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Preliminary screening and its therapeutical application of *OCIMUM TENUIFLORUM* Buffer Extract (OTBE)

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Abstract
The existing study deals with the role of OCIMUM TENUIFLORUM Buffer Extract (OTBE) on its therapeutical applications. OCIMUM TENUIFLORUM seeds were subjected to Soxhlet-extraction using phosphate buffer and the obtained final extract was termed as OTBE [OCIMUM TENUIFLORUM Buffer Extract]. OTBE was initially screened for its phytochemical content, whereas it shows the presence of terpenoids, tannin, phenolic compounds, Saponin, flavonoids and carbohydrates in the extract. Interestingly, OTBE elutes 17 peaks in GC-MS analysis, whereas in HPLC analysis it elutes only 4 peaks. The chromagraphy technique peaks including HPLC and GC-MS analysis conforms that OTBE withholds micro and macro biomolecules in the extract. Furthermore, OTBE exhibit anti-microbial property by inhibiting only E.Coli by found to be shown 1.9 Minimum Inhibition Concentration (MIC) value. Similarly, when OTBE incubated with S.aureus pathogenic organism it was unable to create zone of inhibition. Fortunately, OTBE found to exhibit non-toxic property as it was unable to degrade packed RBC.

Key words: *OCIMUM TENUIFLORUM Buffer Extract (OTBE), GC-MS, RP-HPLC, Anti-microbial property and Non- toxic property.*

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I. Introduction

The herb "Thulasi" is considered the most sacred by Hindus in India. The scientific name for this herb is Ocimum, belonging to the Lamiaceae family [1]. It is characterized by its erect, softly hairy, and aromatic nature, and is predominantly grown in both temple grounds and domestic settings as a sacred plant [2]. Globally, there are reported to be 63 species of Ocimum. Within India, there are two primary species cultivated: Ocimum sanctum, commonly known as Sritulasi, Ramatulasi, or Lakshmitulasi, with its leaves a vibrant green, and Ocimum tenuiflorum, recognized by its purple leaves, referred to as Shyamatulasi, Krishnatulasi, or Karuntulasi [3]. This plant is native to India and exhibits a broad distribution, spanning the entire Indian subcontinent to elevations of up to 1,800 meters in the Himalayas, and as far as the Andaman Nicobar Islands [4]. Its leaves, stems, flowers, roots, and seeds are believed to have healing benefits. It's a key ingredient in traditional medicine for various illnesses, including colds, headaches, and heart disease [5]. Common uses include making herbal teas, powders, or fresh leaves. It's also been suggested to treat conditions like bronchitis, malaria, and arthritis, among others [6]. O. tenuiflorum is unique in its variety of chemical profiles, with some variations being rich in certain chemicals. Its oil is known for its floral aroma, hinting at cloves [7]. The field of phytomedicine, which involves plant-based pharmaceuticals, is diverse and includes major groups of compounds such as alkaloids, terpenoids, coumarins, tannins, flavonoids, saponins, quinones, phenolic acids, and phenolics [8]. These nitrogen-containing compounds act as natural defenses for plants against pathogens and herbivores, with their powerful biological activities leading to various uses as poisons, narcotics, stimulants, and pharmaceuticals [9]. These compounds, which are essential for plant health, are categorized into phenolic acids, flavonoid polyphenolics (including catechins, xanthones, flavones, and flavanones), and non-flavonoid polyphenolics [10]. Among these, caffeic acid is the most common phenolic compound found in various plants.

II. Materials and Methods

All the chemicals used were of analytical grade.

Preparation of OTBE

OCIMUM TENUIFLORUM was purchased from local market. It was subjected to Soxhlet extraction using PBS buffer. The finally obtained extract was termed as *OCIMUM TENUIFLORUM* Buffer Extract (OTBE) and it utilized for further assays.

Preliminary phytochemical screening of OTBE

OTBE was screened for terpenoids, phytosterol, tannin, phenolic, glycoside, saponins, flavonoids, carbohydrates, proteins and steroids [11].

Reverse Phase High Performance Liquid Chromatography analysis of OTBE

OTBE was subjected to RP-HPLC using C_{18} column (150mm×3mm, particle size 2.7µm) with VWD detector in Agilent 1260-infinity II. The column was pre-equilibrated with HPLC water and Acetonitrile and sample was eluted at the flow rate of 1ml/min in linear gradient mode [12].

GC-MS analysis of OTBE

OTBE was analyzed in GC-MSD, model number 5977B, Agilent Make on single quadrupole mass spectrometers in the Electron Impact Ionization Total Ion Chromatography (EITIC) mode with capillary column (30m lengthX0.25mm ID, 0.25µm film thickness, composed of 5% Phenyl methyl poly siloxane). Helium (99.999%) gas was used as carrier gas at the flow rate of 1ml/min and the injection volume of 2µl. Split ratio of 10:1, temperature program was set as follows, injector temperature 350°C; Auxiliary temperature 250°C, oven temperature initially 50°C (5min hold) with an increase in temperature of 10°C/min to 150°C (5min hold), thereafter 20°C/min to 200°C (5min hold), 25°C/min ramp to 250°C (5min hold), 30°C/min ramp to 280°C (5min hold). Total run time 40.5 min. Sample was analyzed in GC-MSD, model 5977B Agilent Make. Mass spectrum was taken at 70ev; a scan interval of 2.92s [13].

Direct hemolytic activity of OTBE

Direct hemolytic activity was determined by using washed human erythrocytes. Briefly, packed human erythrocytes and Phosphate Buffer Saline (PBS) (1:9v/v) were mixed; 1mL of this suspension was incubated independently with the various concentrations of OTBE (100µL and 200µL) for 1hr at 37°C. The reaction was terminated by adding 9mL of ice cold PBS and centrifuged at 1000g for 10min at 37°C [14]. The amount of hemoglobin released in the supernatant was measured at 540nm. Activity was expressed as percent of hemolysis against 100% lysis of cells due to the addition of water (positive control), whereas PBS served as negative control.

Antimicrobial assay of OTBE

The bacterial cultures (E. coli and S. aureus) were grown in Muller Hinton nutrient agar medium that contain peptone (1%), beef extract (1%) and NaCl (1%) at pH 6.8. Sterile nutrient agar petri plates were prepared and 0.1mL of the overnight grown bacterial culture was spread on the solidified agar plates evenly with the help of a glass spreader. Wells were made on the solidified agar using a cork borer. The test solution was made by dissolving 50mg of OTBE in 1.0mL of water to get 50mg/mL concentration followed by sonication for 2min. The 100µL of this test solution containing 5mg of OTBE added into the respective wells. The standard antibiotic drug Amoxycillin was kept as positive control and tested against both the pathogens. These plates were incubated at 37°C for 24hr. The diameter of 'zone of inhibition' at each well was measured and recorded [15]. The minimum inhibitory concentration (MIC) assay was carried out in triplicate and the average values were reported.

III. Results and Discussion

Physical and chemical Characterization of OTBE

The extracted OTBE was found to be present of terpenoids, tannins, phenolic compounds, saponins, flavonoids and carbohydrates (Table 01). In addition OTBE withholds minerals such as Aluminium, Boron, cadmium, copper, iron, manganese and zinc in the extract (Table 02).

SL. No	Name of the Phytochemical	Result
1	Terpenoid	+ ve
2	Phytosterol	-ve
3	Tannin	+ve
4	Phenolic compound	+ve
5	Glycosides	-ve
6	Saponin	+ve
7	Flavinoids	+ve
8	Carbohydrates	+ve
9	Protein	-ve
10	Steroids	-ve

Table 01

SL. No.	Name of the Metal	OTBE (ppm)
1	Aluminum	0.46
2	Boron	0.01
3	Barium	0.00
4	Cadmium	0.01
5	Copper	0.02
6	Iron	0.42
7	Manganese	0.02
8	Molybdenum	0.00
9	Nickel	0.00
10	Lead	0.00
11	Zinc	0.05

Table 02

RP-HPLC analysis of OTBE

OTBE shows only 4 major peaks in the HPLC chromatogram at different retention time in reverse phase HPLC attached to Variable Wavelength Detector. Sample was eluted at 295nm at room temperature (Fig.01).

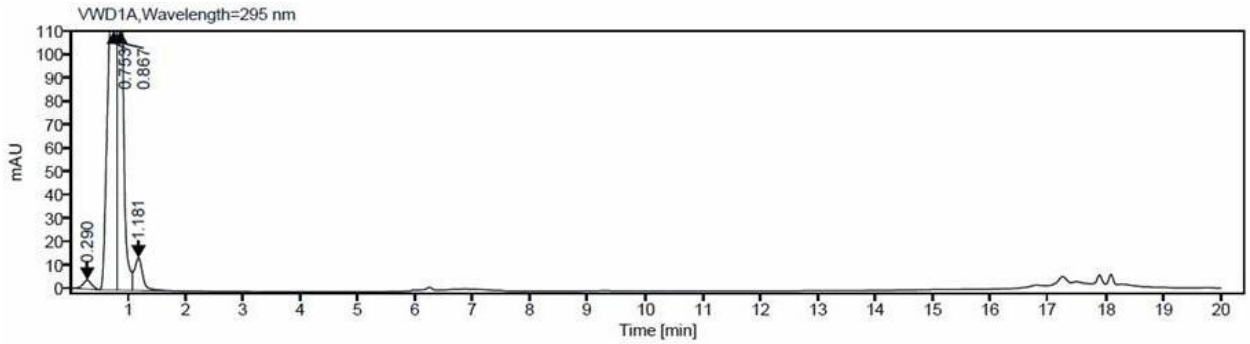

Fig. 01: RP-HPLC Chromatogram of OTBE

GC–MS analysis of OTBE

OTBE found to be presence of 17 different set of compounds as it elutes 17 major peaks at the retention time of 1.7, 2.8, 15.7, 21.7, 22.1, 23.3, 23.9, 24.1, 24.11, 26.7, 26.9, 27.3, 29.1, 29.5, 29.8, 30.5 and 32.0 respectively (Fig.02).

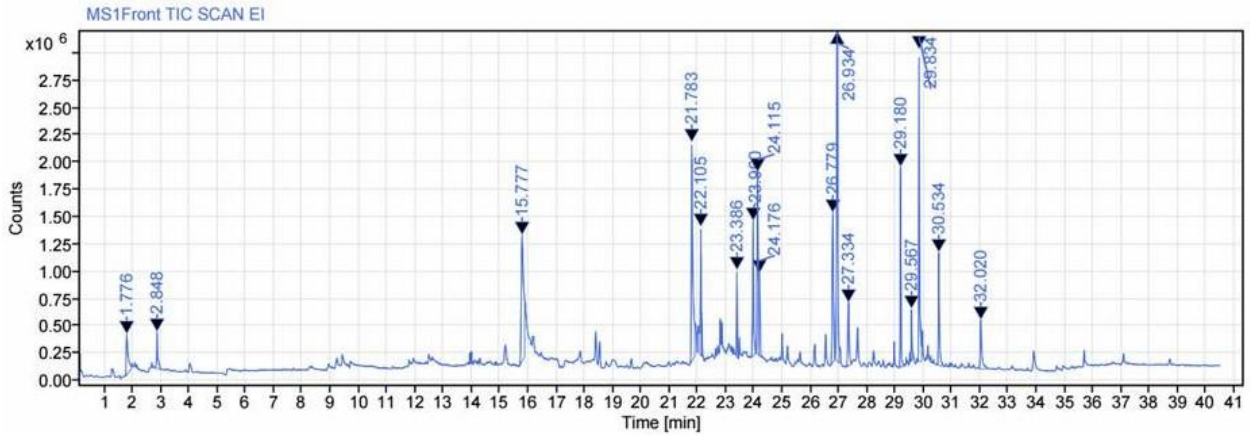

Fig. 02: GC-MS Chromatogram of OTBE

Moreover, OTBE did not hydrolyze RBC suggested its nontoxic property (Fig.03).

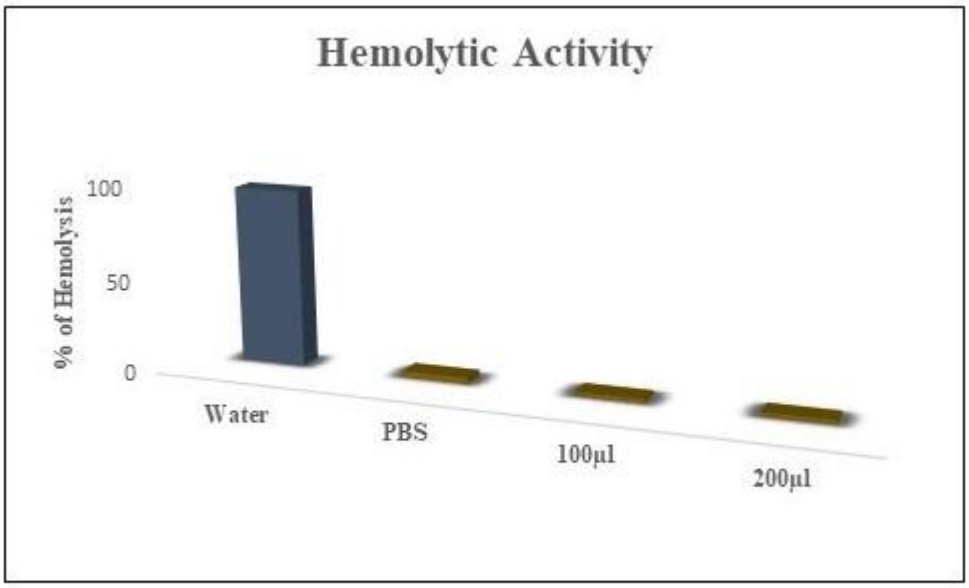

Fig.03: Hemolytic Activity of OTBE

Antimicrobial activity of OTBE

OTBE was subjected to anti-microbial activity assay using with both gram negative bacteria (*E.coli)* and gram positive bacteria (*S.* aureus). Unexpectedly, OTBE found to be show zone of inhibition against only on gram negative bacteria (Fig.04). Anti-microbial property plays a pivotal role in the treatment of several infectious diseases caused by many pathogenic organisms [16]. Lots of antimicrobial drugs are available in the market in varieties of form which includes as an ointments, tablets and intravenous injections [17]. As long as we used antibiotic drug in the form of tablet it may leads to several health disorders. Thus, identify the novel anti-microbial drug from herbal source play a major role in herbal medicinal field [18].

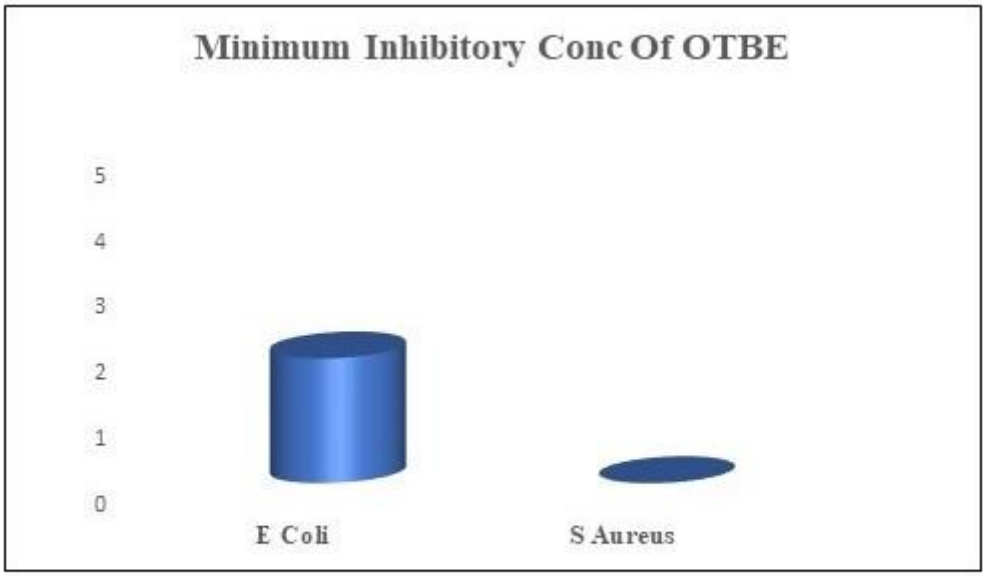

Fig.04: Anti-Microbial Property of OTBE

IV. Conclusion

In conclusion, this study demonstrates the preliminary screening of OTBE and its anti-microbial property which is very specific to gram negative bacteria but not inhibiting the growth of other pathogenic organisms.

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Declaration of Conflict of Interest

The authors declared no potential conflict of interest with respect to the authorship and publication.

Bibliography

[1]. Bast F, Rani P, Meena D. Chloroplast DNA phylogeography of holy basil (Ocimum tenuiflorum) in Indian subcontinent. ScientificWorldJournal 2014;2014:847‑482.
[2]. Mahajan N, Rawal S, Verma M, Poddar M, Alok S. A phytopharmacological overview on Ocimum species with special emphasis on Ocimum sanctum. Biomed Prev Nutr 2013;3: 185-92.
[3]. Pattanayak P, Behera P, Das D, Panda SK. Ocimum sanctum Linn. A reservoir plant for therapeutic applications: An overview. Pharmacogn Rev 2010; 4:95-105..
[4]. Mondal S, Mirdha BR, Mahapatra SC. The science behind sacredness of Tulsi (Ocimum sanctum Linn.). Indian J Physiol Pharmacol 2009;53:291-306.
[5]. Panda VS, Naik SR. Evaluation of cardioprotective activity of Ginkgo biloba and Ocimum sanctum in rodents. Altern Med Rev 2009;14:161‑71.
[6]. Shivananjappa M, Joshi M. Aqueous extract of tulsi (Ocimum sanctum) enhances endogenous antioxidant defenses of human hepatoma cell line (HepG2). J Herbs Spices Med Plants 2012;18:331-48.
[7]. Manikandan P, Murugan RS, Abbas H, Abraham SK, Nagini S. Ocimum sanctum Linn. (Holy Basil) ethanolic leaf extract protects against 7,12‑dimethylbenz (a) anthracene-induced genotoxicity, oxidative stress, and imbalance in xenobiotic-metabolizing enzymes. J Med Food 2007;10:495‑502.
[8]. Sharma MK, Kumar M, Kumar A. Ocimum sanctum aqueous leaf extract provides protection against mercury induced toxicity in Swiss albino mice. Indian J Exp Biol 2002;40:1079‑82.
[9]. KaramalaSK, SrilathaC, AnjaneyuluY, ChandraSekharaRaoTS, Sreenivasulu D, Pidugu AP. Hematobiochemical changes of lead poisoning and amelioration with Ocimum sanctum in wistar albino rats. Vet World 2011;4:260-3.
[10]. Lahon K, Das S. Hepatoprotective activity of Ocimum sanctum alcoholic leaf extract against paracetamol-induced liver damage in Albino rats. Pharmacognosy Res 2011;3:13-8.
[11]. Edeoga, Okwu, Mbaebie. (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 4:685-8.
[12]. Abhishek Gowda N, Bhaskara S, Sunil PE, Sangita Roy, Bhanuprakash KS, Ashwini S, Deepa G, Sharath Kumar MN, "Study the Role of Guizotia Abyssinica Secondary Metabolites [GASM] on Gram Negative Bacteria and Human Citrated Plasma," International Journal of Multidisciplinary Research and Publications (IJMRAP), Volume 6, Issue 3, pp. 105-108, 2023.

[13]. Justesen U, Knuthsen P, Leth T. (1998). Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. Journal of Chromatography A, 799: 101–110.
[14]. Sowmyashree G, Bhagyalakshmi M, Girish KS, Kemparaju K, Rangaiah SM, Jane HP. (2015). Anticoagulant and Antiplatelet activities of Jackfruit seed extract. I J of Phar and Pharmaceutical sciences. 7(1): 187-191.
[15]. Marulasiddeshwara MB, Dakshayani SS, Sharath Kumar MN, Chethana R, Raghavendra Kumar P, Devaraja S. (2017). Facile-one pot-green synthesis, antibacterial, antifungal, antioxidant and antiplatelet activities of lignin capped silver nanoparticles: A promising therapeutic agent. Materials Science & Engineering C.
[16]. Chaieb K, Kouidhi B, Jrah H, Mahdouani K, Bakhrouf A. Antibacterial activity of Thymoquinone, an active principle of Nigella sativa and its potency to prevent bacterial biofilm formation. BMC Compl Altern Med 2011; 11: 29.
[17]. Bita A , Rosu AF, Calina D, Rosu L, Zlatian O, Dindere C, et al. An alternative treatment for Candida infections with Nigella sativa extracts. Eur J Hosp Pharm 2012; 19: 162.
[18]. Halamova K, Kokoska L, Flesar J, Sklenickova O, Svobodova B, Marsik P. In vitro antifungal effect of black cumin seed quinones against dairy spoilage yeasts at different acidity levels. J Food Prot 2010; 73(12): 2291-2295.