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EXTRACTION, PHYTOCHEMICAL SCREENING AND EVALUATION OF PLANT OCIMUM SANCTUM AND OCIMUM BASILICUM ACTING AGAINST CNS DEPRESSANT

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Abstract:
Ocimum sanctum and Ocimum basilicum L which has many therapeutic uses, the plant is used as an antibacterial, dysentery, diarrhoea, persistent fever, cough, stomachache, wounds, cuts, burns, fractures, general weakness, antispasmodic, diureticetc, rheumatic, etc. from the earliest times. The plants contain quercetin, essential oil, tannin and various phytosterols, etc. So far, there has been no substantial work on the medicinal use of these plants. Consequently, the current study has been planned to discover the CNS activity of Ocimum sanctum and Ocimum basilicum L. The total phenolic content 0.214 mg/100mg of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentrations 0.452 mg/100mg, expressed as quercetin equivalents. Phytochemical Test of prepared extract was performed by standard method. Data for total phenolic and total flavonoid content, data shows that moderate amount of total phenols and total flavonoids are present in the extract and the amounts are 0.352mg Gallic acid /100mg dry wt and 0.620 mg Quercetin/100mg dry wt of dried extract of Ocimum basilicum respectively. The effect Ocimum basilicum shows better results as compaired to that of Ocimum sanctum. The climbing time significantly increased only in Ocimum basilicum as compared to vehicle control. In the TST model in mice there was a significant decrease in immobility time in all the groups as compared to vehicle control. The anti immobility effect of Ocimum basilicum was significant as comparable to that of Ocimum sanctum. Ocimum sanctum and Ocimum basilicum may be developed as an antidepressant drugs. Further research is required to gain closer insights into the exact mechanism of its action.

Key words: Ocimum sanctum and Ocimum basilicum, CNS Activity,

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INTRODUCTION:

Since the early days of mankind, herbal medicines that have created the cornerstone of health care around the world are still widely used and are of great importance in international trade. Even though this varies widely between countries, the identification of their clinical, pharmaceutical and economic importance is still rising. For pharmacological research and drug production, medicinal plants are noteworthy, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for drug mixtures or as models for pharmacologically active compounds. Therefore, the regulation of consumption and exports, along with international cooperation and coordination, is important for their security in order to ensure their future availability. According to the United Nations Biological Diversity Conference, the conservation and sustainable use of biological diversity is crucial to addressing the food, health and other needs of the increasing world population, which is why access to and sharing of both genetic resources and technologies is important.

Legislative controls have not evolved into a formal control model in respect of medicinal plants. There are various ways in which medicinal plants, herbs or products derived from them are specified by countries, and countries have adopted a variety of licencing, dispensing, manufacturing and trading approaches to ensure their security, quality and effectiveness [1]. Only a moderately limited number of plant species have been researched for possible medicinal uses, considering the use of herbal medicines over several centuries. For an even smaller number of plants, their extracts, and the active ingredients and preparations comprising them, protection and efficiency data are given [2]. In approximately any culture, herbal medicines (defined as an arrangement derived from plants and fungi, e.g. by alcoholic extraction or decoction, used for the prevention and treatment of diseases) are an essential part of traditional medicine. Herbal medicines and supplements are an important market in developing countries.

In the use of herbal arrangements sold as medicines, a number of countries such as Germany have a long history and figures for prescriptions and sales are steady or even decreasing [3]. Herbal medicinal output is marketed as "food supplements" or "botanical medicines" in the US and the UK. Sales of such goods have been rising strongly in these countries in recent years. Herbs are largely used by traditional healers in the Third World [4].

Herbal medicine is still the pillar of primary health care for about 75-80 percent of the world's population, mainly in developing countries. This is largely due to the universal perception that herbal medicines are available with no side effects as well as being inexpensive and close [5]. The use of herbal remedies worldwide exceeds that of predictable medications by two to three times, according to the World Health Organization (WHO).

The use of plants predates human history for medicinal purposes and forms the derivation of a great deal of modern medicine. A lot of traditional drugs derived from plant sources: most of the lowly effective drugs were developed a century ago. Aspirin (willow bark), digoxin (foxglove), quinine (cinchona bark), and morphine (opium poppy) are examples [6]. Medical literature from the beginning of time is packed with accounts of people who have used herbs to make humanity sick. Nevertheless, we saw the rise of allopathic medicine parallel to the onset of the industrial revolution. Herbal medicine was also an important way of healing, but was seen less excitedly.

In the universal herbal revolution, medicinal plants have immense market potential across the globe. High-quality phytomedicinal products may be expected to provide safe and reliable medication. In India, there are a large number of herbal remedies consisting of ayurveda , siddha, unani, etc. Being used from the earliest times and making their future therapeutic claims with specification of excellence regulation. However, these medications suffer from a lack of standardisation criteria and scientific screening-based documentation. The assessment of these drugs is often focused on phytochemical, pharmacological and related methods, including a variety of chromatography, spectroscopy, and microscopy-like instrumental techniques. There are so many examples of herbal drugs which have strong CNS activity like opium, cannabis, cocain bhrami, hypercicum, cinigrin, ephedra, physostigma, pilocarpus, belladonna, datura, coffee, nux-vomica, labelia, camphor, hyoscyamus, codeine and poppy latex.

The plants are used as prophylactic and a variety of cure diseases from earliest times. *Ocimum sanctum* and *Ocimum basilicum* L which has many therapeutic uses. The plant is used as an antibacterial, dysentery, diarrhoea, persistent fever, cough, stomachache, wounds, cuts, burns, fractures, general weakness, antispasmodic, diureticetc, rheumatic, etc. from the earliest times. The plants contain quercetin, essential oil, tannin and various phytosterols, etc. So far, there

has been no substantial work on the medicinal use of these plants. Consequently, the current study has been planned to discover the CNS activity of *Ocimum sanctum* and *Ocimum basilicum* L.

MATERIAL AND METHODS:

Extraction by maceration process

Defatted dried powdered of *Ocimum sanctum* and *Ocimum basilicum* L has been extracted with hydroalcoholic solvent (Ethanol: water, 80:20) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C [7-8].

Determination of percentage yield

The extraction yield is a measure of how well a solvent extracts bioactive component from natural plant samples, and it is defined as the amount of plant extracts recovered in mass following solvent extraction versus the initial quantity of plant samples. Following extraction, the yield of the obtained plant extracts was determined in grams and then translated to a percentage. The percentage yield of chosen plant components was calculated using the formula below.

Percentage yield = Weight of Extract/ Weight of powdered drug x 100

Phytochemical Screening

Phytochemical screening is a method for quickly extracting, identifying, and screening phytochemicals from a wide range of therapeutic plants. These phytochemicals are used as precursors in the production of a variety of novel medicines. As a result, plant extracts produced by water and ethanol extraction of all chosen plant samples were submitted to several qualitative screenings in order to determine the presence of plant bioactive components. Preliminary phytochemical screening is crucial for determining the profile of a particular extract in terms of the chemical compounds generated by the plant. The following conventional techniques were used to conduct phytochemical analyses on extracts [9].

1. Detection of alkaloids: Extract were dissolved individually in dilute Hydrochloric acid and filtered.

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

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

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

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

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates 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.

3. Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for 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.

4. Detection of 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.

5. Detection of 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.

6. Detection of tannins

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

7. Detection of 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.

8. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of 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.

Quantitative estimation of bioactive compound

Total Phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method [10]. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method [10]]. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

In vivo* pharmacological screening of hydroalcoholic extracts of *Ocimum sanctum* and *Ocimum basilicum

Animals

Animal's Swiss albino mice (males; 20–25 g) were used in the present study. They were provided normal diet and tap water ad labium and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee.

Acute oral toxicity study

Adult Swiss albino mice of either sex, weighing between 20 and 25 g, fasted overnight and were used for acute toxicity study, as per the Organization for Economic Co-Operation and Development (OECD 423) guideline. Four groups of mice of both sexes were fasted overnight. The first control group mice received 0.5% carboxymethyl cellulose (CMC) suspension in distilled water while the other three groups received Hydroalcoholic extracts of *Ocimum sanctum* and *Ocimum basilicum* suspended in 0.5% CMC at doses of 200, 600, and 2000 mg/kg. Animals were observed closely for first 4 hours, for any toxicity manifestation, like increased motor activity, salivation, convulsion, coma, and death. Subsequently observations were made at regular intervals for 24 h. The animals were under further investigation up to a period of 14 days and no mortality was reported within the study period.

In vivo CNS activity of hydroalcoholic extracts of *Ocimum sanctum* and *Ocimum basilicum* by using various animal Models:

- Anti-anxiety activity by plus maze model
- Anti-anxiety against forced swimming model
- Antidepressant activity by tail suspension model

Elevated plus Maze model

The plus maze apparatus consisted of two open arms, measuring 16 × 5 cm, and two closed arms, measuring 16 × 5 × 12 cm, connected to a central platform (5 × 5 cm). The maze was elevated to a height of 25 cm above the floor. Each mouse was placed individually at the center of elevated plus maze with its head facing toward an open arm and observed for 5 min to record the number of entries into open arm, closed arm and time spent in each arm. In EPM test, the percent time spent on the open arms was determined as follows [11]:

$$\textbf{Time spent}\ \% = \frac{\textbf{Number of seconds spent on open arms}}{\textbf{300 total seconds (5 min observation time)}}\ \textbf{x100}$$

Forced swimming test (FST) model

Forced swimming test in glass jar FST in glass jar was performed as described by Porsolt et al. with few modifications. This test consists of two parts, an initial training period of 15 min followed by actual test for 5 min duration 24 h later. Mice were individually forced to swim inside a vertical borosilicate glass cylinder (height: 40 cm; diameter: 15 cm; containing 15 cm hight of water maintained at

25 ± 1OC). Mice placed in the cylinder for the first time were initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom [11].

After 2-3 min, activity began to subside and was interspersed with phases of immobility or floating of increasing length. After 5-6 min, immobility reached a plateau where the mice remained immobile for approximately 80% of the time.

After 15 min in the water, the mice were removed, wiped with dry cloth and allowed to dry before being returned to their home cages. The cylinders were emptied and washed thoroughly after testing for each mouse. The mice were again placed in the cylinder 24 h later after three doses of drug and their activity was recorded from above for 5 min using a digital camera.

The recordings were later analyzed by a rater who was blinded to the treatment condition, to find the duration of immobility, swimming behavior and climbing behavior in the 5 min test period using stopwatch. An animal was judged to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose just above the surface, with no additional activity other than that necessary to keep its head above water. Swimming was defined as active movement throughout the swim chamber, which included crossing into another quadrant. Climbing activity (also termed thrashing) consisted of upward directed movements of the forepaws along the side of the swim chamber

Tail suspension test model

TST was done as described by Steru *et al.*, 1985 [12] After three doses of drugs, mice were suspended on a string held by a metal stand, by an adhesive tape placed 1 cm from the tip of the tail. This string was 58 cm above the table top. The activity of the mice was recorded using a digital camera for a period of 5 min. During the experiment, each animal under test was both acoustically and visually isolated from other animals. The videos were analyzed by a rater blinded for treatment condition to find the duration of immobility in seconds. Mice were considered immobile when they hang passively and completely motionless.

RESULTS AND DISCUSSION:

The total phenolic content 0.214 mg/100mg of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentrations 0.452 mg/100mg, expressed as quercetin equivalents (Table 1). Phytochemical Test of prepared extract was performed by standard method and results shown in table 2 & 3. Data for total phenolic and total flavonoid content has been summarized in Table 4. Data shows that moderate amount of total phenols and total flavonoids are present in the extract and the amounts are 0.352mg Gallic acid /100mg dry wt and 0.620 mg Quercetin/100mg dry wt of dried extract of *Ocimum basilicum* respectively. The effect *Ocimum basilicum* shows better results as compaired to that of *Ocimum sanctum.* The climbing time significantly increased only in *Ocimum basilicum* as compared to vehicle control. In the TST model in mice there was a significant decrease in immobility time in all the groups as compared to vehicle control. The anti immobility effect of *Ocimum basilicum* was significant as comparable to that of *Ocimum sanctum*. *Ocimum sanctum* and *Ocimum basilicum* may be developed as an antidepressant drugs. Further research is required to gain closer insights into the exact mechanism of its action (Table 4, 5 & 6).

Table 1: % Yield of leaves extract of *Ocimum sanctum* and *Ocimum basilicum*

S. No.	Hydroalcoholic extract	% Yield (w/w)
1.	*Ocimum sanctum*	5.63%
2.	*Ocimum basilicum*	10.24%

Table 2: Phytochemical screening of extract *of Ocimum sanctum*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Dragendroff's test Hager's test	 -ve +ve
2.	**Glycosides** Legal's test	 +ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
4.	**Phenol** Ferric chloride test	 +ve
5.	**Proteins** Xanthoproteic test	 +ve
6.	**Carbohydrates** Fehling's test	 +ve
7.	**Saponins** Foam test	 +ve
8.	**Diterpenes** Copper acetate test	 -ve
9.	**Tannins** Gelatin Test	 -ve

+ve (Present), -ve (Absent)

Table 3: Phytochemical screening of extract *of Ocimum basilicum*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Dragendroff's test Hager's test	 -ve -ve
2.	**Glycosides** Legal's test	 -ve
3.	**Flavonoids** Lead acetate Alkaline test	 -ve +ve
4.	**Phenol** Ferric chloride test	 +ve
5.	**Proteins** Xanthoproteic test	 -ve
6.	**Carbohydrates** Fehling's test	 +ve
7.	**Saponins** Foam test	 +ve
8.	**Diterpenes** Copper acetate test	 -ve
9.	**Tannins** Gelatin Test	 +ve

+ve (Present), -ve (Absent)

Table 4: Total phenolic and total flavonoid content of *Ocimum sanctum*

S. No.	Total phenol content	Total flavonoid content
1.	***Ocimum sanctum***	
	0.214 mg/100mg	0.452 mg/100mg
2.	***Ocimum basilicum***	
	0.352mg/100mg	0.620 mg/100mg

Table 5: Effect of different treatments on the time spent by mice behavior in elevated plus maze

Treatments	Dose (i.p.)	Time spent in open arm (seconds)	Entries in open arm
Saline	1 ml/kg	36.83 ± 4.57	5.33 ± 0.61
Diazepam	0.5 mg/kg	128.5 ± 5.2	12.33 ± 0.33
Ocimum sanctum	100 mg/kg	101.83 ± 10.19	10.16 ± 1.57
Ocimum basilicum	100 mg/kg	80 ± 18.34	12.00 ± 1.438

Table 6: Effect of drugs on immobility, swimming and climbing time in forced swimming test in mice

Treatment group	Dose (mg/kg)) *per os*	Immobility time (s) (mean ± SEM)	Swimming time (s) (mean ± SEM)	Climbing time (s) (mean ± SEM)
Vehicle control	2.5 mL/kg	220.7 ± 13.8	42.0 ± 5.5	35.3 ± 11.8
Diazepam	0.5 mg/kg	126.3 ± 15.9*	128.8 ± 10.8*	46.8 ± 9.4
Ocimum sanctum	100 mg/kg	174.5 ± 18.4	69.7 ± 10.7	55.2 ± 9.7
Ocimum basilicum	100 mg/kg	112.7 ± 8.6*	88.0 ± 13.7	96.3 ± 16.0*

Statistical analysis of data was carried by one-way ANOVA followed by Tuckey-Kramer multiple comparisons test. * $p < 0.05$ as compared to control; n = 6 in each group

Table 7: Effect of drugs on immobility time in tail suspension test and total counts of locomotor activity in photo actometer in mice

Treatment group	Dose (mg/kg)) *per os*	Immobility time (s) (mean ± SEM)	Total counts (mean ± SEM)
Vehicle control	2.5 mL/kg	151.8 ± 17.9	114.7 ± 11.1
Diazepam	0.5 mg/kg	41.7 ± 13.0*	117.8 ± 22.1
Ocimum sanctum	100 mg/kg	71.8 ± 14.5*	118.3 ± 16.4
Ocimum basilicum	100 mg/kg	68.7 ± 21.7*	113.5 ± 14.2

Statistical analysis of data was carried by one-way ANOVA followed by Tuckey-Kramer multiple comparisons test. * $p < 0.05$ as compared to control; n = 6 in each group

CONCLUSION:

In this CNS activity all the test groups, except Diazepam in, showed a significant decrease in immobility time as compared to the vehicle control group. The effect *Ocimum basilicum* shows better results as compaired to that of *Ocimum sanctum*. The climbing time significantly increased only in *Ocimum basilicum* as compared to vehicle control. In the TST model in mice there was a significant decrease in immobility time in all the groups as compared to vehicle control. The anti immobility effect of *Ocimum basilicum* was significant as comparable to that of *Ocimum sanctum*. *Ocimum sanctum* and *Ocimum basilicum* may be developed as an antidepressant drugs. Further research is required to gain closer insights into the exact mechanism of its action.

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