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Research Article

ETHANOLIC AND HYDRO ALCOHOLIC EXTRACT OF *OCIMUM KILIMANDSCHARICUM* FOR ANTI-OXIDANT ACTIVITY BY USING *IN-VITRO* METHOD

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Abstract

According to the Ayurveda, plants have been used for the treatment of so many diseases. Herbal drugs are easily available and have fewer side effects. So, many people are attracted towards the herbal drugs. Ocimum kilimandscharicum is one of a few types of basil that is perennial. It is a well-known plant in Indian traditional system of medicine.The genus of *Ocimum* belongs to the family Labiatae and one of the most popular culinary herbs known for its medicinal properties. In this present study ethanolic, hydro alcoholic extract of *Ocimum kilimandscharicum Linn* was studied for Anti-oxidant using *in-vitro* model. The study revealed that different concentrations of the extract exhibited significant Anti-oxidant activities in a dose dependent manner at a concentration of 400mg/ml respectively and well compared with standard drug. Thus, it could that due to the presence of chemical constituents present in the extracts have well prospective for the management of Oxidation.This Knowledge will be useful in finding more potent above all those activitie from the natural resources for the clinical development of activities and therapeutics.

Keywords: *Ocimum kilimandscharicum Linn*, Ethanolic extract, Hydro alcoholic extract, Anti-oxidant activity.

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INTRODUCTION

The plant *Ocimum kilimandscharicum* (fig no .2) valued as home remedy for cough and colds, skin diseases, coryza, fever, headache, rhinitis, pharyngitis, arthralgia, neuralgia, abdominal colic, nausea, vomiting, dyspepsia and diarrhea [1].

Lamiaceae, or the Mint family was found to be cosmopolitan in distribution comprising of about 252 genera and almost more than 7000 species and is well known since ancient civilization for its medicinal properties in folklore medicine and also as an important edible herb in culinary practices [2].

OXIDATIVE STRESS, DISEASES AND ANTIOXIDANTS

Free radicals are the molecules fragments containing one or more unpaired electrons in the outer orbit. Reactive oxygen species (ROS) and reaction nitrogen species (RNS) are the by-products of cellular redox process responsible for playing aboth deleterious and beneficial effect in the body. The reactive oxygen and nitrogen radicals includes superoxide (O_2), hydroxyl (OH), Peroxyl (ROO), lipid peroxyl (LOO), alkyl (RO), nitric oxide (NO) and nitrogen dioxide (NO_2) radical. Though, hydrogen peroxide (H_2O_2), ozone (O_3),singlet oxygen (O_2),hypochlours acid (HOCI), nitrous acid (HNO_2), Peroxynitrite ($ONOO^-$), di nitrogen trioxide (N_2O_3), lipid peroxide (LOOH) are not radicals in nature but can lead to free radicals reaction in living organisms. It has been estimated that 5% of inhaledoxygen is transformed in to several harmful ROS species like O_2, OH, $H_2O_2$by equivalent reduction of oxygen [3].

EFFECTS OF OXIDATIVE STRESS ON THE HUMAN BODY

Oxidation is a normal and necessary process that takes place in your body. Oxidative stress, on the other hand, occurs when there's an imbalance between free radical activity and antioxidant activity. When functioning properly, free radicals can help fight off pathogens. Pathogens lead to contaminations [3]. When there are more free radicals present than can be kept in balance by antioxidants, the free radicals can start doing damage to fatty tissue, DNA, and proteins in your body. Proteins, lipids, and DNA make up a large part of your body, so that damage can lead to a vast number of diseases over time. These include:

- diabetes
- atherosclerosis, or the hardening of the blood vessels
- inflammatory conditions
- high blood pressure, which is also known as hypertension
- heart disease
- neurodegenerative diseases, such as Parkinson's and Alzheimer's
- cancer

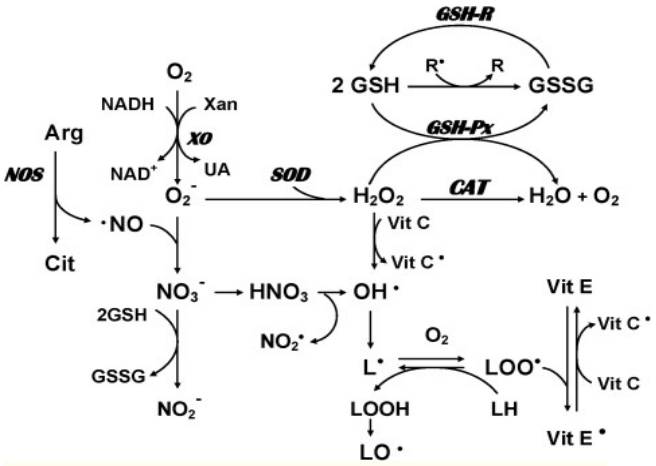


Fig 01: possible mechanisms involving production and removal of oxygen and nitrogen free radicals in mammalian cells

Molecular oxygen can be converted to superoxide radicals (O_2^-•) in the presence of xanthine oxidase (XO). Subsequently, superoxide dismutase (SOD) catalyzes the conversion of superoxide radicals to hydrogen peroxide (H_2O_2). Catalase (CAT) and glutathione peroxidase (GSH-Px) convert hydrogen peroxide to water. Glutathione (GSH) is utilized by GSH-Px to yield the oxidized form of glutathione (GSSG), which is converted back to GSH by glutathione reductase (GR). Hydrogen peroxide is susceptible to autoxidation to form hydroxyl radicals (OH•), particularly in the presence of metal catalysts such as iron. In addition, nitric oxide (NO), which is the product of a five-electron oxidation of the amino acid L-arginine, can also produce hydroxyl radicals as well as nitrogen dioxide radical.(fig 01) .On the other hand, α-tocopherol (vitamin E) has the ability to inhibit lipid peroxidation as a chain-breaking antioxidant. Vitamin E radicals can be recycled back to their native form by ascorbic acid (vitamin C).

DIFFERENT TYPES OF ANTIOXIDANTS:

There are three primary types of antioxidants:-

- Phyto-chemicals
- Vitamins and
- Enzymes

Most of them are found in plants.

PHYTOCHEMICALS:

Phytochemicals are type of antioxidants that are produced by plants to protect themselves against free radicals. For example: Carotenoids, Flavonoids, Allyl sulphides, Polyphenols etc[4].Studies show that humans who eat sources of phytochemicals also benefit from the antioxidant properties of the plant.

Vitamins

The human body does not produce vitamins. So, it is essential to include them in our daily food through foods or supplements. Common antioxidant vitamins include vitamins A, C, E, folic acid, and beta-carotene. Vitamins need to be supplemented every day, without fail [4].

Enzymes

Enzymes are antioxidants that are synthesized in our body. For example: superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, and catalases. They are made from the protein and minerals in the food we eat. It is important to have good quality protein and minerals in our daily food.

Plant name: -*Ocimum kilimandscharicum Linn* [5]

Fig 02: Ocimum kilimandscharicum Linnplant

BOTANICAL CLASSIFICATION [6]

Kingdom Plantae
Division Angiosperms
Class Eudicots
Sub class Asterids
Order Lamiales
Family Lamiaceae
Genus *Ocimum*
Species *kilimandscharicum*

VERNACULAR NAMES

Sanskrit Karpoorthulasi
Malayalam Karpoorthulasi
English Camphor basil
Hindikapurtulsi
Kannada karupuratulasi
Tamil karupuratulasi

Table 01: Chemical constituents of *Ocimum kilimandscharicum*

NAME OF THE PART	CHEMICAL CONSTITUENTS
Seed oil	α – pinene (1.23%), camphene (7.32%), β – myrcene (1.58%), ethylamyl carbinol (0.88%), l – phellandrene(0.26%), α – terpinene (0.33%), p – cymene (0.62%), dl – limonene(13.56%), 1,8 –cineole (0.85%), β – ocimene (2.00%), γ – terpinene (0.88%), trans- sabinene hydrate (0.49%), α – terpinolene (1.33%), linalool (1.70%), cis – sabinene hydrate (0.47%), camphor (56.07%), 4 – terpineol (3.50%), myrtenol (1.24%), trans – caryophyllene (0.33%), germarcrene-d(0.43%)[7].
Essential oil of aerial parts	α-pinene(1.23%), camphene(7.32%), β-myrcene(1.58%), α-phellandrene (0.26%), α-terpinene(0.33%), p-cymene(0.62%), DL-limonene (13.56%), 1,8-cineole (0.85%), β-ocimene (2.00%)γ-terpinene (0.88%), cis-sabinene hydrate (0.47%), α-terpinolene (1.33%), trans-sabinene hydrate (0.49%), linalool (1.70%), camphor (56.07%), terpinen-4-ol (3.50%), myrtenol (1.24%), trans-caryophyllene (0.33%), germacrene D (0.43%) as their constituents [7].
Leaves	Camphor, 1,8-cineole, limonene, trans caryophyllene, camphene, 4-terpeneol, myrtenol, α-terpineol, endo-borneol ,linalool . Leaves also contain flavonoids, tannins, saponins, sterols, carbohydrates, proteins and triterpenoids

CLAIMED MEDICINAL USES

- ✓ In this plant is used as a traditional medicine, treatment of various ailments including colds, coughs, abdominal pains, measles and diarrhoea.
- ✓ The leaves are used for treat congested chest, cough and cold.
- ✓ Infusion of leaves is a cure for measles.
- ✓ Essential oils possess is used as a biologically active constituents that act as

insect repellents, particularly against mosquitoes and storage pests.

- ✓ Some local farmers also mix stored foodstuffs with dry leaves of *Ocimum kilimandscharicum* for protection against insect pest damage in storage.
- ✓ The plant shows antibacterial and antioxidant activity [8].
- ✓ It also used in viral infections, foul ulcers, anorexia and for healing wounds. *Ocimum kilimandscharicum* in boiled water in a pot or saucepan to generate an aroma.
- ✓ It is also used for the Mediterranean area in interesting forms for decorative purposes.

PLANT COLLECTION AND AUTHENTICATION

The leaves of *Ocimum kilimandscharicum*plant was collected from Sri Venkateswara University of Tirupati-517502, A.P., India, in the month of March and authenticated by Dr. K. Madhava chetty, Assistant professor Department of Botany, the plant voucher number 2127.

PREPARATION OF LEAVES POWDER FOR EXTRACTION

The leaves of *Ocimum kilimandscharicum* plant were separated, cleaned and well dried at room temperature to avoid the degradation of phytoconstituents. The dried leave part of the crude drug was ground in to a coarse powder to use for the study.

PROCESS OF EXTRACTION

About 70 gram of *Ocimum kilimandscharicum* crude drug leaves powder was extracted separately with 500 ml of solvents of increasing polarity viz. Ethanol, Hydroalchol successively by continuous hot percolation method by using Soxhlet extraction apparatus at a constant temperature of 30-45°C. The crude powder was extracted with each solvent for three consecutive days. After extraction, the extracts were collected and dried under air at room temperature to get a well dried extracts. Then the dried extracts were weighed and the percentage yield of each solvent extract was calculated from the weighed powder of each plant. The percentage yield of Ethanol, hydroalchol extracts were 10 % w/w 8.65 % w/w respectively. These extracts are further used for the evaluation of *in vitro* activities.

PRELIMINARY PHYTOCHEMICAL SCREENING

Various chemical tests were performed using dried ethyl acetate, ethanol and aqueous extracts to detect the presence of phytoconstituents like carbohydrates, alkaloids, glycosides tannins, flavonoids and saponins(Table 02, 03)

TESTS FOR ALKALOIDS

Extract was dissolved in dilute Hydrochloric acid and filtered.

Mayer's Test

Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test

Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test

Filtrate was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test

Filtrate was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

TESTS FOR CARBOHYDRATES

Extract was dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test for the presence of carbohydrates.

Molisch's Test

Filtrate was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Benedict's test

Filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test

Filtrate was hydrolysed with dil. Hcl, neutralized with alkali and heated with Fehling's A & B solutions.

Formation of red precipitate indicates the presence of reducing sugars.

Tests for glycosides

Extract was hydrolysed with dil. Hcl and then subjected to test for glycosides.

Modified Borntrager's Test

Extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test

Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

TESTS FOR SAPONINS

Froth Test

Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

TESTS FOR PHYTOSTEROLS

Salkowski's Test

Extract was treated with chloroform and filtered. The filtrate was treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

LiebermannBur chard's test

Extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

TESTS FOR PHENOLS

Ferric Chloride Test

Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

TESTS FOR TANNINS

Gelatin Test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

TESTS FOR FLAVONOIDS

Alkaline Reagent Test

Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test

Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

TESTS FOR PROTEINS AND AMINO ACIDS

Xanthoproteic Test

The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin Test

To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

TESTS FOR DITERPENES

Copper acetate Test

Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

ESTMASTION OF TOTAL PHENOLIC CONTENT

Principle

The Folin-Ciocalteu method is an electron transfer (ET) based assay and gives reducing capacity, which has normally been expressed as phenolic content. The oxidation of phenols by molybdictungstate reagent yields a coloured product(Table 04).

$Na_2WO_4/Na_2MoO_4 \longrightarrow (phenol\text{-}MoW_{11}O_{40})^{-4}$

$Mo\ (VI) + e^- Mo\ (V) \longrightarrow$

(Yellow) (Blue)

The conditions to be followed for reliability of the results include proper volume ratio of alkali and F-C reagent, optimum reaction time and temperature for colour development and monitoring of optical density at 765nm .Gallic acid is used as reference standard.(fig 03)

REAGENTS

- 2.5 ml of 10% v/v Fc reagent
- Sodium Carbonate (20%w/v) prepared in distilled water i.e., is 2 g in 100 ml of distilled water

METHOD

The amount of total phenolic in the crude extracts was determined by using the Folin-Ciocalteu reagent by using the colorimetric method [9].The ethanol solution of each extract(0.5 ml,1.0 mg/ml) were added to the test tubes containing 2.5 ml of 10% v/v Folin-Ciocalteu reagent and 2.0 ml 2% w/v sodium carbonate. The tubes were shaken thoroughly and incubated at 45^{o}C for 15 minute with intermittent shaking. Absorbance was observed at 765 nm using UV-Vis Spectrophotometer. Gallic acid was used as a standard to get a calibration curve, and results were expressed as Gallic acid equivalents in milligram per gram (mg GAE/g) of dried extract. The estimation was carried out in triplicate.

ESTIMATION OF TOTAL FLAVONOID CONTENT

REAGENTS

- 75 µl of 5 % Sodium nitrate solution was prepared in 5 g in 100 ml of distilled water
- 150 µl of Aluminium tri chloride 10%
- 1M Sodium hydroxide

METHOD

Total flavonoid content of the ethanolic extract of *Ocimum kilimandscharicum* leaves were determined according to a modified colorimetric method. Briefly, 1.5 ml of plant extract was taken and 75 µl of 5% $NaNO_2$ solution was added. After 6 min, 150 µl of 10% $AlCl_3.6H_2O$ was added to the mixture, which was kept at room temperature for 6 more minutes, followed by the addition of 0.5 ml of 1M NaOH and the total volume was made up to 2.5 ml with the addition of deionised water. The resulting solution was mixed well and immediately, the absorbance was measured at 510 nm on a UV-VIS spectrophotometer. For the blank, the extracts were replaced with an equal volume of deionised water.

A standard calibration curve was prepared with different concentrations of quercetin (in deionised water) [10]. (fig 04)

*IN VITRO*EVALUATION OF ANTIOXIDANT ACTIVITY

HYDROGEN PEROXIDE SCAVENGING ASSAY

Scavenging activity of Hydrogen peroxide (H_2O_2) by the plant extract was determined by the method of Ruch. Plant extract (4 ml) prepared in distilled water at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min[11]. (**Fig 05, 06)** The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using following equation(Table 05,06).

% Inhibition = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

Where

$A_{control}$ = absorbance of the blank control

A_{sample} =absorbance of the test sample

REDUCING SUGAR ASSAY

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu6 The extract (0.75 mL) at various concentrations was mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium hexacynoferrate [$K_3Fe(CN)_6$] (1%, w/v), followed by incubating at 50oC in a water bath for 20 min. The reaction was stopped by adding 0.75 mL of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 3000 r/min for 10 min. (Fig.no 7,8) 1.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power (Table.no:7,8)

% Inhibition = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

Where

$A_{control}$ = absorbance of the blank control

A_{sample} =absorbance of the test sample

RESULTS AND DISCUSSION

PHYTOCHEMICAL SCREENING OF EEOK

Table 02: Phytochemical screening of EEOK

S. No	Phytochemical tests	Results
1	Amino acids: Ninhydrin test	+
2	Carbohydrates: Molisch's test Barfoeds test Selivanoff's test Pentoses test	- - - -
3	Flavanoids: shinoda test Alkaline reagent test Lead acetate test Ferric chloride test	+ + + +
4	Tannins: Ferric chloride test Chlorogenic test	+ +
5	Proteins: Warming test Trichloroacetic acid Biuret test	- - -
6	Steroids: Libermann-burchard test salkowaski test	+ +
7	Glycosides:General test Born tragers test Modified born tragers test Hydroxy anthraquinones	+ + + +
8	Cardiac glycosides: Baljet's test Libermann burchard test Keller killiani test	+ + +
9	Reducing sugars: Benedicts test	+
10	Phenolic compounds: Ferric chloride test Lead acetate test	+ +
11	Alkaloids: Dragendorffs test Mayer's test Tannic acid test	- - -

(+) present, (-) absent

Table 03: Phytochemical Screening of Hydro Alcoholic Extract

S. No	Phytochemical tests	Results
1	Aminoacids:Ninhydrin test	+
2	Carbohydrates: Molisch's test	+
3	saponins : froth formation test	+
4	Flavanoids: Shinoda test Alkaline reagent test	+ +
5	Tannins: Ferric chloride test Chlorogenic test	+ +
6	Proteins: Warming test Trichloroacetic acid Biuret test	- + +
7	Glycosides:General test Borntragers test Modified Born tragers test Hydroxy anthraquinones	+ + + +
8	Steroids: Libermann-burchard test Salkowaski test	+ +
9	Cardiac glycosides: Baljet's test Libermann Burchard test Keller killiani test	- + +
10	Reducing sugars: Benedicts test	+
11	Phenolic compounds: Ferric chloride test Lead acetate test	+ +
12	Alkaloids: Hagers test Tannic acid test	+ +

(+) present, (-)absent

Table 04: Total Phenolic and Total Flavonoid Content of Eeok and Haeok

S.No	EXTRACTS	Total phenolic content(GAE mg/g of dry material)	Total flavonoid content(QE mg/g of dry material)
1	EEOK	103 .01 ±0.10	57.86 ± 0.124
2	HAEOK	91 .2 ± 0.05	46.78 ± 0.06

Results were expressed on Mean ± SEM (n=3)

Fig 03: Total phenolic content estimated by standard Gallic acid

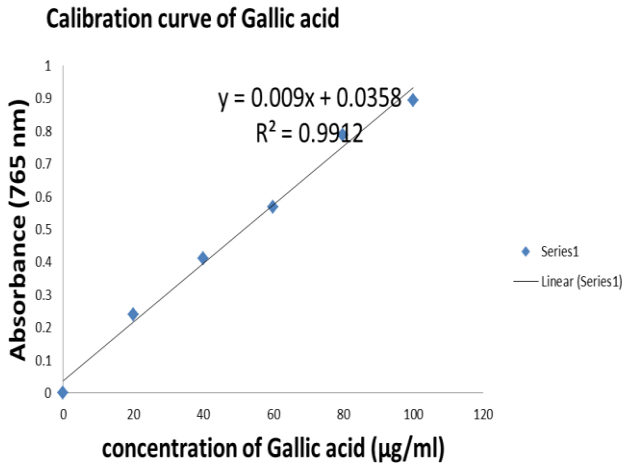
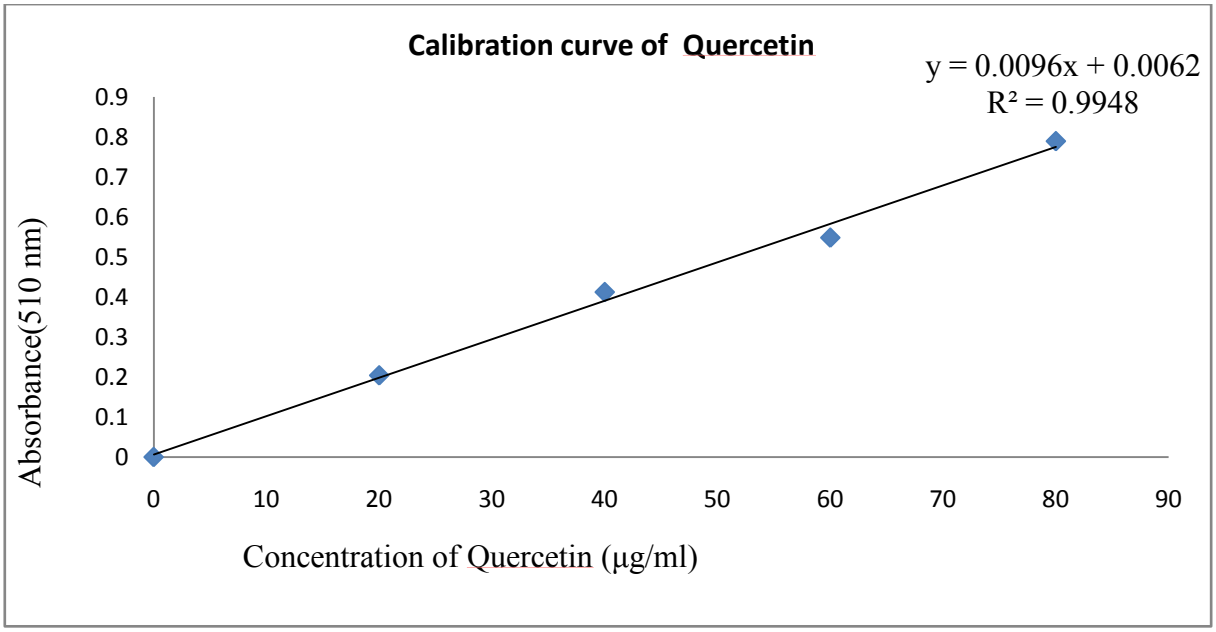


Fig 04: total flavonoid content estimated by standard Quercetin



Tab 05: Evaluation of *in vitro* Hydrogen Peroxide scavenging activity

S. No	Extract	Concentration(µg/ml)	% inhibition (mean ± SD) EEOK	IC_{50}(µg/ml)	% inhibition (mean ± SD) HAEOK	IC_{50}(µg/ml)
1	EEOK	100	22.52 ±0.13	343.51±0.22	19.66 ±0.18	538.77±0.22
2		200	38.77 ±0.24		32.04 ±0.26	
3		300	59.66 ±0.18		43.66 ±0.17	
4	HAEOK	400	70.70 ± 0.27		60.01 ±0.3	

Results were expressed on Mean ± SEM (n=3)

Tab 06: Evaluation of Hydrogen Peroxide scavenging activity of Ascorbic acid

S. No	Concentration(µg/ml)	% inhibition of Ascorbic acid (mean ± SD)	IC_{50}(µg/ml)
1	12.5	22.04 ±0.18	178.31±0.22
2	25	31.02 ±0.29	
3	50	32.77 ±0.11	
4	100	54.81 ±1.02	

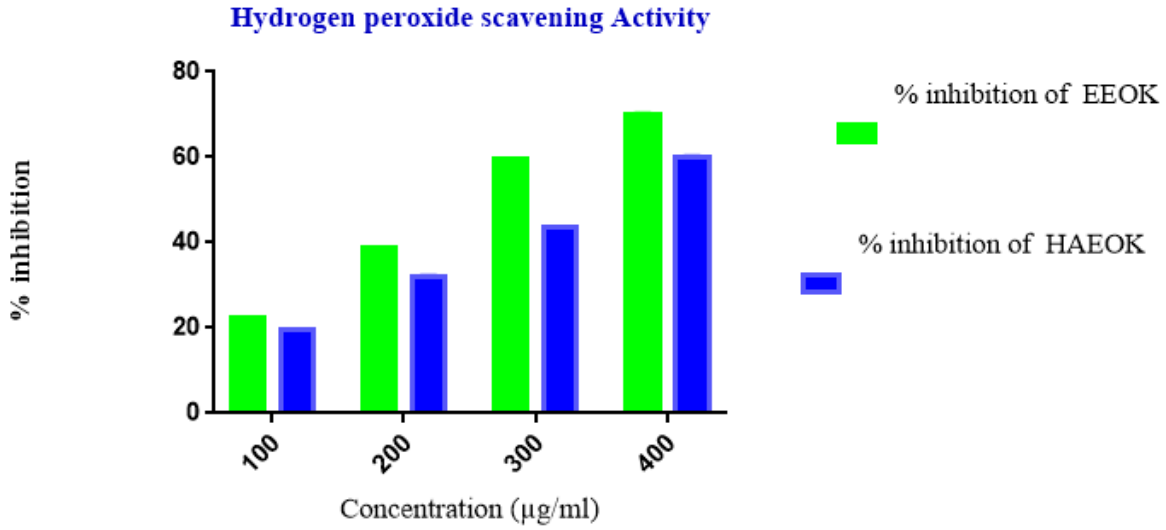


Fig 05: % inhibition of Hydrogen peroxide scavenging assay by EEOK, HAEOK

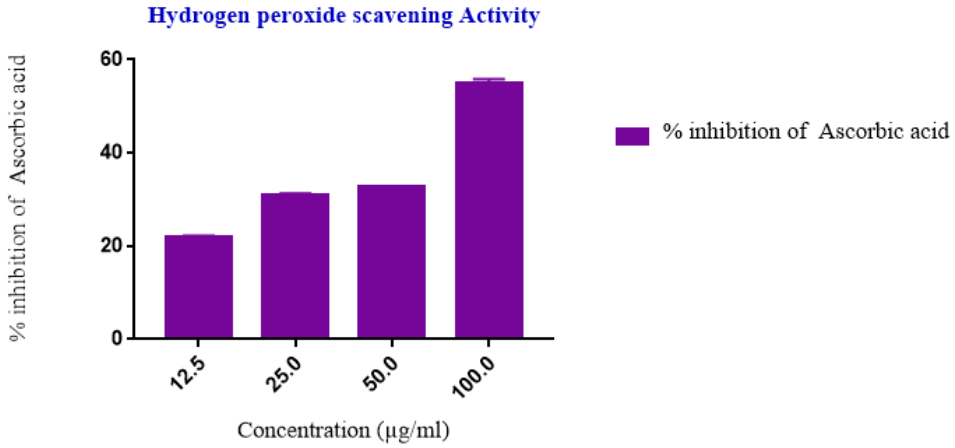


Fig 06: % inhibition by standard Ascorbic acid

Tab 07: Evaluation of *in vitro*Reducing sugar Assay

S. No	Extracts	Concentration(µg/ml)	%inhibition of EEOK	%inhibition of HAEOK
1	EEOK	100	0.43 ±0.01	0.40 ±0.01

2	HAEOK	200	0.55 ±0.02	0.49 ±0.02
3		300	0.76 ±0.02	0.66 ±0.02
4		400	0.89 ±0.03	0.76 ±0.01

Tab 08: Evaluation of *in vitro*Reducing sugar Assay by standard Ascorbic acid

S. No	Concentration(µg/ml)	%inhibition of Ascorbic acid
1	12.5	0.22 ±0.01
2	25	0.36 ±0.03
3	50	0.59 ±0.02
4	100	0.87 ± 0.01

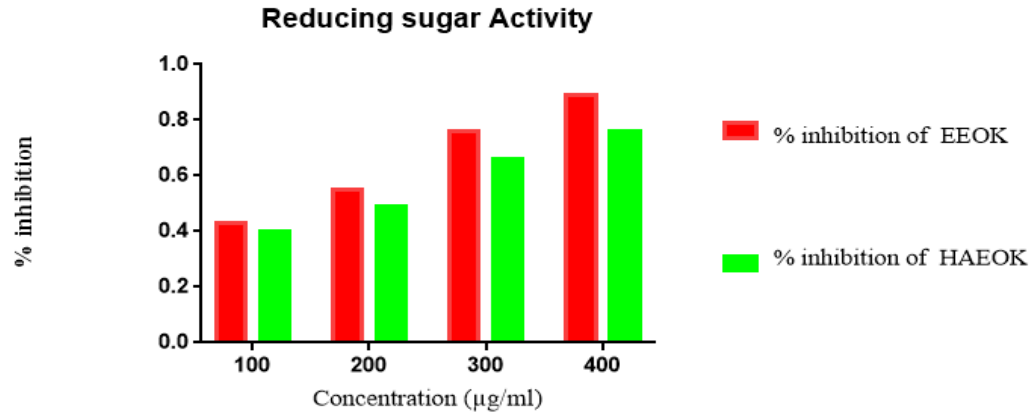


Fig 07: % inhibition of reducing sugar by EEOK, HAEOK

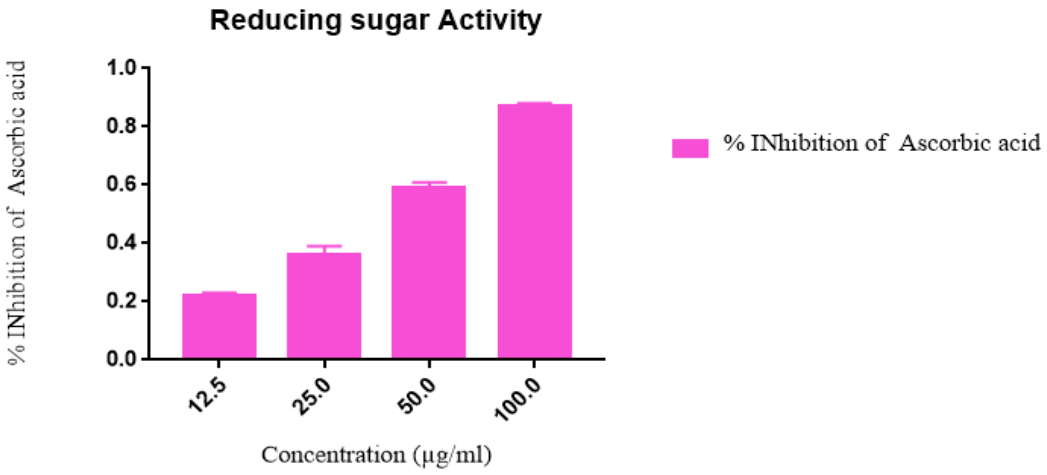


Fig 08: Reducing sugar inhibition by standard Ascorbic acid

PRELIMINARY PHYTOCHEMICAL SCREENING

The In the present study of *O. kilimandscharicum*, Preliminary phytochemical analysis –of Ethanol, hydroalchol reveals the presence of pharmacologically active Ethanol soluble constituents, such as tannins, organic acids, amino acids, and steroids flavonoids, phenolic compounds, and mono-saccharide. Phytochemical screening of *O. kilimandscharicum*, are shown in Table1and Table 2.the *Ocimum kilimandscharicum* plant leaves were investigated in preliminary phytochemical screening then I have done in some of the pharmacological activity.

*IN-VITRO*ANTI-OXIDANT ACTIVITY

The main aim of our present work was to evaluate anti-oxidant activity of plant extracts with that of standard. Generation of free radicals or reactive oxygen species during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress.Reactive oxygen species and reactive nitrogen species includes free radicals and other nonradical reactive derivatives. Reactivity of radicals is generally stronger than non-radical species though radicals are less stable.

HYDROGEN PEROXIDE ASSAY AND REDUCING SUGAR ASSAY

Our finding reveals that *Ocimum kilimandscharicum Linn* effeciently inhibit the free radical scavenging assay by *in-vitro.* The result of experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against free radicals. The ethanolic extract, hydroalcoholic extract (100-400µg/ml) of the plant. The extract showed inhibitory activity of EEOK from 22.52±0.13 to 70.70 ± 0.27, HAEOK from 19.66 ±0.18 to 60.01 ±0.3.Ascorbic acid shown results in at a concentration of (12.5-100) free radical inhibition from 22.04 ±0.18 to 54.81 ±1.02. A comparison of Anti-oxidant inhibitory activity between the standard drug and plant extracts has been depicted in fig. 1. In our study, the ethanolic extract of the plant showed maximum Anti-oxidant inhibitory activity (IC50 = 343.51±0.22 µg), hydroalcoholic extract(IC50=538.77±0.22).the anti-oxidant free radical assay involved in reducing sugar assay of EEOK concentration at(100-400µg/ml) the inhibition from 0.43 ±0.01to 0.89 ±0.03,HAEOK same concentration we taken from0.40 ±0.01to 0.76 ±0.01.and comparison of standard one that is also from 0.22 ±0.01to 0.87 ± 0.01 in which could be attributed to the presence of polyphenol and flavonoids because polyphenols are not only capable of reducing oxidative stress.

CONCLUSION

Historical accounts of certain medicinally relevant herbs reveals contain pharmacologically active substances in sufficiently high concentrations to have a drug like effect when consumed in reasonable quantity. The in-vitro anti- oxidant activitie carried on the two different extracts viz, the ethanolic and hydroalcoholic extracts of Ocimum kilimandscharicum. The study revealed that the hydroalcoholic extract has promising results relative to ethanolic extract. However these studies are not sufficient to claim and hence rigorous, stringent battery of pharmacological, phytochemical and bio analytical studies followed by observational studies in humans are to be carried to support folklore claim of the stated activities.

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