracts, for the radiance and resistance of the skin and slowing down of the aging process by maintaining estrogen levels [15].

4. CONCLUSION

This investigation suggest that the flower ectract of *Ocimum basilicum* possess antioxidants compounds, phenols,polyphenols and show peroxidation.So,it is concluded *Ocimum basilicum* flower depicts as satisfactory results as leaf extract [16] like an standard antioxidant itself.On basis of antioxidant potential flower extract is recommended for inhibition of diseases like obesity and metabolic disorder.

In future essential oils(linalool, estragole, methyl cinnamate, bicyclosesquiphellandrene, eucalyptol, α -bergamotene, eugenol, ɣ-cadinene and ger-macrene) [17] can be extracted from flower extract like leaves and stem.

DISCLAIMER

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.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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