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Research Article

## Evaluation of preliminary phytochemical screening, acute toxicity and antioxidant profile of *Ocimum kilimandscharicum*

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### ABSTRACT

Nature has offered us diverse curative herbs having with powerful antioxidant phytochemicals. *Ocimum* (Lamiaceae) is a notable source of volatile oils and flavouring agents in general and primarily of eugenol, methyl eugenol, linalool, methyl chavicol, etc. *Karpoora Thulasi* is a member of this genus; nevertheless, not much literature has been reported on its safety and antioxidant potential. In this investigation, we did a pre-clinical safety assessment of concentrate of *O. kilimandscharicum* on Sprague Dawley rodents. Toxicological concordat of the *O. kilimandscharicum* concentrate was carried out following OECD guidelines 423. Further, to verify the traditional efficacy and elucidate the mechanism, the present study compared in-vitro antioxidant activity of the plant by DPPH, ABTS and Hydroxyl radical scavenging method using ascorbic acid as the standard. In acute oral toxicity, no treatment-related death or toxic signs were observed. Moreover, the study revealed that the *O. kilimandscharicum* extract could be well tolerated up to the dose 2000 mg/kg body weight and could be classified as Category 5. Moreover, ABTS free radical activity of the extract was 79.2148 while that of DDPH Inhibition potential was found to be 70.72758. Our findings present substantiation that the crude extracts of *O. kilimandscharicum* is a likely source of natural antioxidants, and this justified its long-established uses.

**Keywords:** *Ocimum kilimandscharicum*, Acute Toxicity, In-vitro Antioxidant Activity DPPH, ABTS**Article Info:** Received 24 Jan 2019; Review Completed 21 March 2019; Accepted 28 March 2019; Available online 31 March 2019**Cite this article as:**

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### INTRODUCTION

Plants are a critical source of medication and assume a fundamental role in world health. The utilisation of herbaceous plant has achieved a dominant role healthcare framework all over the planet. The ratiocination behind this is a direct consequence of their better social acceptability, better concord and pliancy with the human body and lead to lesser reactions.<sup>1</sup>

Plant metabolites show a voluminous variety of chemical structures with accompanying activities that have pharmaceutical potential. In nature, these specialised secondary metabolites are involved in the interactions of plants with their environments in roles such as signalling hormones, conferring resistance against pests and diseases, attracting pollinators, and defending against pathogens or herbivores. Moreover, herbs utilised in the traditional system of medicine like Ayurveda, Traditional Chinese medicine, folk medicine etc. are obvious contenders for lead substances with novel structures, along with mechanisms of action.<sup>2,3</sup>

Natural antioxidants shield from oxidative trauma, and allied maladies, therefore, play an important role in health care. Plant foods are the primary source of natural antioxidants. Fruits and vegetables are important dietary sources of antioxidant polyphenols to humans. In nature, these specialised secondary metabolites are involved in the interactions of plants with their environments in roles such as signalling hormones, conferring resistance against pests and diseases, attracting pollinators, and defending against pathogens or herbivores.<sup>4,5</sup>

*O. kilimandscharicum* (Figure 1.) is a perennial aromatic plant, with pubescent quadrangular branchlets and stem hirsute with sessile glands, and grows up to 1m. This plant is easily identified by its shrubby habit and has attracted interest as a source of camphor. It has a green coloured slightly bitter leaves. Leaves are simple, elliptic-ovate decussate, and 3 to 4 cm in length with acute apex and serrate margin. The leaves are pubescent with white hairs on both sides. The inflorescence is dense. Fruits are nutlet, black ovoid, smooth, producing mucilage when wet.



Figure 1: kilimandscharicum plant

*O. kilimandscharicum* reported to exhibiting excellent anti-oxidant potentials. It has been known that triterpenes flavonoids and eugenol are important compounds found in leaf extract of basil. Camphor Basil is unique in possessing terpenoids while other *Ocimum* species are popular for phenylpropanoids as major components of essential oil.<sup>6,7</sup>

Methanol and aqueous extracts of *O. kilimandscharicum* inhibit HIV-1 reverse transcriptase in vitro.<sup>8</sup> Due to wholehearted demand in ethnomedicine in modern society, logical proof for the usefulness of numerous herbal remedies for various conditions is required and has been reported for a few of the phytomedicines.<sup>9,10</sup> This study, hence we explored the phytochemical compositions, acute toxicity as well as in-vitro free radical scavenging potential of *O. kilimandscharicum* plant.

## MATERIALS AND METHODS

### Plant material

*Ocimum kilimandscharicum* leaves utilised in this study was procured from the local bazaar Ballimaran, Delhi, India and was authenticated by Dr H B Singh (Scientist, NESCAIR, New Delhi). Chemical reagents

All the chemicals used in this study were obtained from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), and SD Fine-Chem. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

### Preparation of plant extracts

The crude extracts were prepared by cold maceration technique<sup>11</sup>. The extraction was done by refluxing 300 g of the drug in 2400ml of methanol (98%) and placing the mixture on an orbital shaker (at 160rpm) for 72 hrs. The mixture was concentrated in a rotary evaporator. The resulting *Ocimum kilimandscharicum* extract (OKE) was lyophilised and stored in an amber coloured airtight container at 4°C until further use.

### Preliminary Phytochemical Study

For the identification of various phytochemical constituents, the OKE extract was subjected to qualitative tests as per the standard procedure.<sup>12,13</sup>

### Selection and Procurement of animals

#### Experimental animals

For acute oral toxicology assessment, (total of Fifteen), age (6 weeks) and weight (129.1 to 140.2 gm) female Sprague Dawley (SD) pathogen-free rats were used for an acute oral toxicity test. The toxicity study was put into effect to fulfil in-toto OECD test guideline-423.<sup>14</sup> All animals were kept in the wire-bottomed cages at 25 ± 3o C temperature, 50–60%

humidity, and a 12h light-dark cycle. Rats were acclimatised to the laboratory environment for seven days ahead of the experiments. They were granted free to passage rat pellet diet (Lipton India Ltd, Mumbai, India) and water ad libitum. The bedding materials of the enclosure were changed every day. All the experimental trial was carried out in conformity with the CPCSEA guidelines. The study design was approved by the Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) (Reg No. CPCSEA Reg. No. 1283/c/09/CPCSEA). Bhopal (MP), India. Protocol approval reference no. PBRI/11/IAEC/PN-209.

### Acute toxicity

Animals were randomly divided in the group (n=3), a control group (Group A0) and dose levels (Groups A1, A2, A3 and A4, administered 50mg, 500mg, 1500mg and 2500 mg OKE / kg body weight respectively). OKE was given once by oral gavage at a weight of 10 mL/kg body weight. The control animals (A0) have been treated with distilled water in the same volume. Animals were attended for three hours after dose administration changes in behaviour. The rats were weighed, and visual observations for mortality, behavioural pattern (salivation, fur, lethargy, and sleep), changes in physical appearance, injury, pain, and any signs of illness were conducted once daily during the subsequent period of 24 and 48 hours.

### In-vitro Antioxidant Evaluation

DPPH (2,20 -diphenyl-1-picrylhydrazyl) assay was conducted according to the Brand-Williams, Cuvelier, and Berset (1995) technique with some modifications.<sup>15</sup> The stock solution was prepared by mixing 2.5 mg of DPPH radical with 100 mL of methanol. The solution absorbance was adjusted at 0.7 ± 0.02 in 515 nm using an UV-VIS Spectrophotometer (Shimadzu, Tokyo, Japan). 3.9 mL of DPPH radical were placed in a test tube and 100 µL of the sample extract or standard were added (methanol was used as blank). The decrease in absorbance at 515 nm was measured at 1 min intervals for the first 10 min, and then at 5 min intervals until stabilization. Calibration curve was prepared using ascorbic acid as standards and results (AOC) are expressed as ascorbic acid equivalents in mg/100 g of fresh weight. All measurements were done in triplicate.

The ABTS (2,20 -Azinobis-3-ethylbenzotiazoline-6-sulfonic acid) assay was conducted according to Miller, Rice-Evans, Davies, Gopinathan, and Milner (1993).<sup>16</sup> ABTS+ cation was generated through the interaction of 19.2 mg of ABTS dissolved in 5 mL of HPLC-grade water and 88 µL of 0.0378 g/mL potassium persulfate (K2S2O8) The cation was incubated in the dark at room temperature for 16 h. The ABTS activated radical was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. After the addition of 30 µL of sample or standard to 2970 µL of diluted ABTS solution, absorbances were recorded 6 min after mixing. Calibration curve was prepared using ascorbic acid as a standard and results (AOC) are expressed as ascorbic acid equivalents in mg/100 g of fresh weight. All measurements were done in triplicate.

### Statistical analysis

The results were expressed as mean ± SEM (n = 5). Data acquired as of hematology analysis, serum biochemistry, as well as the body and organ weight measurements were articulated as mean + SD and evaluated by unpaired t test (2-tailed P value) to compare the mean of the treated group with the control. Data were analysed using Student's t-test, and results were considered significant when p < 0.05.

Table 1: Phytochemical Analysis of *O. kilimandscharicum* extract (OKE)

S. No.	Phytochemical	Result
	Alkaloids	+
	Amino acid	++
	Carbohydrate	+
	Coumarin glycosides	-
	Flavanoids & phenols	+++
	Fats & Oils	-
	Glycosides	+
	Protein	+
	Saponin	+
	Steroids	++
	Tannin	++

Table 2: General appearance and behavioural observations for control and treated groups in Acute toxicity study of DCE (n = 3)

Observation	3 Hours					24 hours					48 hours				
	A0	A1	A2	A3	A4	A0	A1	A2	A3	A4	A0	A1	A2	A3	A4
Behavioural patterns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Coma	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Diarrhea	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Eyes	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Lethargy	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Mucous	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Salivation	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Skin and fur	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Sleep	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Tremors	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Dosing Groups A0=Control  
A4=2000mg/kg group

group A1=5mg/kg group  
\*= Normal

A2=50mg/kg group  
--= not normal

A3=300mg/kg group

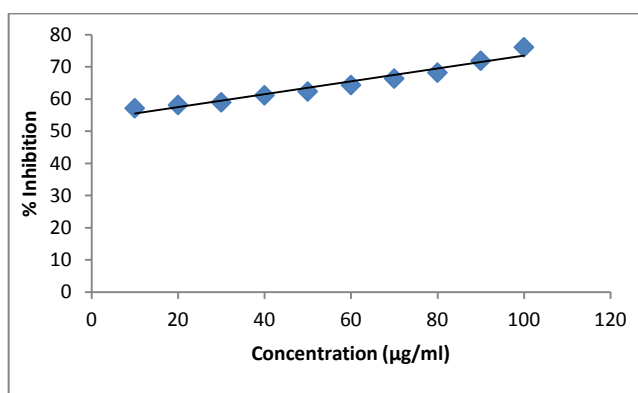
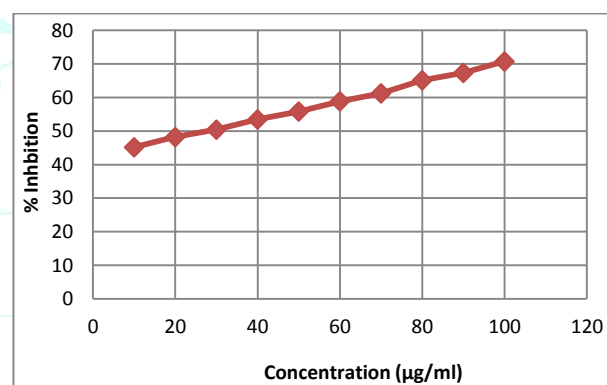
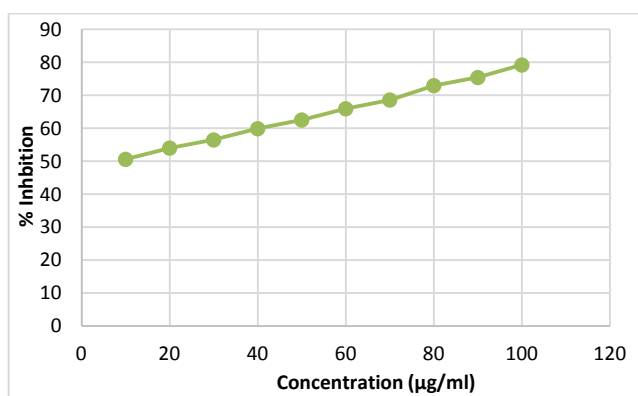


Figure 2: Standard curve of Ascorbic acid

Figure 4: DPPH Inhibition Potential of *O. kilimandscharicum* ExtractFigure 3: ABTS Inhibition Potential of *O. kilimandscharicum* Extract

## RESULTS

### Preliminary Phytochemical Analysis

The results of the study showed a number of secondary metabolites (Table 1). It was observed that extracts of *O. kilimandscharicum* contained a significant concentration of secondary metabolites like Saponins, Flavonoids, Glycosides, Phytosterol, Tannins. The phenolic compounds found in fruits and vegetables have attracted much interest due to their potential as antioxidants.<sup>17,18</sup>

### Acute Toxicity Profile:

Safety evaluation of plant extracts used in folk medicine has generated considerable interest in the scientific community in an attempt to identify those that can potentially cause toxicity to humans.<sup>19</sup> In this context, the objective of the present study was to investigate the acute of extract of extracts of *O. kilimandscharicum*. Toxic signs and the

severity, onset, progression and reversibility of the signs have been observed and recorded in relation to dose and time. The animals were observed continuously for 24 to 48 hr after dosing. The results of the acute toxicity are shown in Table 2. Neither mortality nor any abnormal clinical signs were observed in rats treated at the dose level of 2500 mg kg<sup>-1</sup> body weight in the studies.

#### Antioxidant Studies:

The antioxidant property of plant products has to design by combining two or more different in vitro test to get suitable data, because of the multifarious character of phytochemicals. Each of these tests is based on one feature of the antioxidant activity, such as the ability to scavenge free radicals, or the metal ion chelation. The ABTS method is generally indicated for evaluating the antioxidant activity of hydrophilic compounds and the DPPH method is commonly used for aqueous/organic extracts with hydrophilic and lipophilic compounds (Rufino et al., 2010).<sup>20</sup> VCEAC is described as the antioxidant capacity equivalent to vitamin C concentration (mg/L). The antioxidant capacity of vitamin C was designated at a value of 100 mg/L. The higher the VCEAC value of the test compound, the more effective the antioxidant. VCEAC value greater than 100 indicates that the corresponding compound is a more effective antioxidant than vitamin C.<sup>21</sup> Figures 2, 3 and 4 we give the values of free radical scavenging potential of *O. kilimandscharicum* extract with reference to the standard antioxidant. VCEAC values obtained in this work for OKE with both methods (DPPH and ABTS) overlap. A high positive correlation was obtained between the two assays (ABTS and DPPH) used to measure antioxidant activity of OKE under study ( $R = 0.82$ ,  $P < 0.05$ ), indicating that the extract demonstrate comparable activities in both assays. However, it is important to note that the DPPH assay presented lower values of antioxidant activity (approximately 50%) in comparison with the ABTS assay; this result concurs with other studies carried out by Almeida et al. (2011)<sup>17</sup>

#### CONCLUSION

These findings suggest that OKE is comparatively safe and does not possess acute untoward effects. Further antioxidant activity for all assay indicate that extracts of this plant scavenged free radicals in a dose-dependent manner. These findings are also supported by earlier reports that plant metabolites such as flavonoids, tannins, catechins and other phenolic compounds possess antioxidant activity.<sup>22</sup> Nevertheless, data collected during this study is insufficient to reach at a definite conclusion since trial in humans is required to establish safety and efficacy of any drug.

**CONFLICT OF INTEREST STATEMENT:** We declare that we have no conflict of interest.

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