International Journal of Pharmacological Research
ISSN: 2277-3312 (Online)
Journal DOI: https://doi.org/10.7439/ijpr **Research Article**

Comparative antioxidant, cytotoxic & analgesic studies of two varieties of *Ocimum gratissimum*

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Abstract

Ocimum gratissimum is an aromatic, perennial herb, 1-3 m tall; stem erect, round-quadrangular, much branched, glabrous or pubescent, woodrat the base, often with epidermis peeling in strips. Leaves opposite; petiole 2-4.5 cm long, slender, pubescent; blade elliptical to ovate, 1.5-16 cm x 1-8.5 cm, membranaceous, sometimes glandular punctate, base cuneate, entire, margin elsewhere coarsely crenate-serrate, apex acute, puberulent or pubescent. Inflorescence a verticillaster, arranged in a terminal, simple or branched raceme 5-30 cm long; rachis lax, softly pubescent; bracts sessile, ovate, 3-12 mm x 1-7 mm, acuminate, caducous; pedicel 1-4 mm long, spreading or ascending, slightly curved; flowers in 6-10-flowered verticillate, small, hermaphrodite; calyx 2-lipped, 2-3 mm long, in fruit 5-6 mm, pubescent, upper lip rounded and recurved, reflexed in fruit, lower lip with4, narrow, pointed teeth, central pair of teeth minute and much shorter than the upper lip; corolla campanulate, 3.5-5 mm long, 2-lipped, greenish-white, pubescent outside, upper lip truncate, 4-fid, lower lip longer declinate, flat, entire; stamens 4, declinate, in 2 pairs, inserted on the corolla tube, filaments distinctly exerted, upper pair with a bearded too that the base; ovary superior, consisting of 2 carpels, each 2-celled, style 2-fid.Fruit consisting of 4, dry, 1-seeded nutlets enclosed in the persistent calyx (the lower lip closing the mouth of the fruiting calyx); nutlet subglobose,1.5 mm long, rugose, brown; outer pericarp not becoming mucilaginous in water.

Keywords: Phytochemical Screening, Cytotoxicity determination, Antioxidant Activity Evaluation, Analgesic Activity.

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***Article History:**
Received: 28/11/2019
Revised: 30/12/2019
Accepted: 03/01/2020
DOI: https://doi.org/10.7439/ijpr.v10i1.5309

QR Code

How to cite: Azam S. Comparative antioxidant, cytotoxic & analgesic studies of two varieties of *Ocimum gratissimum*. *International Journal of Pharmacological Research* 2020; 10(01): e5309. Doi: 10.7439/ijpr.v10i1.5309
Available from: https://ssjournals.com/index.php/ijpr/article/view/5309

1. Introduction

Herbal medicines involve the integration of several therapeutic experiences and practice of indigenous system of medicine that may span many previous generations, which often provide valuable guidelines to the selection, preparation and application of herbal formulation with a view to providing therapeutic benefits. Treatment of illness and maintenance of health using herbal medicines is the oldest and most popular form of healthcare practice known to humanity that has been practiced by all ages throughout the history of civilization.

Medicinal plants have been used since ancient times for the treatment and management of diabetic mellitus (DM) in traditional medicine systems of many cultures throughout the world. Recently, the World Health Organization (WHO) recommended the use medicinal plants for the management of diabetic mellitus and further encouraged the expansion of the frontiers of scientific evaluation of the hypoglycemic activities of vast number of plants products have been evaluated and confirmed in animal models as well as human beings. In some cases, the bioactive principles of the medicinal plants have been isolated and identified. In developing countries, the use of anti-diabetic herbal remedies has been on the decline since the introduction of insulin and synthetic oral hypoglycemic drugs during the early part of 20th century.

However, recently in the developed countries, there has been the resurgence of interest in medicinal plants that exhibit hypoglycemic property. The renew interest in herbal antidiabetic remedies in developed countries to be

motivated by several factors that include adverse reaction, secondary failure rates and cost of conventional remedies. Recently, the World Health Organization (WHO) recommended the use of medicinal plants for the management of DM and further encouraged the expansion of the frontiers of scientific evaluation of hypoglycemic properties of diverse plant species. Consequently, current estimates showed that over 70% of the global population applies resources derived from traditional medicine for the management and alleviation of DM and its complication. Ethno-pharmacological surveys indicate that more than 1200 plants are used in traditional medicine system following claims of their hypoglycemic properties. The hypoglycemic activity of a large number of plants products have been evaluated and confirmed in animal models as well as in human beings. In some cases, the bioactive principle of the medicinal plants has been isolated and identified. Nevertheless, the mechanisms of action of most of these antidiabetic principles are not well defined and remain largely speculative. However, reports suggest that the array of antidiabetic bioactive principles in medicinal plants may act in synergy to exert glycemic control through interference with one or more process involved in glucose metabolism and metastasis. *Ocimum gratissimum* (**Family:** Lamiaceae) is an aromatic, perennial herb found in along lake shores, in savannah vegetation, in sub montane forest, and disturbed land at elevations from sea level.

Aim of this article briefly focuses on the potential phytochemicals and pharmacological activity of *Ocimum gratissimum*. Various parts of plant including seeds, bark, leaves and fruits had been studied and investigated for various pharmacological properties. The plant has been reported for significant antioxidant, analgesic and others properties. The plant is very well known for their pharmacological properties science ancient age. [01]

2. Materials and Method

2.1 Collection and Identification of plant

The whole plant of two varieties of *Ocimum gratissimum* was collected from Gazipur, Bangladesh at first with the help of a comprehensive literature review *Ocimum gratissimum* from Lamiaceae family was selected for the comparison. The whole plants were collected from Gazipur, in 2019 and identified by the taxonomist of the National Herbarium of Bangladesh, Mirpur and Dhaka. The voucher specimens of the plants have been deposited in the herbarium.

2.2 Drying and grinding

The leaf of the plants was taken and selected to be studied. They were first shed dried for several weeks, crushed and dried again. Then the crushed parts of the plants were reduced coarse powder with the help of a mechanical grinder. The powders of the plant were stored into an airtight container and kept in a cool, dark and dry place until analysis commenced.

2.3 Extraction of the dried powdered sample

The powered plant material (500 mg) was used for extraction by Soxhlet apparatus at elevated temperature using methanol consecutively (2L of solvent). After each extraction the plant material was dried and use again for the next extraction. Extraction was considered to be complete when the plant material becomes exhausted of their constituents that were confirmed from cycles of colorless liquid siphoning in the Soxhlet apparatus. Extracts of each part were filtered individually through fresh cotton bed and then by Whatman filter paper. The filtrates obtained were dried at reduced pressure using rotatory evaporator to have gummy concentrate of the crude extracts. Each extract as kept in suitable container with proper labelling and stored in cold and dry place. Percentage yield of extracts of green tulsi and red tulsi is 3 % and 2.7 % respectively.

2.5 Phytochemical Screening:

A preliminary phytochemical screening of methanol extract of *Ocimum gratissimum* as carried out. The crude plant extracts were subjected to different qualitative tests to find out the presence of chemical constituents. These were identified by characteristic colour changes using standard procedure.

2.6 Brine Shrimp Lethality Bioassay

Cytotoxicity determined by Brine Shrimp lethality bioassay described by Meyer et al. Brine Shrimp lethality bioassay is a rapid and comprehensive bioassay for the bioactive compounds of natural and synthetic origin. By this method, natural product extracts, fractions as well as the pure compounds can be tested for their bioactivity. The method utilizes *in vivo* lethality in a simple zoological organism (*Brine nauplii*) as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products.

The Brine Shrimp assay has advantages of being rapid (24 hours), inexpensive, and simple (e.g., no aseptic techniques are required). It easily utilizes a large number of organisms for statistical validation and requires no special equipment and a relatively small amount of sample (2-20 mg or less). Furthermore, it does not require animal serum as is needed for cytotoxicity's.

2.6.1 Preparation of sea water

38 gm sea salt (without iodine) was weighed, dissolved in one litre of distilled water and filtered off to get clear solution.

2.6.2 Hatching of Brine Shrimp

Artemia salina leach (brine shrimp eggs) collected from pet shops was used as the test organism. Seawater was taken in the small tank and shrimp eggs were added to one

side of the tank and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through the hatching time. The hatched shrimps are attracted to the light (phototaxis) and so nauplii free from egg shell was collected from the illuminated part of the tank. The nauplii was taken from the fish tank by a pipette and diluted in fresh clear sea water to increase visibility and 10 nauplii were taken carefully by micropipette.

2.6.3 Preparation of test solutions with samples of experimental plants

32 mg of each of the test samples were taken and dissolved in 200µl of pure dimethyl sulfoxide (DMSO) and finally the volume was made to 20 ml with sea water. Thus, the concentration of the stock solution was 1600µg/ml. Then the solution was serial diluted to 800, 400, 200, 100, 50, 25, 12.5, 6.25 µg/ml with sea water. Then 2.5 ml of plant extract solution was added to 2.5 ml of sea water containing 10 nauplii.

2.6.4 Preparation of control group

Control groups were used in cytotoxicity study to validate the test method and ensure that the results obtained were only due to the activity of the test agent and the effects of the other possible factors were nullified. Two types of control groups were used: Positive control and Negative control.

2.6.5 Preparation of the positive control group

Positive control in cytotoxicity study is a widely accepted cytotoxic agent and the result of the test agent is compared with the result obtained for the positive control. In the present study vincristine sulfate was used. As vincristine is a very cytotoxic alkaloid.

2.6.6 Preparation of the negative control group

50 µl of DMSO was added to each of three premarket test tubes containing 4.95 ml of simulated sea water and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds.

2.6.7 Counting of nauplii

After 24 hours, the test tube was inspected using a magnifying glass against a black background and the number of survived nauplii in each tube was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The mortality was corrected using Abott's formula

$$P_t = [(P_o - P_c)/ (100 - P_c)] \times 100$$

Where, P_o= Observed mortality

P_c= Control mortality

The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}). This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure time and determined by linear regression method from plotting % mortality against correspondent log of concentration.

2.7 Antioxidant Activity Evaluation

2.7.1 Determination of Total Phenolic content [02]

The content of total phenolic compounds in plant methanolic extracts was determined as described previously using the Folin-Ciocalteu Reagent (FCR). The Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent or Folin-Denis reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants. It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent.

However, this reagent does not only measure total phenols and will react with any reducing substance. The reagent therefore measures the total reducing capacity of a sample, not just the level of phenolic compounds, Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo(VI):

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

2.7.1.1 Preparation of 7.5% Sodium carbonate solution

7.5 gm of Na_2CO_3 was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

2.7.1.2 Preparation of Standard solution

The stock solution was prepared by taking 0.025 gm of galic acid and dissolved into 5 ml of Absolute Ethanol. The concentration of this solution was 5µg/µl of galic acid.

2.7.1.3 Preparation of Extract solution

0.025 gm of each plant extracts were dissolved into 5 ml of Ethanol to make the concentration of each solution 5µg/µl of plant extract. These solutions were considered as stock solutions.

2.7.1.4 Experimental Procedure

1) 1.0 ml of plant extract (200 µg/ml) or standard of different concentration solution was taken in a test tube.
2) 5 ml of Folin-Ciocalteu (Diluted 10-fold) reagent solution was added to the test tube.
3) 7.5% Sodium carbonate solution (4 ml) was added to the same test tube and mixed well.
4) Test tubes containing standard solutions were incubated for 30 minutes at 20°C to complete the reaction but the test tubes containing extract solution were incubated for 1 hour at 20°C to complete the reaction.

5) Then the absorbance of the solution was measured at 765 nm using a spectrophotometer against blank.
6) A typical blank solution contained the solvent used to dissolve the plant extract.
7) The Total content of phenolic compounds plant extracts of Gallic acid equivalents (GAE) was calculated using the following equation:

C = (c x V)/m, Where;

C = total content of phenolic compounds, mg/gm plant extract, in GAE

c = the concentration of gallic acid established from the calibration curve (mg/ml)

V = the volume of extract in ml

m = the weight of crude plant extract in gm

2.7.2 Determination of Total Flavonoids Content

Total flavonoid was determined using the Aluminum chloride colorimetric method described by Wang and Jiao (2000). The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids, In preliminary experiments, the wavelength scans of the complexes of 15 standards with aluminum chloride showed that the complexes formed by flavonols with C-3 and C-5 hydroxyl groups, such as galangin, morin and kaempferol, as well as those with extra ortho-dihydroxyl groups, such as rutin, quercetin, quercitrin and myricetin, had maximum absorbance at 415-440 nm[03]. However, the λ_{max} of the complexes formed by chrysin and apigenin which have only the C-5 hydroxyl and C-4 keto groups were at 395 and 385 nm, respectively. Another flavone compound investigated, luteolin, which has the C-5 hydroxyl group and the ortho-dihydroxyl groups in B ring formed a complex that showed a strong absorption at 415 nm. In compromise, therefore, the wavelength 415 nm is chosen for absorbance measurement.

2.7.2.1 Preparation of 10% Aluminium chloride ($AlCl_3$) solution

10 gm of $AlCl_3$ was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

2.7.2.2 Preparation of 1M Potassium acetate solution

9.815 gm of potassium was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

2.7.2.3 Preparation of Standard solution

Quercetin stock solution of concentration 5µg/µl was prepared by dissolving 0.025 gm of quercetin into 5 ml of ethanol.

2.7.2.4 Preparation of Extract solution

0.025 gm of each plant extracts were dissolved into 5 ml of Ethanol to make the concentration of each solution 5µg/µl of plant extract. These solutions were considered as stock solutions.

2.7.2.5 Experimental Procedure

1) 1.0 ml of plant extract (200 µg/ml) or standard of different concentration solution was taken in a test tube.
2) 3 ml of methanol was added to the test tube.
3) Then 200µl of 10% aluminium chloride solution was added into the same test tube.
4) Followed by the addition of 200 µl of 1M potassium acetate solution into the test tube.
5) Finally, 5.6 ml of distilled water was mixed with the reaction mixture
6) The reaction mixture then Incubated for 30 minutes at room temperature to complete the reaction.
7) Then the absorbance of the solution was measured at 415 nm using a spectra photometer against blank.
8) Methanol served as blank.
9) The Total content of flavonoid compounds in plant methanol extracts in quercetin equivalents was calculated by the following formula equation

C = (c x V)/m, where;

C = total content of flavonoid compounds, mg/gm plant extract, in quercetin equivalent

c = the concentration of quercetin established from the calibration curve in mg/ml

V = the volume of extract in ml

m = the weight of crude plant extract in gm

2.7.3 Analgesic Activity

2.7.3.1 Experimental Animals

For the experiment Swiss albino mice of either sex, 6-7 weeks of age, weighing between 25-30g, were collected from the animal research lab in the department of pharmacy Jahangirnagar University, Savar, Dhaka. Animals were maintained under standard environmental conditions (temperature: 27.0±1.0°, relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

2.7.3.2 Acute Toxicity Study

Acute toxicity describes the adverse effects of a substance which result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). The acute toxicity study was conducted to find out LC_{50} of the test samples. The test samples were administered orally to the test animals at different concentrations (100, 250, 500, 1000, 2000, 3000 and 4000 mg/kg body weight). After administration of the extract

solutions mortality or sign of any toxicity was observed for 1 hour. Then the test animals were observed every 1 hour for next 5-6 hours. The animals were kept under observation for 1 week [04].

2.7.3.3 Analgesic Activity:

Pain is an unpleasant feeling often caused by intense or damaging stimuli. The international Association for the study of Pain's widely used definition states: '' Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is the most common reason for physician consultation in most advanced countries such as the United States. It is a symptom in many medical conditions, and can significantly interfere with a person's quality of life and general functioning. Nociceptive pain is cost by stimulation of peripheral nerve fiver that respond only to stimuli approaching or exceeding harmful intensity and may be classified according to the mood of moxious stimulation. The most of the common categories being '' thermal '' (e.g. heat or cold), '' mechanical'' (e.g. crushing, shearing, etc.) and '' chemical '' (e.g. iodine in a cut, chili powder in the eyes). It is also the protective attempt by the organism to the injurious stimuli as well as initiate healing process for the tissue and considers being the major cause of rheumatoid arthritis. Analgesics relive symptoms of pain, but hardly affect it is underlying causes.

Currently available analgesic drugs such as opiates and NSAIDs are not useful in on cases due to they are adverse effects. Scientific and methodical investigation of herbal plants has become a potential source for the discovery of lead compounds of high therapeutic value in terms of analgesic activities. Ethno- the pharmacological studies have become increasingly in valuable in the development of modalities for the management of pain and related disorders as. Thus, green pharmaceuticals have now received considerable attention and popularity in this area due to its availability, less side effect and economic feasibility compared to the orthodox medicine.

2.7.3.4 Analgesic Activity by Hot Plate Method:

The analgesic activity of *Ocimum gratissimum* was assessed using hot plate method of Eddy and Leimbach (1953). The temperature was maintained at 55± 0.2C. Animals licked their limbs mouse were divide into three different groups, five in each group. These mice were treated as follows; control group received normal water.

The test group received 250, 375 &500 mg / kg of *Ocimum gratissimum* the standard group received Diclofenac 100 mg / kg by the oral route. Half hour after dosing group specific drugs, mice were placed on the hot plate and the time until both licking and jumping occurs was recorded by a stop watch. The latency period was recorded of 0min, 60min, 120min,180min, following oral administration a reading was taken which is considered as blank reading and its helpful to differentiate between normal latency time and latency time after drug administration. The cut off time of 12 second was employed for hot plate test

2.8 Statistical analysis:

Results are presented as mean ±SEM statistical analysis for the experiments was carried out using two-way ANOVA followed by tukey test. The results obtained were compared with the control group p values <0.05 considered to be statistically significant.

3. Result & discussion

3.1 Phytochemical Screening

Preliminary phytochemical screening of the crude extracts of whole plant *Ocimum gratissimum Schott* revealed the presence of different kind of chemical groups that are summarized in Table 1.

Table 1: Result of phytochemical screening of various plant extracts of *Ocimum gratissimum* (Green tulsi).

Constituent	Green tulsi	Red tulsi
Carbohydrate	+	+
Flavonoid	+	+
Glycoside	+	+
Steroids	_	_
Saponin	+	_
Tanins test	+	+
Alkaloids test	+	+

N: B (+) sign means present and (-) sign means absence

Different crude extracts of *Ocimum gratissimum* have been shown to possess phytoconstituents including carbohydrates (Monosaccharides, reducing and mixed-reducing sugars), alkaloid, glycosides, flavonoid, tannins and saponin. No steroid was detected. These phytoconstituents present in the extracts may account for their various pharmacological activities shown in other investigations.[06]

3.2 Assessment of antioxidant potential (*in-vitro*)

3.2.1 Total Phenol Content Determination

Total phenolic content of the crude extracts of *Ocimum gratissimum* was determined by using the Folin-Ciocalteu reagent and were expressed as Gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents of the test fractions were calculated using the standard curve of Gallic acid ($y= 0.0147x + 0.1275$; $R^2 = 0.9881$).

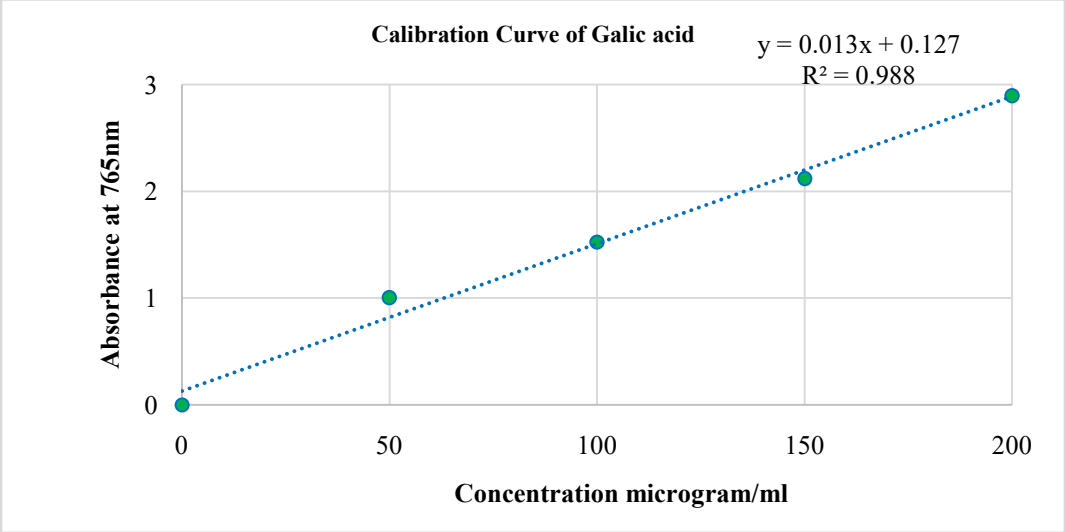

Figure 1: Calibration Curve of Gallic Acid

Table 2: Total phenol contents of the Methanol extracts of *Ocimum gratissimum*

Extract	Total phenol content (mg Gallic acid equivalents/gm Extract)
green tulsi	4.35
red tulsi	5.10

Phenolics, ubiquitous to the plant kingdom, are composed of several classes of compounds including flavonoids (flavones, isoflavones, and flavanones), anthocyanin and catechins. They possess an ideal structural chemistry for free radical scavenging activity. Anti-oxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors which can stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction)[07]**.** Oxidation reactions have been reported to play a central role in atherogenesis and epidemiological studies have shown an association between cardiovascular diseases and low plasma concentrations of ascorbate, tocopherol and β-carotene[08]. Furthermore, there is an inverse correlation between quercetin intake and plasma LDL cholesterol concentration.[09] Polyphenols have been shown to block LDL oxidation, decrease the formation of atherosclerotic plaques and reduce arterial stiffness, leaving arteries more responsive to endogenous stimuli of vasodilation.[10] Moreover, polyphenols have been shown to exert anti-carcinogenic effects by modulating enzyme systems that metabolize carcinogens or pro-carcinogens to genotoxins by converting them to less reactive compounds before they react with DNA. Polyphenols have been shown to inhibit the Cytochrome P_{450} superfamily of enzymes that metabolizes many pro-carcinogens to reactive compounds before they react with DNA and induce malignant transformation, thus reducing the formation of reactive intermediates.[11]Glutathione reductase activity in rats has also been shown to be induced by certain polyphenols (quercetin, flavones, flavanones and tangerine). An induction of this enzyme is generally considered to reflect an increase in cellular protection, ensuring that potential toxins are conjugated and excreted more rapidly from the body. In addition, they have been shown to inhibit lipoxygenase and cyclogeneses activity leading to lower aggregation of platelets and a reduction of thrombotic tendency.[12] This ability is believed to be mainly due to their redox properties,[13] which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. The results strongly suggest that phenolics are important components of the tested plant extracts. Among the extracts showed maximum potency in phenolic content determination assay and although a crude extract it possesses considerable amount of gallic acid equivalent substances (polyphenolic compounds).

3.2.2 Total flavonoid contain test

Total flavonoid content of the crude extracts of *Ocimum gratissimum* was determined by using the Aluminum chloride colorimetric method described by Wang and Jiao (2000) and were expressed as Quercetin equivalents per gram of plant extract. The total flavonoid contents of the test fractions were calculated using the standard curve of Gallic acid (y= 0.009x - 0.0364; R^2 = 0.9724).

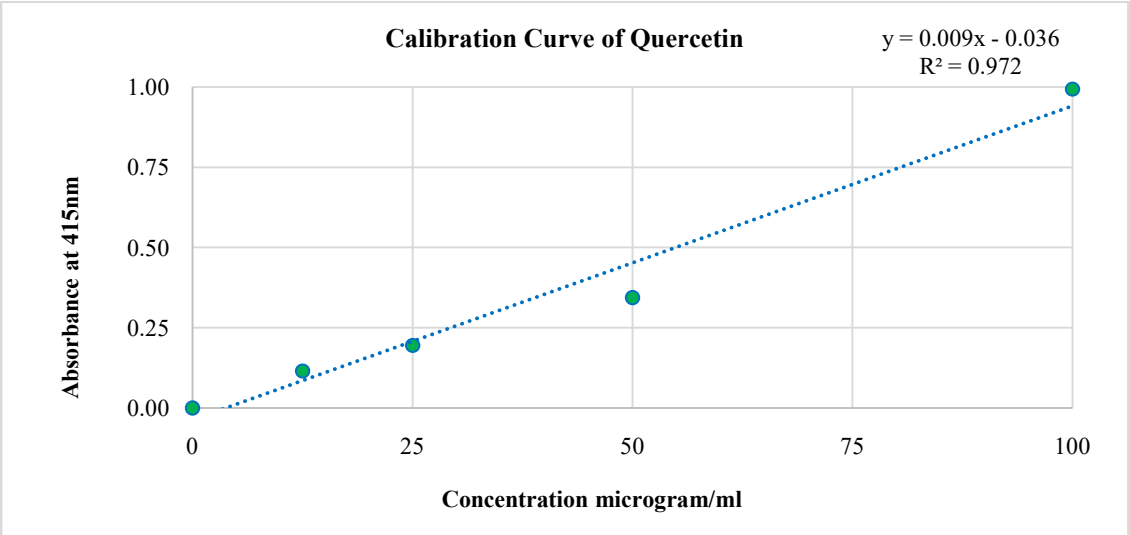

Figure 3: Calibration Curve of Quercetin

Table 3: Total flavonoid contents of the Methanol extracts of *Ocimum gratissimum*

Extract	Total flavonid content (mg Gallic acid equivalents/gm Extract)
green tulsi	8.31
red tulsi	7.69

3.3 Acute Toxicity Study

Acute toxicity describes the adverse effects of a substance which result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). The acute toxicity study was conducted to find out LC_{50} of the test samples. The test samples were administered orally to the test animals at different concentrations (100, 250, 500, 1000, 2000, 3000 and 4000 mg/kg body weight). After administration of the extract solutions mortality or sign of any toxicity was observed for 1 hour. Then the test animals were observed every 1 hour for next 5-6 hours. The animals were kept under observation for 1 week.

3.4 Result of Brine Shrimp Lethality Bioassay for Cytotoxic Activity

The methanolic extract of *Ocimum gratissimum* (Green tulsi) Found to be considerably lethal to brine shrimp nauplii, with **LC_{50} value 359.904 µg/ml** & The methanolic extract of *Ocimum gratissimum* (Red tulsi). Found to be considerably lethal to brine shrimp nauplii, with **LC_{50} value 507.56 µg/ml** where's anticancer drug vincristine sulfate showed **LC_{50} value 3.65 µg/ml**

Table 4: Data for Brine Shrimp lethality bioassay for cytotoxic activity of Vincristine sulphate & Data for Brine Shrimp lethality bioassay for cytotoxic activity of *Ocimum gratissimum*

Conc. (µg/ml)	No. of nauplii taken (N_0)	No. of nauplii dead	No. of nauplii alive (N_1)	% Mortality $\%M = \frac{N0-N1}{N0} \times 100$	LC_{50} (µg/ml)
0.079	10	1	9	10	
0.157	10	2	8	20	
0.313	10	2	8	20	
0.625	10	3	7	30	3.65
1.25	10	3	7	30	
2.5	10	4	6	40	
5	10	7	3	70	
10	10	10	0	100	

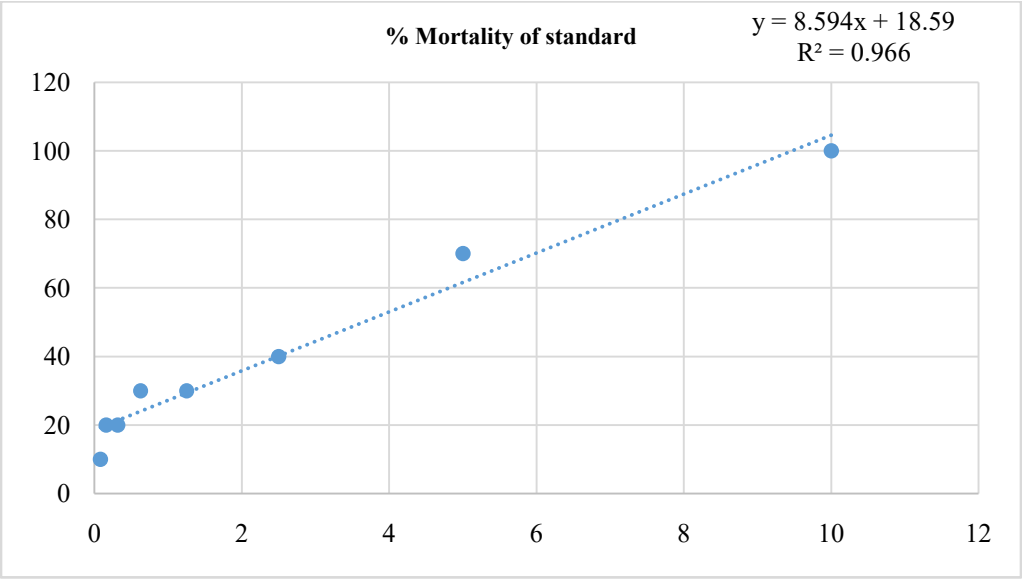

Figure 4: Calculation curve for % mortality vs concentration of Vincristine sulfate

Table 5: Data for Brine Shrimp lethality bioassay for cytotoxic activity of Vincristine sulphate & Data for Brine Shrimp lethality bioassay for cytotoxic activity of *Ocimum gratissimum*

Conc. (µg/ml)	No. of nauplii taken (N_0)	No. of nauplii dead (green tulsi)	No. of nauplii dead (red tulsi)	No. of nauplii alive (N_1) (green tulsi)	No. of nauplii alive (N_2) (red tulsi)	% Mortality of green tulsi % M = $\frac{N0-N1}{N0}\times100$	% Mortality of red tulsi % M = $\frac{N0-N2}{N0}\times100$	LC_{50} of green tulsi (µg/ml)	LC_{50} of red tulsi (µg/ml)
6.25	10	1	2	9	8	10	20	359.904	507.56
12.5	10	2	3	8	7	20	30		
25	10	3	3	7	7	30	30		
50	10	4	3	6	7	40	30		
100	10	4	3	6	7	40	30		
200	10	5	4	5	6	50	40		
400	10	6	5	4	5	60	50		
800	10	7	6	3	4	70	60		

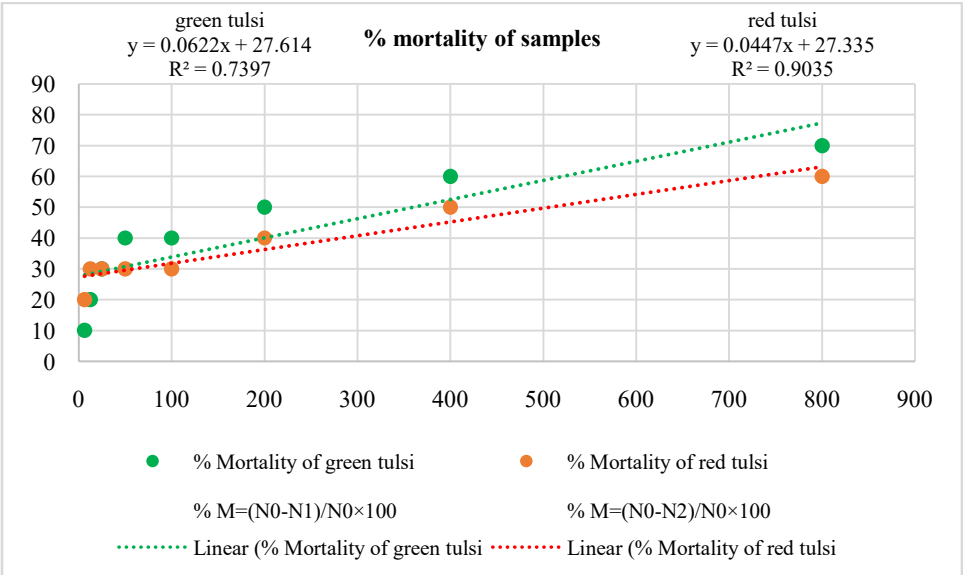

Figure 5: Calibration Curve for % mortality vs concentration of *Ocimum gratissimum*

Table 6: Result of *Ocimum gratissimum* Brine Shrimp lethality bioassay

Sample/Standard	LC_{50}(µg/ml)
ME of *Ocimum gratissimum* (Green tulsi).	359.904
ME of *Ocimum gratissimum* (Green tulsi).	507.56
Vincristine sulphate (standard)	3.65

3.5 Analgesic Activity

The positive control (Vincristine sulfate) showed expected cytotoxicity with LC_{50} value of µg/mL. Although the extract wasn't as Vincristine sulfate, the LC_{50} value of the extract is well below 1000 μg/mL, which make them promising candidates for sophisticated anti-cancer study. [14]

3.5.1 Analgesic Activity by Hot Plate Method

In hot plate analgesic test, diclofenac used as standard and showed statistically significant result increase in the reaction time.[15]

Table7: Effect of methanolic extract of plant *Ocimum gratissimum* (Green tulsi& Red tulsi) latency period in hot plate test (250mg/kg)

Group	0 min	60 min	120 min	180 min
Control	11.88	10.58	8.32	5.36
standard	15.42	17.9	14.72	12.2
Sample(green tulsi)	4.86	7.68	6.38	7.88
Sample (red tulsi)	7.12	10.68	10.9	7.82

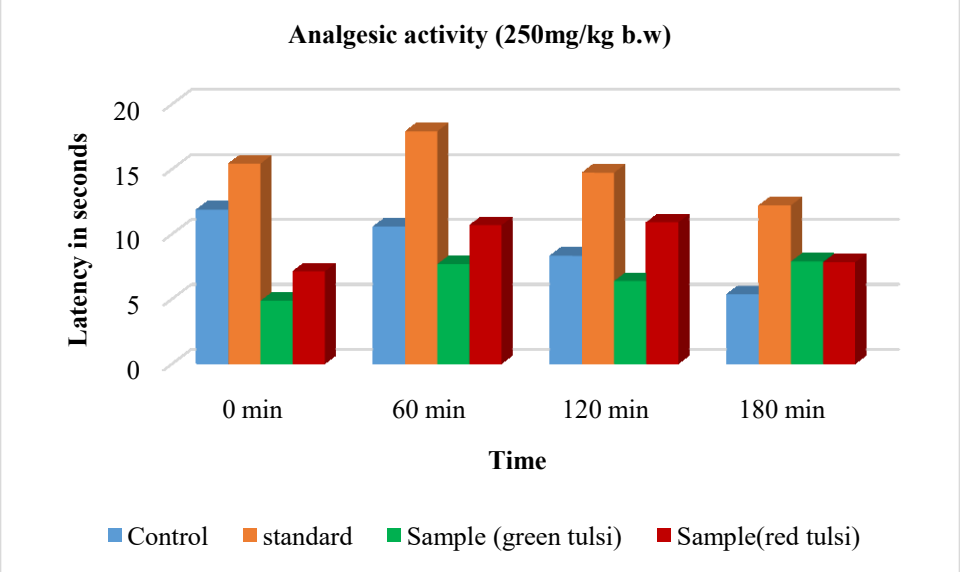

Figure 6: Analgesic effect of methanolic extract of plant *Ocimum gratissimum* (Green tulsi& Red tulsi) (250mg/kg) hot plate test

Table 8: Effect of methanolic extract of plant *Ocimum gratissimum* (Green tulsi& Red tulsi) latency period in hot plate test (375mg/kg)

Group	0 min	60 min	120 min	180 min
Control	11.88	10.58	8.32	5.36
standard	15.42	17.9	14.72	12.2
Sample (green tulsi)	7.24	10.6	8.88	6.78
Sample (red tulsi)	5.78	9.1	9.9	7.46

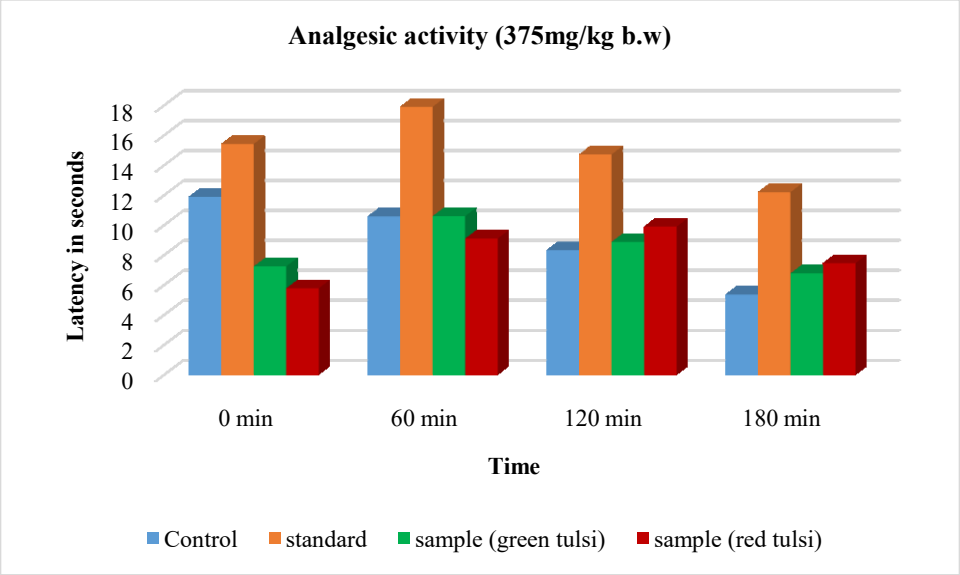

Figure 7: Analgesic effect of methanolic extract of plant *Ocimum gratissimum* (Green tulsi & Red tulsi) (375mg/kg) hot plate test

Table 9: Effect of methanolic extract of plant *Ocimum gratissimum* (Green tulsi) latency period in hot plate test (500mg/kg)

Group	0 min	60 min	120 min	180 min
Control	11.88	10.58	8.32	5.36
standard	15.42	17.9	14.72	12.2
Sample (green tulsi)	9.98	11.68	8.78	9.84
Sample (red tulsi)	5.3	9.8	6.88	6.4

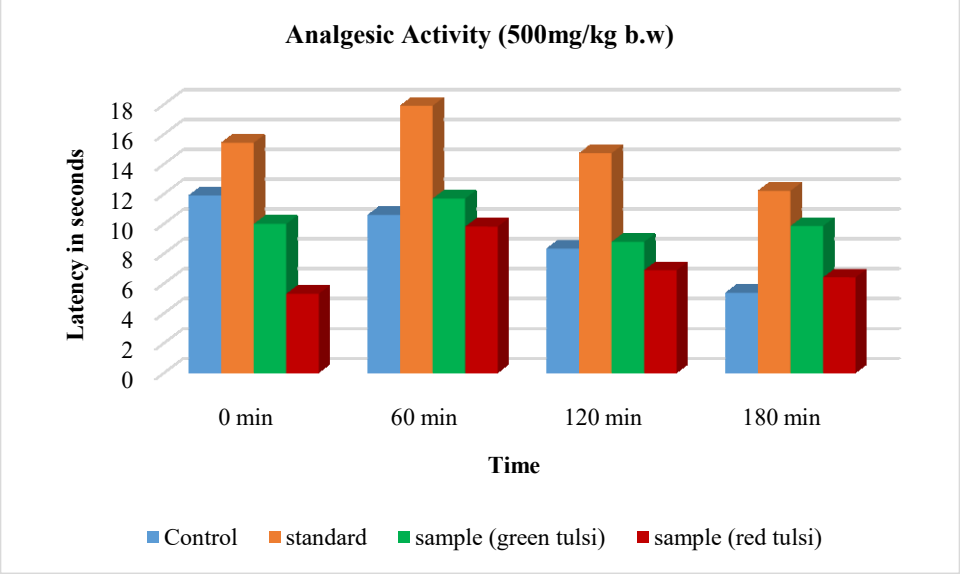

Figure 8: Analgesic effect of methanolic extract of plant *Ocimum gratissimum* (Green tulsi& Red tulsi) (500mg/kg) hot plate test

Ocimum gratissimum was subject to hotplate test in mice and elicited the response depicted by the figures. The figures show the latency time change in different time interval. At one hour's standard showed highly significant ($P<0.01$) increase in a latency time.

4. Conclusion

Two varieties of *Ocimum gratissimum* (Green tulsi& Red tulsi)were subjected to vigorous Antioxidant, Cytotoxic and Analgesic to compare therapeutic activities between them. The phytochemical screening revealed

chemical constituents that form the foundation of their pharmacological activity. The leaf part of both of the plant exhibited potential antioxidant and analgesic activity. This part of *Ocimum gratissimum* (Green tulsi & Red tulsi) has a reputation of being antioxidant which has been substantiated by oxidative effect. And there is no notable different between them.

5. Future Direction

All the conducted experiments in the present study are based on crude extract and are considered to be preliminary and more sophisticate research is necessary to reach a concrete conclusion about the findings of the present study. Elaborate phytochemical investigation must be arranged that might lead to isolation and characterization of chemical constituents present in the crude extracts. Subsequently, isolated phytoconstituents must be subjected to all the present plus some additional more advanced pharmacological, both in vivo and in vitro, tests to claim a particular chemical constituent to be responsible for a specific biological activity.

Antioxidant testing methods undertaken were all in vitro. In vivo antioxidant testing methods like TBARS (Thiobarbituric acid reactive substance), erythrocyte membrane stabilization assay, measurement of NO and antioxidant enzyme levels in brain, heart and liver samples may confirm the antioxidant activity of the plant parts.

The extracts must be examined in various other neuropharmacological studies such as elevated plus maze, Y-maze, Hole cross, Whole board and so on to substantiate the present data.

Therefore, further chemical and pharmacological studies with extracts of *Ocimum gratissimum* for isolating new bioactive compounds and evaluation of their exact mode of action and chronic toxicity profile might be the next steps to be followed to eventually find new lead compounds.

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