

# World Journal of Current Medical and PHARMACEUTICAL RESEARCH

www.wjcmpr.com

ISSN: 2582-0222

Extraction of Kapura Tulasi (Ocimum kilimandscharicum) leaves: Phytochemical studies and Anti diabetic activity of the Ethanolic Extracts

Motte.Sushma<sup>1</sup>, Lahari.Sidde<sup>2</sup>, Bandi.Jayanthi<sup>3</sup>.

- <sup>1</sup>Department of Pharmacology, Balaji college of Pharmacy, JNTUA, Anantapur, Andhra Pradesh,515001.
- <sup>1</sup>Department of Pharmaceutical Chemistry, Balaji college of Pharmacy, JNTUA, Anantapur, Andhra Pradesh, 515001.
- <sup>2</sup>Department of Pharmacy Practice, Balaji college of Pharmacy, JNTUA, Anantapur, Andhra Pradesh, 515001..

### ABSTRACT

The leaves of Ocimum kilimandscharicum (privately known as Kapura Tulasi) have for some time been being used in the indian arrangement of medication for its enemy of diabetic properies. Could be a short herb, nearby to india it is used to treat various afflictions checking colds, hacks, stomach torments, measles and the runs.ocimum kilimandscharicum, has a spot to family lamiaceae .The current work, meant to assess in vitro enemy of diabetic action. Against diabetic appraisal was done by the boundaries, for example, glucose take-up by yeast cells, estimation of hemoglobin glycosylation, extricate impact at physiological glucose fixation. The standard medication utilized was Metronidazole and gallic corrosive. It indicated that EEOK in portion of 250µg/ml demonstrated high level of glucose take-up and hindered the hemoglobin glycosylation by expanding the grouping of hemoglobin. It additionally showed the hindrance of hemoglobin glycosylation at various physiological convergences of the glucose where the concentrate diminishes the development of the glucose-hemoglobin complex and in this manner measure of free hemoglobin increments. In view of the outcomes, it has been demonstrated that the EEOK displayed hostile to diabetic movement.

Key words:

Anti-diabetic activity, Metronidazole, gallic acid, Ethanolic extraction of Ocimum kilimandscharicum, Glycosylation, Hypotonicity.

Article History:

Received On: 25.03.2020 Revised On: 18.06.2020 Accepted On: 22.06.2020 \*Corresponding Author

Name: Lahari.Sidde

Email: lahari.sidde@gmail.com

DOI: https://doi.org/10.37022/wjcmpr.vi.141

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# INTRODUCTION

Man relied on the healing properties of medicative plants, before the introduction of chemical medicines. Medicative plants, the "back bone" of ancient medication which implies over 3.3 billion folks within the less developed countries utilize medicative plants on an everyday basis [1]. An herb could be a plant or plant structure valued for its medicative, aromatic or savory qualities. Flavorer plants turn out and contain a spread of chemical substances that impact the body. Flavorer preparations known as "phytopharmaceuticals", "phytomedicinal" or "phytomedicine", square measure preparations made up of completely different components of herbs or plants. They are available in several formulations and dose forms together with tablets, capsules, elixirs, powder, tincture, cream and parenteral preparations [2,3].



Figure 01: Vegetative twig of O.Kilimandscharicum

# **COMMON NAMES [3]**

Sanskrit: Kapura Tulasi.

English: African Basil, Camphor Basil, Camphor-Scented Basil,

Perennial Basil, Fever plant. German: Kampfer basilikum Hindi:Kapur Tulsi, Kapuri Tulsi [4].

French: Basilic Camphré, Basilic Camphre, Basilic Du Mont

Portuguese: Basilicão-Canforado, Basilicão-Canforado (Brazil)

Russian: Bazilik Kamforrnyi.

Thai: Ka Phrao India (Prachin Buri), Ka Phrao Khaek (Bangkok) [5].

Taxonomy hierarchy	
[19]: Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Super division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae – Mint family
Genus	Ocimum L. – basil
Species	Ocimum kilimandscharicum
	Guerke – hoary basil <sup>19</sup>

Ocimum kilimandscharicum Guerke (Syn. Ocimum camphora Guerke) (Fig.1) belongs to family Lamiaceae. It is a native of Kenya and distributed in East Africa, India, Thailand, Uganda and Tanzania. It is extensively grown in the Tropics. In India it is cultivated on a small scale especially in West Bengal, Assam, Tamil Nadu, Karnataka, Kerala and Dehradun. Commonly the plant is called as camphor Basil, African blue basil and in Ayurveda as Karpura Tulasi [6].

Morphologically it is a perennial aromatic evergreen undershrub with pubescent branchlets having pale green leaves which are glandular, ovate or oblong in shape, base is acute, deeply serrated, pubescent on both surfaces, oppositely arranged and about 3-7 cm in length including petioles which are 4 to 12 mm long, 1 to 2.5 cm wide; indumentum of long white adpressed hairs or sometimes glabrous above; petiole 4-10 mm [7]. Inflorescence is vertical, flowers are purplish white in simple or much branched racemes [8]; bracts usually deciduous, forming a small coma, ovate, entire, cuspidate; pedicel 3 mm, erect, slightly curved [9].

### **CHEMICAL CONSTITUENTS**

Seed oil of *Ocimum kilimandscharicum* contains  $\alpha$  – pinene (1.23%), camphene (7.32%),  $\beta$  – myrcene (1.58%), ethylamyl carbinol ( 0.88%), l – phellandrene( 0.26%),  $\alpha$  – terpinene (0.33%), p – cymene (0.62%), dl – limonene(13.56%), 1,8 – cineole (0.85%),  $\beta$  – ocimene (2.00%),  $\gamma$  – terpinene (0.88%), trans-sabinene hydrate (0.49%),  $\alpha$  – 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%) [10].

The essential oil of aerial parts of Ocimum kilimandscharicum contains  $\alpha$ -pinene(1.23%), camphene(7.32%),  $\beta$ -myrcene(1.58%),  $\alpha$ -phellandrene (0.26%),  $\alpha$ -terpinene(0.33%), p-cymene(0.62%), DL-limonene (13.56%), 1,8-cineole (0.85%),  $\beta$ -ocimene (2.00%) $\gamma$ -terpinene (0.88%), cis-sabinene hydrate (0.47%),  $\alpha$ -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 there constituents [10].

Aqueous extract of leaves of *Ocimum kilimandscharicum* Aqueous extract of leaves of *Ocimum kilimandscharicum* contains camphor, 1, 8-cineole, limonene, Trans caryophyllene, camphene, 4-terpeneol, myrtenol,  $\alpha$ -terpineol, endo-borneol, linalool [11]. Leaves also contain flavonoids, tannins, saponins, sterols, carbohydrates, proteins and triterpenoids [12]. These chemical constituents are mainly responsible for various biological activities.

### TRADITIONAL USES

The plant has traditionally been used in different parts of the world for various ailments. *O. kilimandscharicum* is employed as an indigenous medicine for a variety of ailments like cough, bronchitis, viral infections, foul ulcers, anorexia and wounds [12]. The leaves of *O. kilimandscharicum* are acrid, thermogenic, aromatic, insecticidal, antiviral, appetizing and deodorant and are useful in cough, bronchitis, foul ulcers and wounds, opthalmopathy and vitiated conditions of 'vata'. The plant has reported to have various central nervous system (CNS) activities. The plant has shown neurotoxic, antineuralgic, CNS stimulant, tranquilizer, anti-alzhemerian and sedative effects [13].

### MATERIALS AND METHODS

### PLANT COLLECTION AND AUTHENTICATION

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

# PREPARATION OF POWDERED LEAF FOR EXTRACTION

The collected leaves of *Ocimum kilimandscharicum* are cleaned and dried under room temperature in order to avoid degradation of chemical constituents. These leaves are then grined into a coarse powder and used for the study.

### PROCEDURE FOR EXTRACTION

70 Gms of leaf powder was taken and extracted with 500 ml of ethanol as solvent by using continuous hot percolation with the help of soxhlet apparatus (Fig.2)at a temperature of 45°C. This extraction process was carried out untill the solvent becomes colourless. The crude extract obtained was then shade dried and percentage yield of the extract was calculated. The extract was then used for further *in-vitro* evaluation studies.



Figure 02: Extraction of Ocimum kilimandscharicum Ethanolic Extract

### PHYTOCHEMICAL SCREENING

Chemical tests were done to identify the presence of chemical constituents such as carbohydrates, flavanoids, tannins, glycosides, saponins, steroids, triterpenoids, and alkaloids(Table no:1)

# **ESTIMATION OF TOTAL FLAVANOID CONTENT**

Total flavonoid content of the ethanolic extract of  $Ocimum\ kilimandscharicum\$ leaves was determined according to a modified colorimetric method. Briefly, 1.5 ml of plant extract was taken and 75  $\mu$ l of 5% NaNO2 solution was added. After 6 min, 150  $\mu$ l of 10% AlCl3.6H2O 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(Table no.7). 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).

### Thin layer chromatography [15]

TLC is a chromatography technique used to separate non volatile mixtures. Thin layer chromatography (TLC) plate is prepared by application of uniform layer of adsorbent on the glass . Dimensions of the plate:  $20\text{cm} \times 5\text{cm}$ . The application of

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the adsorbent can be done by pouring the slurry. The slurry is made by dissolving Silica gel G in water (1:2). Now the plate was activated at  $100^{\circ}\text{C}$  - $105^{\circ}\text{C}$  for 30 minutes in an oven. There after the sample (2-5  $\mu$ l) was applied.The plate was developed by inserting it into the tank where the mobile phase solvent is present. The mobile phase we have chosen is n- butanol: acetic acid: water in the ratio of 4:1:5. After the development of the chromatogram the compounds are identified by spraying 0.1% Alcl³ solution. Yellow colour spot was appeared which confirms the presence of flavanoids.

R<sub>f</sub> value was calculated by using the formula.

 $R_f = \frac{ \quad \ \ \, \text{Distance moved by the compound} }{ \quad \, \, \text{Distance moved by the solvent} }$ 

# **COLUMN CHROMATOGRAPHY [16]**

Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids. Column chromatography is advantageous over most other chromatographic techniques because It can be used to determine the number of components of a mixture and as well as the separation and purification of those components. Stationary phase: silica gel Mobile phase: Hexane (100) "Fig 03", hexane: ethyl acetate (80:20) "Fig 04", hexane: ethanol (70:30) "Fig.5", hexane: methanol (50:50) "Fig.6". The coloured bands that are obtained was collected and chemical tests was done to detect the presence of flavanoids. For ethanolic fractions, flavanoids are present.

### IN-VITRO ANTI-DIABETIC ACTIVITY

# **GLUCOSE UPTAKE IN YEAST CELLS [102]**

The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts (50- 2000  $\mu$ g/mL)) were added to 1mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100  $\mu$ L of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. (Table no:2) After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and amount of glucose was estimated in the supernatant. Metronidazole was used as standard drug. All the experiments were carried out in triplicates. (Table 03)

# THE PERCENTAGE INCREASE IN GLUCOSE UPTAKE BY YEAST CELLS WAS CALCULATED USING THE FOLLOWING FORMULA

INCREASE IN GLUCOSE UPTAKE (%) = ABS SAMPLE - ABS CONTROL X 100/ABS SAMPLE

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample.

# **EVALUATION OF HAEMOGLOBIN GLYCOSYLATION** [103]

### PREPARATION OF HAEMOGLOBIN

The blood was collected from a healthy human volunteer and transferred into a blood bottle containing an anticoagulant. Hemolysate was prepared based on the principle of hypotonic lysis . The red blood collected were washed thrice with 0.14M NaCl solution and one volume of red blood cells suspension was lysed with two volumes of 0.01M phosphate buffer, pH 7.4 and 0.5 volume of CCl4. The haemolysate was then freed from the debris by centrifugation at 2300 rpm for 15 min at room temperature. The haemoglobin rich fraction i.e., the upper layer was separated and dispensed into sample bottle for storage and refrigerated until required for use.

### ESTIMATION OF HAEMOGLOBIN GLYCOSYLATION

1 mL each of haemoglobin fraction was transferred into three test tubes, each containing 1 mL solution of different concentrations (2, 10, and 20 mg/mL) of glucose in 0.01M phosphate buffer (pH 7.4). The contents were incubated at room temperature for 72 hrs. A blank solution in which the addition of glucose solution was omitted was used as the control. The amounts of hydroxymethylfurfural in nanomole released were estimated at different incubation periods of 0, 24 hrs, 48 hrs and 72 hrs which correspond to the degree of glycosylation. (Table 04)

# EFFECT OF EXTRACTS ON HAEMOGLOBIN GLYCOSYLATION

To 1 mL of haemoglobin solution,  $5\mu L$  of gentamycin and  $25~\mu L$  of the plant extracts ( $30\mu g/mL$ ) were added. The reaction was started by the addition of 1 mL of 2% glucose in 0.01M phosphate buffer (pH 7.4) and incubated in the dark at room temperature. The concentrations of glycated haemoglobin at the incubation period of 0, 24 and 72 hrs were estimated spectophotometrically at 443nm. (Table no: 5)

# EFFECT OF EXTRACT AT PHYSIOLOGICAL GLUCOSE CONCENTRATION

To 1 mL of haemoglobin solution, 1 mL of glucose solution (2mg, 10mg and 20mg in 20 mL each of 0.01M phosphate buffer, pH 7.4) and 5µL of gentamycin in 0.01M phosphate buffer (pH 7.4) were mixed and incubated in the dark at room temperature in the presence 30 µg/mL of Gallic acid and plant extracts respectively. Haemoglobin concentrations were estimated over an incubation period of 72 hrs spectrophotometrically at 443 nm, as an index for measuring the degree of haemoglobin glycosylation. The assay was carried out in triplicates.(Table 06)

# RESULTS AND DISCUSSION

Table 01: Phytochemical screening of EEOK

S. NO	NAME OF THE CHEMICAL TEST	NAME OF THE CONSTITUENT	ЕЕОК
1	Ninhydrin test	Amino acids	+ve
2	Warming test Trichloroacetic acid Biuret test	Proteins	-ve
3	Dragendroff's test  Mayer's test  Tannic acid test	Alkaloids	-ve
4	Molisch's test Barfoeds test Selivanoff's test Pentose's test	Carbohydrates	+ve
5	Benedicts test	Reducing sugars	+ve
6	Born tragers test Modified born tragers test Hydroxy anthraquinones	Glycosides	+ve
7	Baljet's test Libermann burchard test	Cardiac glycosides	+ve
8	Ferric chloride test Lead acetate test	Phenolic compounds	+ve
9	Ferric chloride test Chlorogenic test	Tannins	+ve
10	Libermann-burchard test salkowaski test	Steroids	+ve
11	shinoda test Alkaline reagent test Lead acetate test Ferric chloride test	Flavonoids	+ve

# (+) Present (-) Absent

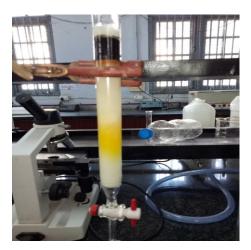


Figure 03: Hexane fraction

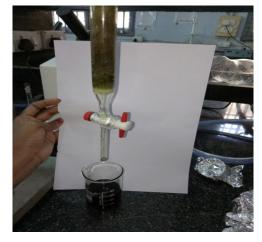


Figure 4: Hexane: ethyl acetate fraction

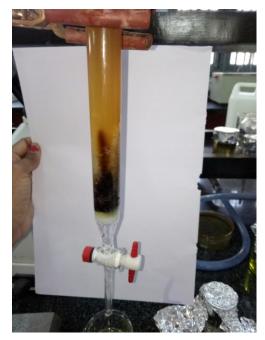


Figure 05: Hexane: ethanol fraction



Figure 06: Hexane: methanol fraction

# THIN LAYER CHROMATOGRAPHY

Distance moved by the compound = 8.6 = 0.71612

Distance moved by the solvent

# Table 02: GLUCOSE UPTAKE IN YEAST CELLS BY EEOK

 $R_f =$ 

TREATMENT (S)	CONC. (MG/M L)	ABSORBANCE AT 443NM (MEAN ± SD)			% INC	REASE IN GI UPTAKE	UCOSE
		5mM 10mM 25mM		5mM	10mM	25mM	
Control	-		•	•		-	
EEOK	50	0.123±0.0 02	0.116±0. 001	0.106±0.0 01	66	67.9	70.7
EEOK	100	0.116±0.0 008	0.122±0. 001	0.113±0.0 004	67.9	66.2	68.7
EEOK	250	0.103±0.0 008	0.105±0. 002	0.135±0.0 02	71.5	70.9	62.7
EEOK	500	0.136±0.0 02	0.127±0. 001	0.166±0.0 01	62.4	64.9	54.1
EEOK	1000	0.125±0.0 01	0.116±0. 002	0.172±0.0 01	65.5	67.9	52.8
ЕЕОК	2000	0.107±0.0 01	0.136±0. 0004	0.142±0.0 02	65.4	62.4	60.2

Table 03: glucose uptake in yeast cells by metronidazole

TREATMENT (S)	CONC. (MG/ ML)	ABSORBANCE AT 443NM (MEAN ± SD)			% INCR	REASE IN GL UPTAKE	UCOSE
		5mM	10mM	25mM	5mM	10mM	25mM
Control	-					-	
Metronidazole	50	0.174±0. 001	0.123±0.0 02	0.155±0.0 01	51.9	61.3	51.2
Metronidazole	100	0.155±0. 001	0.117±0.0 004	0.148±0.0 02	57.1	63.2	53.4
Metronidazole	250	0.166±0. 002	0.132±0.0 02	0.164±0.0 01	54.1	58.4	50.0
Metronidazole	500	0.146±0. 002	0.146±0.0 02	0.156±0.0 01	59.6	54.0	50.9
Metronidazole	1000	0.173±0. 001	0.138±0.0 02	0.141±0.0 02	52.2	56.0	55.6
Metronidazole	2000	0.180±0. 0009	0.176±0.0 001	0.160±0.0 01	50.2	44.6	49.6

# Table 04: ESTIMATION OF HAEMOGLOBIN GLYCOSYLATION OVER THE PERIOD OF 72 HRS

Glucose concentration (mg/ml)	Incubation period (hrs)					
	24 hrs	48 hrs	72 hrs			
2	0.996	0.887	0.924			
10	0.853	0.728	0.583			
20	0.762	0.614	0.547			

# Table 05: EFFECT OF PLANT EXTRACTS ON HAEMOGLOBIN GLYCOSYLATION

Incubation period (hrs)	Gallic acid (std)	ЕЕОК
24	0.829	0.993
48	0.976	1.326
72	1.218	1.395

# Table 06: Effect of extract at physiological glucose concentration

CONC-OF GLUCOSE (MG/ML)	ABSORBANCE AT 24 HRS (MEAN ± SD)		ABSORBANCE AT 48 HRS (MEAN ± SD)		ABSORBANCE AT 72 HRS (MEAN ± SD)	
	ЕЕОК	Gallic acid (std)	ЕЕОК	Gallic acid (std)	ЕЕОК	Gallic acid (std)
2	0.886 ±0.00 2	0.926±0.002	0.982±0.000 9	1.016±0.002	1.192±0.001	1.124±0.008

10	0.925 ±0.00 2	0.916±0.002	1.127±0.000 8	1.085±0.001	1.196±0.000 8	1.168±0.000 8
20	0.995 ±0.00 1	1.293±0.002	1.181±0.001	1.084±0.001	1.196±0.002	1.175±0.001

# **ESTIMATION OF TOTAL FLAVONOID CONTENT**

Table 07: Estimation of total flavonoid content

Extract	Total flavonoid content (QE mg/g of dry material)
EEOK	122.86± 0.38

### DISCUSSION

The calibration curve for 5 sequentially and independently prepared standard solutions of quercetin depicts the concentration of quercetin against the absorbance. The absorbance value increased proportionally upon increasing the concentration of quercetin. A slight deviation from the linearity seemingly occurred at the higher concentration region of quercetin calibration plot. Nevertheless, for our estimation purposes, the calibration plot was employed to ascertain the total flavonoid content of the ethanolic extract. Total flavonoid content in the ethanolic extract of *Ocimum kilimandscharicum* leaves was found to be 122.86 mg/g (Table no:7) equivalent to quercetin (QE), i.e., 1 g of the extract contains 122.86 mg of Quercetin equivalent.

### THIN LAYER CHROMATOGRAPHY

The  $R_f$  value in the chromatographic analysis of Ethanolic extract of *Ocimum kilimandscharicum* was found to be 0.74 which was nearer to the standard  $R_f$  value of isoquercetin(0.716).

# **COLUMN CHROMATOGRAPHY**

The sample containing a mixture of : Hexane (100)"Fig.3", hexane : ethyl acetate (80:20)"Fig.4", hexane : ethanol (70:30)"Fig.5", hexane : methanol (50:50)"Fig.6"The hexane: ethyl acetate fraction eluted from the column as thick green colour. The hexane: ethanol fraction has shown brown colour. The hexane: methanol fraction became colorless .The eluted fractions were collected in test tubes and identified by their distinctive colours. As we needed flavanoids, we has taken the eluted fractions of ethanol and sended it for structural identification by mass and NMR. Finally we identified the compound that was present in the extract was isoquercetin.

Table 08: IR spectra standard values

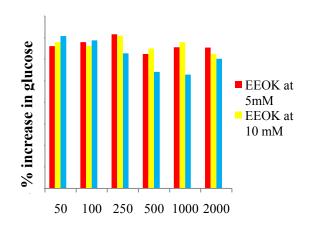
IR spectra standard values:

S.No	Functional group	Standard value	Observed
1.	C=0	1652-1750	1648

2.	ОН	600-700	677.7
3.	C-O-C	1150-1070	1086.91
4.	ArH	3100-3300	3348

### ANTI-DIABETIC ACTIVITY

Figure 07: % increase in glucose uptake by EEOK at 5, 10, 25 mM glucose concentration



Concentration (µg/ml)

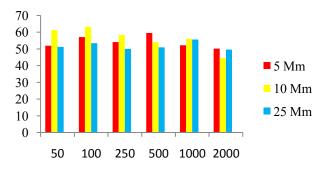


Figure 08: % increase in glucose uptake by metronidazole at 5, 10, 25 mM glucose concentration

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Regulation of glucose level in the blood of diabetic patient can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species. The *in-vitro* assay of the present study indicated that the plant *Ocimum kilimandscharicum*possess good anti diabetic activity.In Yeast (*Saccharomyces cerevisiae*) glucose transport takes place through facilitated diffusion. Type 2 Diabetes is characterised by the deficiency of insulin causing increased amount of glucose in blood.

It was studied that the glucose uptake rate was increased with the increasing concentration of the plant extract and decreased with the increasing extracellular glucose concentration. It is stated that the transport of glucose across yeast cell membrane occurs by facilitated diffusion down the concentration gradient. Hence glucose transport occurs only if the intracellular glucose is effectively reduced (utilized). The data obtained clearly suggests that the EEOK is capable of effectively enhancing glucose uptake which in turn suggests that it is capable of enhancing the effective glucose utilization, thereby controlling blood glucose level.

After the treatment of the yeast cells with the ethanolic plant extract, the glucose uptake was found to increase in a dose dependent manner. (Figure 6) depict the % increase in glucose uptake by the yeast cell at different glucose concentrations i.e.  $25\,\mathrm{mM}$ ,  $10\,\mathrm{mM}$  and  $5\,\mathrm{mM}$  respectively. The ethanolic extract of *Ocimum kilimandscharicum* exhibited significantly higher activity at all glucose concentrations showing the maximum increase at  $5\,\mathrm{mM}$  Glucose concentration i.e.  $71.5\,\%$  increase at  $250\,\mu\mathrm{g/mL}$  of plant extract (Figure 07).

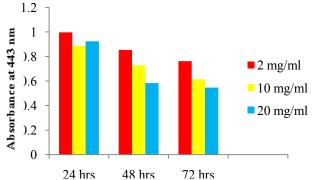


Figure 09: Estimation of haemoglobin glycosylation over the Period of 72 hours

Haemoglobin glycosylation suggests that it is effective in preventing the interaction of glucose with RBCs in blood and it is one of the essential features of any anti-diabetic agent. Increased haemoglobin-glucose level is a serious problem in diabetic patients (hyperglycaemia). Glucose forms a covalent bond with haemoglobin, resulting in increased blood plasma sugar level causes damage to insulin-independent organs like kidney, blood vessels, eye lens, etc.,. Preventing the increase of haemoglobin-glucose level greatly reduces the risk of developing hyperglycaemia associated damage. Plant extract play an important role the inhibition of the glycosylation end products.

An increase in the glycosylation was observed on incubation of hemoglobin with the increasing concentration of the glucose (2mg, 10mg and 20mg) over a period of 72hrs (Figure 09).

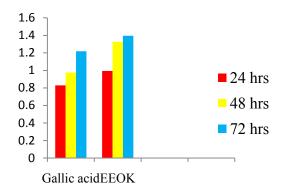


Figure 10: Effect of plant extracts on haemoglobin glycosylation (Values are expressed as mean  $\pm$  SD, n=3)

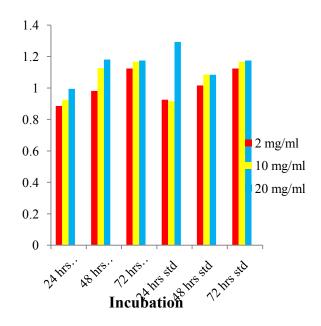


Figure 11: Effect of extract at physiological glucose concentration

The plant extracts also displayed the inhibition of haemoglobin glycosylation at different physiological concentrations of the glucose over the period of 72 hrs, indicating that the plant extract decreases the formation of the glucose- haemoglobin complex and thus amount of free haemoglobin increases (Figure 07,08,09). The results indicate that ethanolic extract of Ocimum kilimandscharicumhave appreciable anti diabetic activity. A non-enzymatic reaction between free amino groups of proteins and reducing sugars is known as glycation. The increased glycation is nothing but additional binding of glucose to hemoglobin which may leads to formation of reactive oxygen species (ROS). Such accelerated haemoglobin glycation can also cause pathogenic conditions like angiopathy, nephropathy and neuropathy in diabetic patients. When oxidation occurs in glycation process, it leads to glycoxidation and formation of advanced glycation end products (AGEs). It is well known that phytoconstituents play an important role in the inhibition of the AGEs.

In present study increased glycosylation was noted when hemoglobin was incubated with glucose over a period of 72 hrs. However, the *Ocimum kilimandscharicum* significantly inhibited the haemoglobin glycosylation which was indicated by presence of increasing concentration of haemoglobin. The

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Ocimum kilimandscharicum also displayed the inhibition of haemoglobin glycosylation at different physiological concentrations of the glucose over the period of 72 hrs, which indicate that the Ocimum kilimandscharicum inhibits formation of the glucose-haemoglobin complex and thus allows haemoglobin to work freely. Results clearly indicate that EEOK has an appreciable anti-diabetic activity.

This observed effect might be attributed to the presence of bioactive compounds like flavonoids and phenols in the *Ocimum kilimandscharicum*. This antidiabetic activity may be attributed to a bioconversion process, which extracts phytoconstituents from the plant materials during fermentation and in addition modify and detoxify certain compounds available in the herbs. Yeast also produces its own vitamins and organic acids, which results in a value added form of medicine. However this needs further investigation of specific bioactive compound responsible for such activities.

#### CONCLUSION

On the basis of the present study, the results finally concluded that the ethanolic extract of *Ocimum kilimandscharicum* possess anti- diabetic activity. These activity may be due to the strong occurrence of polyphenolic compounds such as flavonoids, tannins, steroids, and phenols. This research gives an idea that the compound of the plant *Ocimum kilimandscharicum* can be used as lead compounds for designing potent drugs which can be used for treatment of various diseases such as cancer, neurological disorder, aging and other disorders. However these studies are not sufficient to claim and hence various other pharmacological, phytochemical and bio analytical studies followed by observational studies in humans are to be carried out in order to support the traditional importance of the stated activities.

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