Comparitive Study Of Ocimum Sanctum By Supercritical Carbon Dioxide Method And Microwave Assistance Method With Their Pharmacognostic Acitivities.

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ABSTRACT -

Two significant therapeutic plants from the genus Ocimum, Ocimum basilicum and Ocimum sanctum, have been compared in this study using a variety of phytochemical criteria. Investigations have been conducted on the effects of extraction methods, supercritical carbon dioxide method and microwave assistance method. These analyses are essential for choosing plant leaves based on their composition for the production of herbal medications that can be used for a number of conditions, including diabetes, pharmacological activities, respiratory diseases, etc. The results show that these leaves have the potential to be used in nanotechnology, phytomedicine, and extremely nutritious feed.

Given the plant's numerous pharmacological applications across the globe, they are relevant to nutrition, medicine, and veterinary care.

In microwave-assisted hydrodistillation (MHD), the effects of radiation duration and microwave supplied power on the yield and makeup of the essential oil extracted from Ocimum sanctum. Microwave-delivered power of 1000 W for 3 minutes of microwave radiation and microwave-delivered power of 300 W for 1 hour of microwave radiation at a

spice to water ratio of 1:20 were the ideal circumstances for MHD. A polyherbal mixture of powdered cardamom seeds, bay leaves, and tulsi leaves was extracted using supercritical carbon dioxide (SC-CO2). The highest extract yield and maximum antioxidant potency were achieved using a 20 g powdered mixture of tulsi leaf prepared in an adjusted 1:1:2 ratio.bay leaf: tiny cardamom seed extracted at 608°C, 300 bar, and 90 minutes with a gaseous CO2 flow rate of 2.5 L/min.

KEYWORD: Ocimum sanctum, supercritical carbon dioxide extraction, microwave-assisted hydrodistillation, , pharmacological activities,

INTRODUCTION:

A little plant found all over India, Ocimum sanctum L. (Family Lamiaceae) is also referred to as holy basil in the West and tulsi in India [1].The medicinal plant Ocimum sanctum L. (family Lamiaceae) is used in the food, pharmaceutical, cosmetic, and agricultural industries [2]. The Lamiaceae (Labiateae) family includes the genus Ocimum, which is wellknown for its significance in the perfume business. The number of species in the genus has been reported in various ways. There are between 50 and 60 species, according to certain sources [3, 4].

The "Rigveda," which is believed to have been composed between 4500 and 1600 BC, has the oldest reference to the usage of plants for medical purposes in Hindu culture.[5] It has long been used as a pain reliever, as well as a repellent and to treat rheumatism, diarrhea, high fever, convulsions, diabetes, eczema, and piles [6, 7]. In particular, because of the anticipated benefits of the supercritical extraction method, the use of supercritical fluids in the extraction of volatile oils and extracts has grown since 2000. The manufacturing of highvalue plant extracts for the food, cosmetics, and pharmaceutical industries has drawn more attention to these procedures.[8,9]

The volatile oil found in OS Linn. leaves is 0.7% and contains roughly 71% eugenol and 20% methyl eugenol. Other phytoconstituents included in the OS include UA, carvacrol, caryophyllene, apigenin, lute olin, apigenin-7-O-glucuronide, orientin, and molludistin. [10] Supercritical uid extraction is one of several innovative extraction techniques. [11] Another solvent-free and eco-friendly sample pretreatment method is supercritical fluid extraction. Numerous parameters affecting the yields of basil extract extraction were examined in this study, along with a comparison to traditional extraction methods.

The goal of extraction methods is not just to remove active biocompounds from plant samples. Other techniques have been employed, such as supercritical fluid extraction, which produces extracts and pure compounds by using carbon dioxide (SFE CO2) as the organic solvent. Numerous benefits come with this method, including a brief exposure period to a low-temperature, non-toxic solvent. Additionally, the productivity and quality of the items produced are high. [12]

Since its beginnings, supercritical fluid extraction (SFE) technology has made significant strides and is now the preferred technique in many food processing sectors. SFE has gained

popularity over the past 20 years as a clean, eco-friendly "green" processing method and, in certain situations, as a substitute for organic solvent-based natural product extraction. Comprehensive evaluations of the latest developments in SFE applications in the fields of food science, natural products, by-product recovery, pharmaceuticals, and environmental sciences have been published [13]. Compared to traditional procedures, SFE removes the oil or desired element from the subject material faster. The important components of supercritical fluid extracts are usually kept in their chemically natural state and are usually sanitized and free of contamination [14,15]. SC-CO2 extraction has been used both alone and in combination with other major or minor lipid components to extract phytosterols from plant materials and/or for food purposes [16].

Non-thermal phenomena that have been widely referred to as "microwave effects" have been reported by numerous researchers in recent literature. The increased reaction rates of thermosetting resins during microwave curing are an example of the microwave effect [17]. The quantity of symposia devoted to microwave processing of materials in recent years indicates the growing interest in this field. The American Ceramic Society and the Materials Research Society (MRS) have hosted nine symposia on microwave processing materials thus far.[18,19]

In mythology, Ocimum basilicum is referred to as the "God of Spices," emphasizing its use in cooking. [20] Additionally, a number of dietary products that support and sustain health contain basil. [21] Although this herb is also well-known for its decorative qualities, its wide range of pharmacological activity primarily make it useful for therapeutic purposes.[22,20]

MATERIALS AND TECHNIQUES:

Plant collection site:

Octimum sanctum L. was chosen for these experiments based on its ethnobotanical uses; only the aerial parts of the plant leaves and flowers were used. The plants were extracted using fresh plants within the first three days of acquisition, and the rest were dried in an oven (LSIS-B2V/VC111 1900 W, Cejl, Czech Republic) at 40 C for 72 hours and stored in vacuum packages at -18 C.

The flowers and leaves were ground (IKA WERKE-M20, Staufen, Germany) at low temperatures to prevent loss and thermal degradation of secondary metabolites, and the particle size distribution was assessed using a set of standard sieves.

Characterization of tulsi powder:

Using a sieve shaker, the powder samples were filtered through a series of standard sieves (5, 10, 14, 20, 24, and 44 Tyler meshes) to determine the mean particle diameter of the powder. The particle size distributions were then noted [23].

It was found that the average particle diameter (dp) of tulsi powder was 0.4 mm. AOAC method 930.15 was used to determine the tulsi powder's moisture content [24].

Carbon dioxide extraction of tulsi powder:

According to the procedure, dried tulsi powder was extracted using liquid CO2 [25].

To keep the pump head at 2°C, it is equipped with a modified pump (Speed MAX P/N 7025) and a refrigerated cooling bath. A 50 ml extraction vessel (SS 316) was filled with 20 g of dried tulsi powder.

Comparitive Study Of Ocimum Sanctum

Carbon dioxide Supercritical extraction method:

The concept of supercritical fluid extraction (SFE):

Principle- Supercritical carbon dioxide technology (SC-CO2) is a promising substitute for the traditional thermal processes that would destroy bioactive compounds in food and medicine. It uses pressure and carbon dioxide to kill microorganisms without compromising nutritional value or organoleptic qualities [26].

Supercritical extraction of carbon dioxide Another effective technique for eugenol extraction from ocium sactum is supercritical carbon dioxide (SC-CO2) extraction.[27] In order to extract the volatile oil, a two-stage fractional separation technique was used in a flow apparatus to perform supercritical fluid extraction (SFE) with carbon dioxide (CO2) and 80 g of basil with a mean particle size of 0.6 mm for 2.5 hours at a flow rate of 1.0 kg/h CO2, at 313 K and 9.0 MPa [28,29].

In the first separator, separation was carried out at 7.0 MPa and 263 K, mostly collecting waxes. The volatile oil (SFEO) was extracted from a second separation that was carried out at 2.0 MPa and 273 K. Gravimetric analysis was used to determine the amount of volatile oil collected, with a 0.1 mg margin of error. The supplier of purity CO2 (99.995%) was Air Liquide, located in Lisbon, Portugal.

SFE experiments were conducted in a flow apparatus from Applied Separations, SpeedTM SFE, which permits extraction to be carried out at temperatures up to 393.2 K and pressures up to 60.0 MPa, in order to obtain basil extracts at higher pressures due to the limitations of SFE equipment (maximum pressure of 30.0 MPa) [30,31].

The extract was incubated at ambient temperature to eliminate any remaining CO2. A miscible polar substance, such ethanol, can be added as a modifier to supercritical SC-CO2 to improve its polarity, even if CO2 is the recommended extraction solvent (for extracting nonpolar chemicals) [32]. 6 The extracts produced using this method have minimal concentrations of undesirable chemicals because of the selectivity required in the SFE process [33].

Additionally, after depressurization, SC-CO2 turns gaseous and is easily removed from a flow system.[34] Because it is nontoxic, nonexplosive, affordable, and has the capacity to

dissolve lipophilic materials, supercritical carbon dioxide (SC-CO2) is a desirable substitute for organic solvents. It is also easily extracted from the finished products. [35, 36, 37]

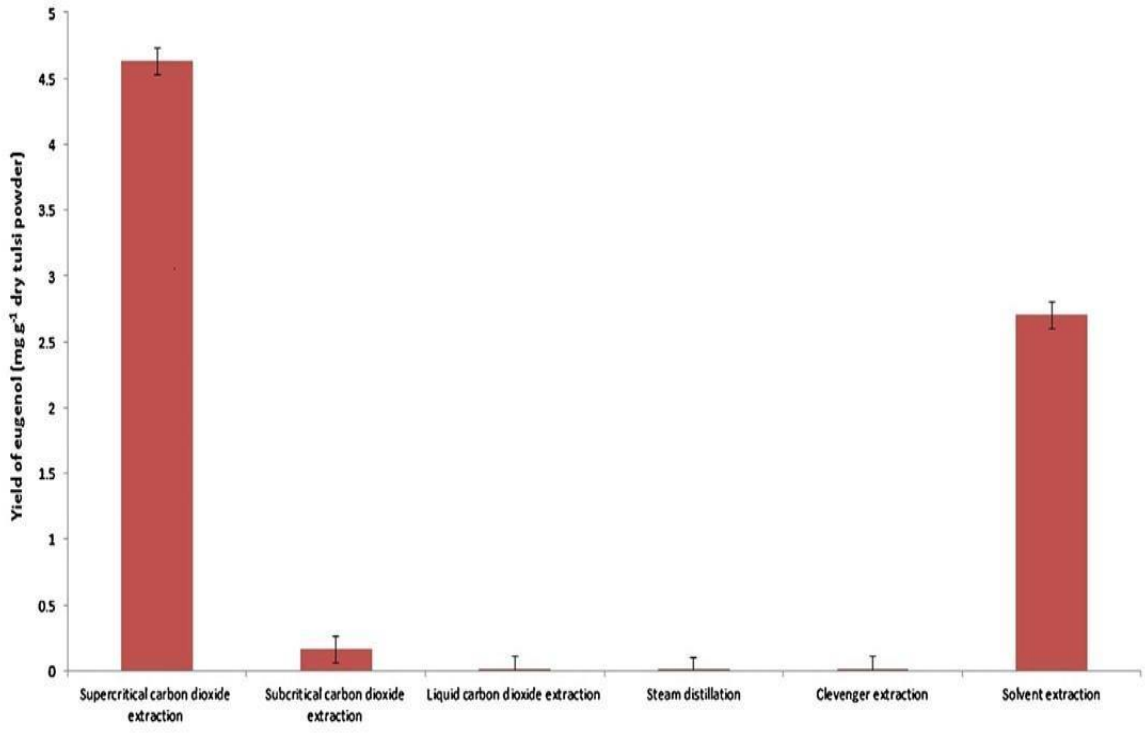


Fig. 1. Comparison of yield of eugenol obtained by different extraction procedure

Application of Supercritical fluid extraction:

The experimental finding that many gases increase their dissolving power when compressed above a certain point serves as the foundation for the use of SFE.[38] Their findings demonstrate that, particularly when a co-solvent is included, the extracts produced by SC-CO2 have a strong antioxidant potential against the DPPH (2,2 diphenyl-1picrylhydrazyl) radical. Because it enables the extraction of thermally labile or readily oxidized chemicals, SF-CO2 is significant for the food sector and natural compound extractions in this context.[39]

These days, SFE is widely employed in a variety of industrial applications, such as the decaffeination of coffee, the refining of fatty acids, and the extraction of flavors and essential oils from natural sources that may find use in functional foods and nutraceuticals.[40]

Gases	Critical Temperature (K)	Critical Pressure (MPa)
Carbon dioxide	304.17	7.38
Ethane	305.34	4.87
Methane	190.55	4.59
Ethylene	282.35	5.04
Propane	369.85	4.24

Table 2. Examples of substances used as supercritical solvents and its corresponding critical temperature and pressure. [41]

Microwave Assitance Method:

The Federal Communications Commission (FCC) has designated two frequencies for industrial, scientific, and medical (ISM) uses, which are frequently utilized for microwave heating. The two frequencies that are most frequently utilized are 2.45 GHz and 0.915 GHz. For material processing, microwave furnaces with tunable frequencies between 0.9 and 18 GHz have recently been created [42].Essential oils can be extracted from plant sources with significant potential using the MAE process.[43].

In a conical flask, around 3g of sample and 200 ml of solvent were combined, and the mixture was microwaved at 700 W for 5 minutes with a 2-minute intermission. Each suspension was pre-leached for ten minutes prior to microwave irradiation. With a duration of 5 minutes for irradiation and 2 minutes for cooling, the microwave irradiation was carried out intermittently, or irradiation-cooling-irradiation. The extraction was done after the sample was treated for five minutes at 700 W in a microwave. Similar procedures to the traditional solvent extraction method were followed for the other steps, such as filtration, concentration, and storage [44].

Because bio-oil contains 15–30 weight percent water and 35–50 weight percent oxygen, it has a poor heating value of 16–18 Mj/kg; also, its high acidity (pH 2-3) adds to its unfavorable characteristics.[45]. Carbon compounds, which are good microwave absorbents with a high ability to absorb and convert microwave energy into heat, have been used in conjunction with microwave heating.[46,47]

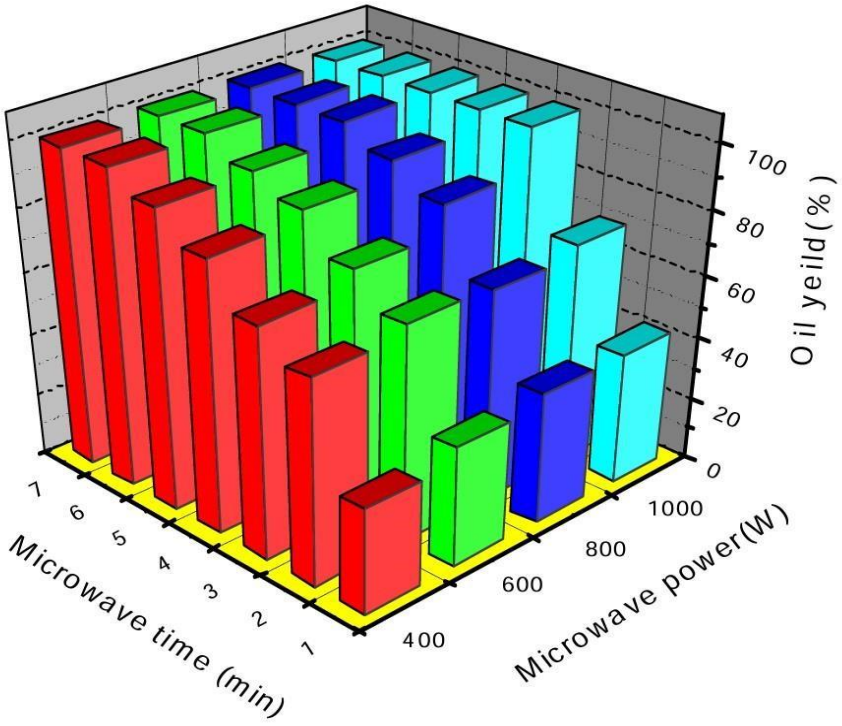


Fig.2. Effect of delivered power and time on oil yield under the MHD conditions

Spice to water ratio: 1:20; microwave conditions: 1000 W of power for three minutes, followed by 300 W of power for one hour.[48].
According to the findings, both methods produced different amounts of oil, but the resulting oil profiles were comparable. Eugenol and methyleugenol, which are the same in other articles, are the primary constituents of essential oils.[49, 50,51]

Microwave-Assisted Simultaneous Distillation and Extraction (MA-SDE) Process:

The MA-SDE apparatus was made up of a modified Likens-Nickerson apparatus for simultaneous distillation-extraction (SDE) and a microwave assisted extraction system that served as the heat source [52].The laboratory modified a home microwave oven (Electrolux, Bangkok, Thailand). The highest The power source was 2450 MHz, and 700 W of power was employed. The microwave oven's internal cavity had measurements of 44.0 × 35.5 × 2.59 cm. In the lab, an oven (Electrolux, Bangkok, Thailand) was modified.

The power source was 2450 MHz, and the maximum power used was 700 W. The microwave oven's internal cavity measured 44.0 × 35.5 x 2.59 cm. The process used for SDE was comparable to that described for MA-SDE. Nonetheless, the process used for SDE was comparable to that described for MA-SDE. However, according to earlier study, the operation was conducted for four hours using an electromantle (Mtops, Seoul, Korea, 500 W) as the heating source [53].

Optimization of MAE based on RSM-BBD

Standard order	Microwave power,X1(W)	Solvent to feed ratio, X2(ml/g)	Extraction time,X3(min)	Total extraction yields ,Y(mg/g)
1	100	30	15	6.38
2	200	30	15	6.47
3	100	70	15	6.99
4	200	70	15	7.07
5	100	50	10	6.17
6	200	50	10	6.70
7	100	50	20	6.62

Table-2 Total extraction yield [54]

Microwave applicators and processing systems:

Single mode-

Because of this, single mode applicators are around one wavelength in size, and these cavities need a microwave source with little frequency output fluctuation to sustain the resonant mode. Single mode applicators feature non-uniform but predictable electromagnetic field distributions, and the regions of high and low electromagnetic field are known due to the ability to determine the electromagnetic field using analytical or numerical techniques.

Single mode cavities typically have a single "hot spot" with a high microwave field strength. There are several clear benefits to being able to design an applicator with known high and low field strength regions. Single mode applicators can be used to concentrate the microwave field at a specific spot with the right design. Ceramics have been joined using this technique [55,56].

The single mode cavity provides a highly regulated setting for cure kinetic investigations, allowing for the best possible coupling between the tiny samples. modest samples utilized for kinetic studies do not constitute a significant coupling load in larger microwave cavities, and controlling the process for modest coupling loads is more

challenging. Monitoring the dielectric characteristics while processing is another benefit of single mode cavities.[57]

Multi-mode applicators*:*

Multi-mode cavities are applicators that have the capacity to support multiple high order modes simultaneously. Microwave ovens at home utilize this kind of applicator. Multimode applicators are frequently built by trial and error, experience, and intuition, in contrast to single mode applicators, which are designed using solutions of the electromagnetic field equations for a certain applicator geometry [58].

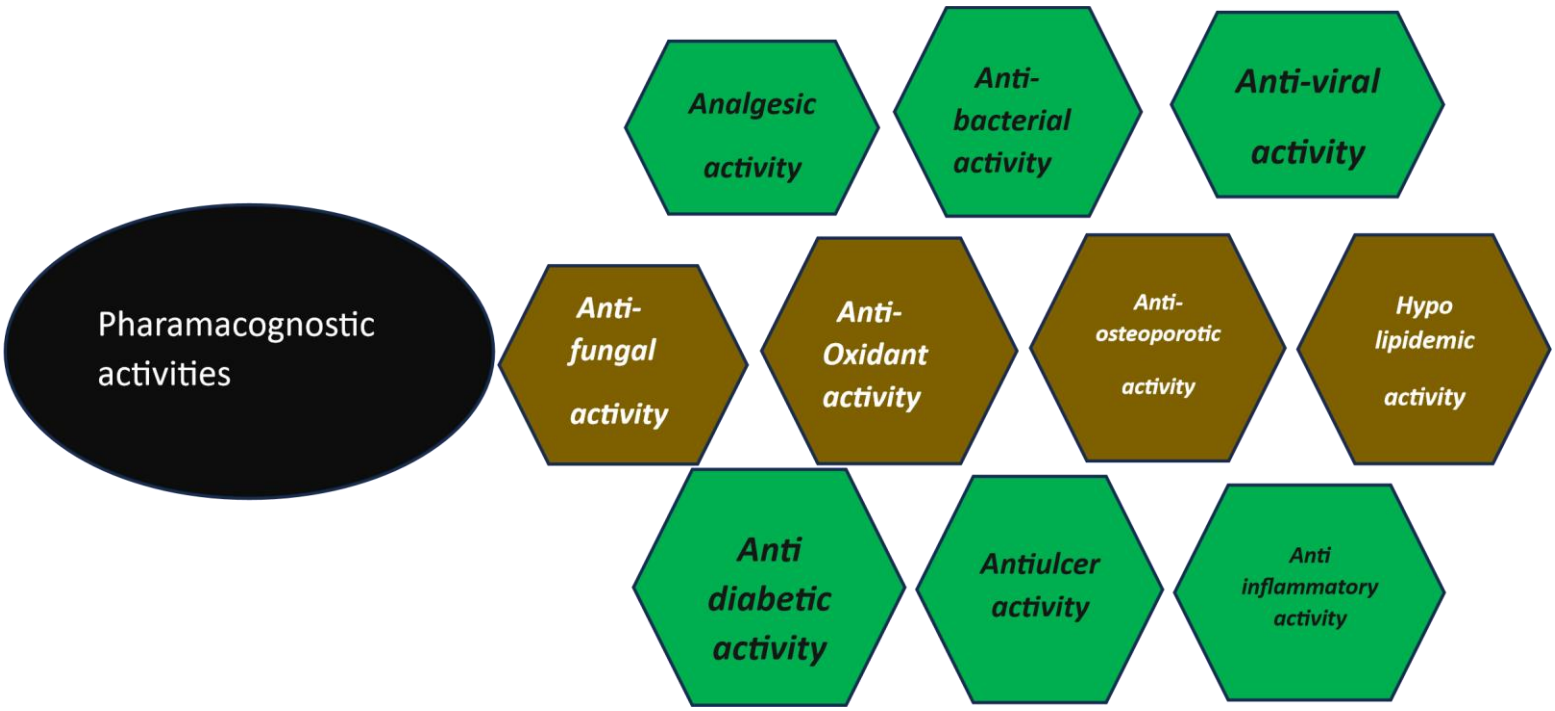
The majority of uniformity-creation methods rely on altering the microwave cavity's electromagnetic field. Hybrid heating is another technique created to provide more consistent heating. Combining microwave heating with traditional heat transfer methods like convection, conduction, or radiation can result in hybrid heating. Researchers at Oak Ridge National Laboratory have successfully employed variations of this technique [59].

Pharmacognostic Activities of Ocimum Santum:

1.Analgesic activity

When tested in Swiss mice using the tail immersion technique, the methanolic extract of O. basilicum was found to have analgesic efficacy. At a concentration of 200 mg/kg, the extract clearly demonstrated a nociceptive impact in comparison to aspirin and the usual medication.[60]

At a dose of 50 mg/kg body weight, the essential oil increased the latency of pain in the hot plate test. The induction of inhibition of cyclo-oxygenase activity and subsequent inhibition of the biosynthesis of pain mediators such as prostaglandin, prostacyclin, and opioid receptor interactions may be the cause of O. basilicum's obvious analgesic effects.[61]



2.Anti-bacterial activity :

The extract of O. basilicum exhibits potential antibacterial action against both Grampositive and Gram-negative bacteria, in contrast to several antibacterial agents. [62,63] O. basilicum essential oil was found to be bactericidal against Gram-negative bacteria including Proteus vulgaris, P. mirabilis, and Pseudomonas aeruginosa; and Gram-positive bacteria including Bacillus subtilis, Micrococcus flavus, M. luteus, Staphylococcus aureus, S. epidermidis, and Streptococcus faecalis. [64] Additionally, the plant's aerial portions shown strong antibacterial properties against Salmonella sp., Escherichia coli, Campylobacter jejuni, and Clostridium perfringens. Furthermore, the volatile oil that was isolated from the plant's aerial parts shown a considerable vulnerability to drug-resistant strains of Enterococcus, Pseudomonas, and Staphylococcus. [65,66]

3.Anti-viral activity:

Eugenol is a powerful natural substance that is being studied extensively to determine its biological activity and potential for use as an antibacterial. Additionally, eugenol and acyclovir work in concert to inhibit the herpes virus in vitro; when used alone, eugenol topical application was shown to slow the progression of herpes virus-induced keratitis in mice models.[67] Eugenol demonstrated virucidal activity against the HSV 1 and HSV 2 viruses, ovicidal activity against the parasite Haemonchus contortus,[68] which inhabits the gastrointestinal tract, and antiprotozoal activity against Leishmania,[69] a group of diseases that cause a wide range of clinical manifestations[70,71]

4.Anti-fungal activity:

Aspergillus fumigatus, A. flavus, Candida albicans, Cryptococcus neoformans, Botrytis fabae, Uromyces fabae, Aureobasidium pullulans, Trichophyton rubrum, Microsporum gypseum, and Trichoderma viride have all been reported to be susceptible to the strong antifungal action of Ocimum basilicum.[72, 73] The morphological changes in the fungal

hyphae, such as hyphal swelling, mycelial asymmetry, sunken, curling, deformed, and broken hyphae, demonstrated that the extract impeded mycelium growth, spore germination, and germ tube extension.[74] The suppression of the transformation of yeast to mycelial form is the mechanism of action. [75] At the in vitro level, the hexane fraction and crude ethanolic extract made from the leaves of the O. basilicum cultivar Maria Bonita both demonstrated anti-cryptococcal efficacy. But the with a low MIC value, the hexane fraction outperformed the ethanolic extract in terms of outcomes. Through the reduction of ergosterol production and capsule size, the extract generated antifungal action. [76]

5. Antioxidant activity:

By raising the amount of antioxidative defense enzymes and significantly lowering lactate dehydrogenase activity and lipid peroxidation, the O. basilicum extract's potent antioxidant activity guards against oxidative stress.[77] The higher stability of extracts suggests that purple basil may be a viable source of stable bioactive components, according to a study done to assess how temperature, light, and copigmentation affect the plant's capacity to scavenge free radicals.[78] This leads to a change in metabolism, which in turn reduces the amount of leaves this plant produces [79,80].

6. Anti-osteoporotic effect :

Basil contains phytoestrogens and flavonoids that stop bone resorption. In postmenopausal women, they also aid in maintaining bone density, which has been shown to have some inhibitory effects on osteoporosis. [81,82] Apigenin, which is found in the volatile oil of O. basilicum, has been shown to induce programmed cell death of mature osteoclasts and limit bone resorption in the rabbit's long bone. [83] The presence of phytoestrogen, which increases the activity of osteoblasts through the estrogen receptor or by increasing the synthesis of insulin 1-like growth factor-1 (IG-F), may be linked to the antiosteoporotic action. [82, 84]

7.Hypolipidemic activity:

Ocimum basilicum is very helpful in preventing hyperlipidemia and the cardiovascular diseases that are associated with it because it has been shown to have hypolipidemic effects through the use of specific polar phytoconstituents. In mice with induced hyperlipidemia, an aqueous extract of basil significantly reduced the levels of triglycerides and total cholesterol in the liver and plasma.[85]

8. Antidiabetic activity:

Experimental animals have demonstrated hypoglycemic effects from O. sanctum leaves [86], [87], [88], and [89]. Blood sugar levels are lowered by decoction made from different plant parts [90]. The physiological pathways of insulin secretion may be stimulated by components of O. sanctum leaf extracts, according to a study [91] on rats. Although Ocimum's antiglycemic qualities have been the subject of numerous investigations, its exact mode of action has not yet been determined [91], [92]. According to a study [93] using tulsi

and neem, diabetic patients benefit more from this combination in terms of decreasing their blood sugar levels.

9.Anti-ulcer activity:

The effectiveness of oral administration of O. basilicum whole plant extract in managing gastric and duodenal ulceration was evaluated in a cysteamine induced duodenal ulceration model, which demonstrated that both ethanolic and aqueous extracts of O. basilicum decreased the ulcer index and the anti-ulcerogenic effects were due to the decrease in pepsin and acid production, which resulted in the subsequent enhancement of gastric mucosal strength.[94]

Significant activity was created in duodenal ulcers by the plant's aqueous extracts; nevertheless, the aqueous extract's activity outperformed that of the ethanolic extract. [95] Such a clear anti-ulcer effect is primarily caused by chemical components such as 1,8cineone, methyl eugenol, linalool, eugenol, and anthocyanins. [96]O. basilicum essential oil at higher doses (200 and 400 µL/kg) reduced ulcer severity, area, and index.

Myeloperoxidase levels and the total colitis index decreased, indicating that O. basilicum has antiulcer properties. [97] Hexane extract from Ocimum basilicum was also shown to have antiinflammatory, antioxidant, and anxiolytic properties that help treat stomach ulcers brought on by aspirin. Therefore, O. basilicum can be used as a medication or nutritional supplement to stop stomach ulcers brought on by aspirin.In individuals receiving aspirinbased chronic therapy. [98].

10. Anti-inflammatory activity:

In rats with acute (carrageenan-induced pedal oedema) and chronic (croton oil-induced granuloma and exudate development) inflammations, methanolic extract (500 mg/kg) and aqueous suspension of OS demonstrated analgesic, antipyretic, and anti-inflammatory properties [99].Due to their ability to inhibit the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism, the fixed oil and linolenic acid exhibit strong antiinflammatory properties against PGE2, leukotriene, and arachidonic acid-induced paw oedema in rats [100].

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