



THE FORMULATION & EVALUATION OF HERBAL TABLETS USING TULSI (*OCIMUM SANCTUM* L.) HAS ANTIBACTERIAL ACTIVITY.

¹Vedant Bachhav, ²Miss. A. D. Sonar, ³Samir Ahire, ⁴Nilesh Zanzane, ⁵Yashkumar Ghogare

Swami Vivekanand Sanstha institute of pharmacy, Mungase, Malegaon, Nashik,
Maharashtra, India, 423201

ABSTRACT-

This article reports the formulation and quality assessment of tulsi tablets that have been formulated for better applicability. Tulsi herbal tablets are formulated by wet and direct granulation compression. The trace properties of the herbal excipient powder blend were determined before tableting, followed by post-compression evaluation. The mixed excipient powder blend exhibits excellent flow properties. The formulations are stable enough as indicated by the results of the stability studies. The prepared tablets are qualitatively satisfactory and contribute to the better applicability of the Tulsi extract. The objective was to construct and evaluate the antimicrobial and antifungal effects of the extracts of Umbrella leaves. *sanctum* against pathogenic bacteria to determine their potential as an antibacterial agent. The leaves were separated from the stem, washed in clean water, and dried until dry enough to grind (dried for 7 days). The dried leaves are pulverized separately in an electric blender until a homogeneous powder is obtained and ethanol, hexane, and chloroform are extracted from the powder. The extraction is done by the "cold extraction method". The activity of Tulsi extract against *K. pneumonia* and *Staphylococcus aureus* was found to be highest at 75% concentration, followed by 50% and 25% respectively. The maximum zone of inhibition was found to be against *Staphylococcus aureus*. The

antibacterial efficacy of *Ocimum sanctum* leaves indicates that the plant has potent antibacterial properties and that *Ocimum* is widespread in India, it can be recommended as a readily available and renewable source of antibacterial agents instead of synthetic chemicals.

KAY WORDS- *Ocimum sanctum*, Tulsi Leaf, Zone of inhibition, Wet granulation, Microwave extraction

INTRODUCTION-

In general, functional food means that despite the remarkable advances in modern medicine, the prevention and treatment of chronic diseases with physiologically active food or beverage ingredients also as functional foods are gaining new interest in today's health-conscious world [1-5].

The concept of applying functional foods and functional foods as an adjuvant therapy has been around for 2,500 years, since Hippocrates, the father of modern medicine said, "Let food be your medicine and let it be your medicine. Let medicine be your food". Tulsi in Hindi or Tulsi in Sanskrit (Holy Basil in English) is a highly revered culinary and medicinal aromatic herb from the family Lamiaceae, native to the Indian subcontinent and used in Ayurvedic medicine for over 3000 years [2].

In Ayurveda, this system is often referred to as the "elixir of life" for its healing powers and is known to treat many common health conditions. In Indian Materia Medica, the tulsi leaf extract is described to treat bronchitis, rheumatism, hay fever, and any bacterial infection. neuralgia, headache, sores, astringent and inflammatory and dental conditions. The sap of the leaves is used as ear drops, while the tea is used to treat stomach and liver disorders [3].

The roots and stems were also traditionally used to treat mosquito and snake bites and for malaria. Three types of tulsi are commonly described. *Ocimum tenuiflorum* (or *Ocimum sanctum* L.) includes 2 botanically and phytochemically distinct cultivars that include Rama or Sri tulsi (green leaves) and Krishna or Shyamatulsi (purplish leaves) while *Ocimum gratissimum* is a third type of tulsi known as Vana or wild/forest tulsi (dark green leaves) [4].

The different tulsi types exhibit great diversity in morphology and phytochemical composition including secondary metabolites, yet they can be distinguished from other *Ocimum* species by the color of their yellow pollen, high levels of eugenol, and smaller chromosome number. Despite being distinct species with *Ocimum tenuiflorum* having six times less DNA than *Ocimum gratissimum* is traditionally used in the same way to treat similar ailments. For consistency, this review uses the term Tulsi to refer to both *Ocimum tenuiflorum* and *Ocimum gratissimum* [5].

EXPERIMENTAL WORK-

Material & methods-

Tulsi leaves were obtained from the medicinal garden of Swami Vivekanand Sanstha College of Pharmacy, Mungase. Leaves are separated from the stem, then washed with clean water, dried until dry, then pounded (dry for 7 days). The dried leaves are pulverized separately in an electric blender until a dry, homogeneous powder is obtained. Organic solvent extracts (ethanol, hexane, chloroform) were prepared from the powder obtained by cold extraction. 300 g *Ocimum sanctum* (Linn.) A fine powder mixed from this extract [6];

Steps-

- (a) *Ocimum sanctum* plant;
- (b) leaves separated and dried;
- (c) leaves ground to powder;
- (d) the extract obtained 100% ethanol and another solvent.

It was then filtered with Whatman filter paper to obtain a clear filtrate. The resulting filtrate was therefore reduced at a low temperature below 60 °C to obtain a solid residue of the *Ocimum sanctum* extract (Linn.) from 300 grams of *Ocimum sanctum* (Linn.) powder dissolved in 1 liter of ethanol and with another solvent, 18 g of extract was obtained (residue), and thus the yield was 6% w/v. Weigh accurately 1 g of each reconstituted extract in 10 ml of the respective solvent to obtain a stock solution. Furthermore, dilutions were made with the respective solvents. Accurately weigh 10 mg of standard gentamycin dissolved in 10 ml of distilled water to give 1 mg/ml. The various dilutions and standards

were pipetted into the labeled dishes. They were incubated at 37°C for 24 h and 25°C for 36 h. After incubation, the zones of inhibition were measured (from the antibiotic zone scale) in mm and compared with the standard. For another crude powder extraction method, microwave extraction was used where 0.5:10 parts Crude of de Energy (Tulsi powder) and organic solvents were weighed and placed in a microwave chamber. The organic solvent (acetone) used as the extraction solvent has a good dispersion coefficient (solubility = 0.5555) that can be heated to a large extent and dissipates microwave energy[6,7]



Fig no 1 – Extraction assembly & Extract photograph

The extraction was performed at different microwave operating powers ranging between 100450 W and different irradiation times of 0.53 min. Samples were microwave irradiated by intermittent cooling irradiation for up to 3 min extraction because longer irradiation time and higher power caused solvent boiling. The solvent was then separated through a 0.45 μ m filter and evaporated under vacuum, the remainder was weighed and dissolved in 10 ml of methanol for HPLC analysis for Phytoconstituent concentrations. The separation of a component by chromatography takes place[8].

Microbiological Assay-

The test organisms included in the study were gram-positive *Staphylococcus aureus*, details obtained from the school's microbiology laboratory [9].

Preparation of Media-

For 100 ml of media, 40 gm of agar is dissolved in 100 ml of distilled water in a 250 ml flask, prepared, and autoclaved at 121°C to 15-20 min at 15 lbs/inch² [10].

Preparation of Disk-

The freshly prepared and sterilized fusion medium is poured into a petri dish inside the Laminar and after pouring, the UV lamp is turned on to prevent contamination of the plate while the medium solidifies. It is left for half an hour to solidify well. Once the medium has solidified, the UV lamp is turned off and 10 µl of the bacterial suspension is pipetted to the plate and gauze. A sterile disc is placed (using clamps) on top of a standard plate. (4 discs were placed on one plate) [11].



Fig no 2- Tulsi leaves serial dilution showing the Zone of inhibition (in mm) against *Staphylococcus aureus* (Gram-positive bacteria).

Various Instruments used in the formulation:

Electronic balance, Bulk density apparatus, Standard sieve 30#, Hot air oven, Tablet compression machine, Friability apparatus, Hardness tester, USP Type I tablet dissolution apparatus, Infrared spectroscopy [12].

FORMULATION OF HERBAL API LOADED TABLETS-**Pre formulation studies:**

Pre-formulation studies are done where the physical, chemical, and mechanical properties of a new drug substance or chemical entities are characterized alone and combined with excipients to develop a stable, safe, and effective dosage form [13].

Parameters-

- 1) Solubility of API
- 2) Melting point
- 3) UV analysis
- 5) FTIR Study[14].

Pre compressional studies-

- 1) The flow properties and compressibility of extract and excipient powder blends for the tablet were evaluated by measuring the Angle of Repose.
- 2) Bulk Density (BD) and Tapped Bulk Density (TBD)
- 3) Carr's Compressibility Index
- 4) Hausner's ratio.

The values obtained after testing are compared with the standard values and inferences were drawn in Table [15]

Method of preparation of Herbal Tablet-

Herbal tablets were prepared by wet granulation, and the wet dough mass of all well-mixed ingredients was passed through sieve no. 16 to get uniform-sized granules. After 3-4 hrs of air drying the granules were further dried in a hot air oven for 20-30 min at 45°C-50°C. Dried granules were further sieved and then magnesium stearate and Talc were added as lubricants. Next tablets were subjected to compression using a Tablet compression machine. Hardness of the tablets was maintained at about a maximum of 4 kg/cm [16,17].

Table no 1 - The formula for the herbal antimicrobial tablet (per 100mg) in mg

Ingredient	F1	F2	F3	F4
Tulsi alcoholic extract	3.50	4.00	4.50	5.00
Chitosan	21.5	22	22.5	23
PVP	7.5	7.5	7.5	7.5
ColloidalSilica / Aerosil	4	4	4	4
Magnesium Stearate	6	6	6	6
Talc	5.5	5.5	5.5	5.5
Mannitol	Q.S to 100 mg	Q.S to 100 mg	Q.S to 100 mg	Q.S to 100 mg

Post Compression Evaluations-

Herbal tablets were prepared & evaluated by the below-mentioned parameters-

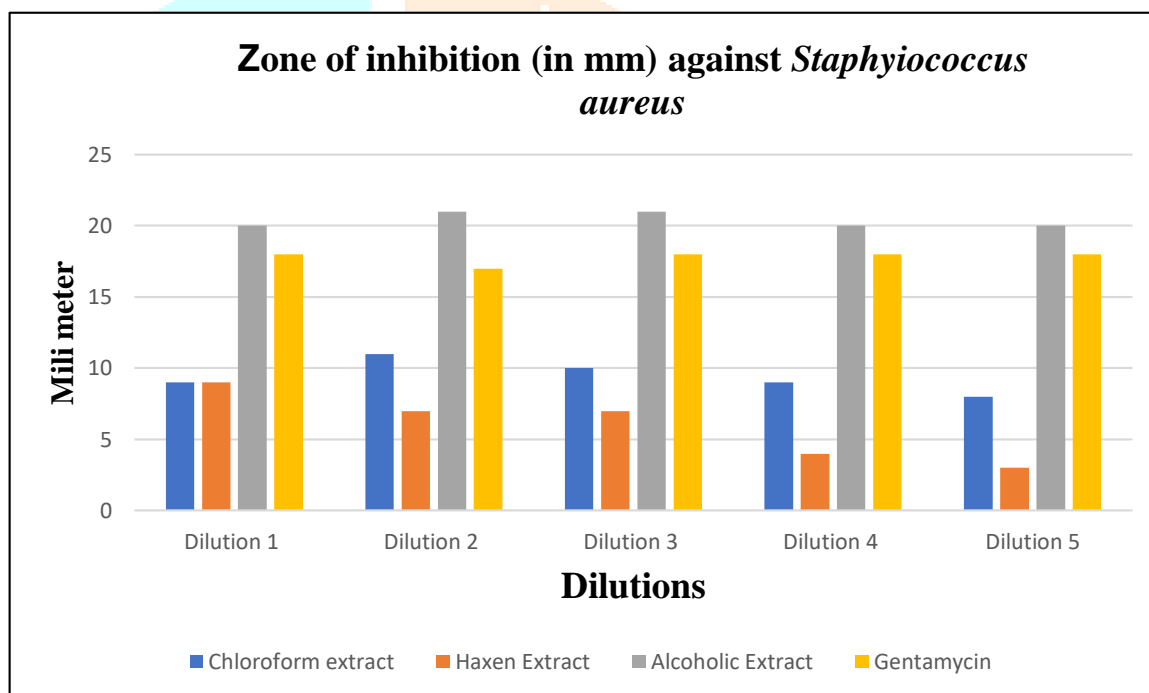
- 1) Hardness was determined using the Monsanto hardness tester.
- 2) Friability was determined using Roche Friabilator.
- 3) Thickness and diameter of the tablets were determined using Vernier calipers.
- 4) Weight variation test was carried out as per official methods with the specification limit.
- 5) Disintegration Test using USP-DT apparatus.
- 6) In-vitro drug release by Dissolution apparatus.

The observation of these all-quality control parameters of the herbal tablet is given in the table [18,19,20].

OBSERVATION-

Table no 2- Tulsi leaves serial dilution showing the Zone of inhibition (in mm) against *Staphylococcus aureus* (Gram-positive bacteria)

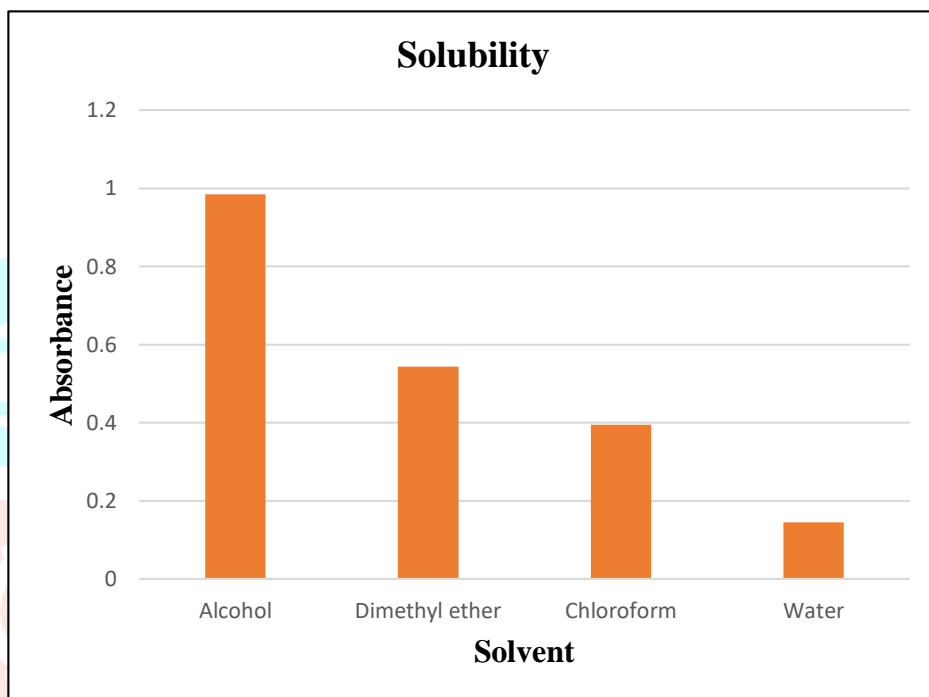
Extract	D1	D2	D3	D4	Dil5
Chloroform extract	9	11	10	9	8
Haxen Extract	9	7	7	4	3
Alcoholic Extract	20	21	21	20	20
Gentamycin	18	17	18	18	18



Graph No1- The Zone of inhibition of various concertation is seen in the graph

Table no 3- Solubility of Tulsi Extract

Sr. NO	Solvent	Absorbance
1	Alcohol	0.984
2	Dimethyl ether	0.544
3	Chloroform	0.095
4	Water	0.045



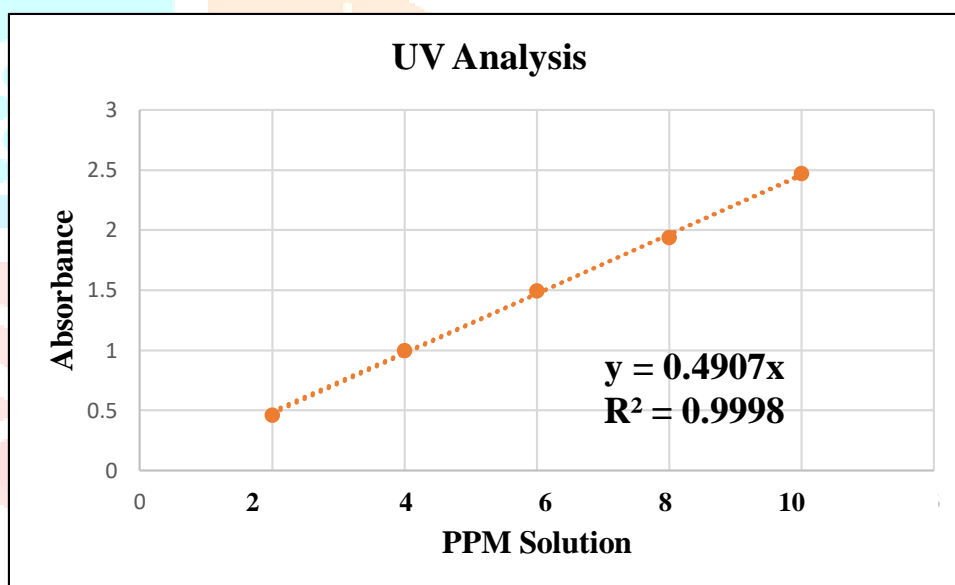
Graph no 2- In this graph shows the solubility of Tulsi exact in various solvents& various concentration

The Solubility of Extract in the Alcohol is the highest whereas the extract has very low solubility

in water

Table no 4 – UV Analysis of Tulsi Extract

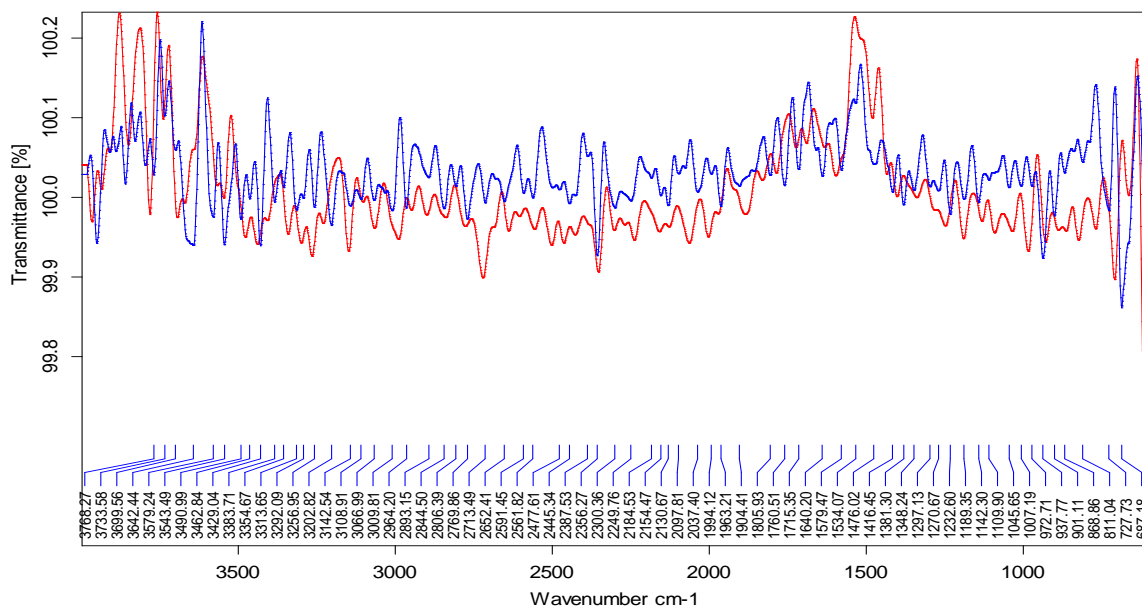
Sr. No	PPM solution	Absorbance
1	2	0.4548
2	4	0.9916
3	6	1.4901
4	8	1.9361
5	10	2.4674



Graph No 3- The graph shows the regression analysis & Slope of related that

Tulsiextract -polymer compatibility studies (FTIR Study):

Compatibility amongst both extract and the excipients used in the formulations was studied by FTIR analysis. An IR spectrum properly blended mixtures of the extract with the excipients were recorded in FTIR spectrophotometer in the scanning range of 1600-15850 & 1500-1400 cm^{-1} with a resolution of 4 cm^{-1} . The basic purpose was to observe any changes in the spectrum pattern of the extract due to polymers and thus identify the chances of any chemical interactions [21,22]



Graph No 4- The various functional group & compatibility of extract & other ingredients shown in this graph.

Table no 5- Pre compressional evaluation of the powder blend

Formula tions	Angle of repose	Bulk Density	Tapped Density	Compressibil ity Index (%)	Hausner"s Ratio
F1	23.56±0.695	0.740±0.002	0.881±0.001	13.521±0.008	1.182±0.002
F2	21.96±1.211	0.729±0.003	0.879±0.003	15.781±0.003	1.198±0.003
F3	23.78±0.473	0.702±0.009	0.809±0.009	13.928±0.007	1.189±0.002
F4	22.84±0.512	0.719±0.001	0.821±0.003	15.127±0.002	1.151±0.004

Table no 6- Post compression evaluation of herbal antimicrobial tablets.

formulations	Hardness (Kg/cm)	Friability	Diameter (mm)	Thickness (mm)	Weight variation (%)	Disintegration (min)
F1	3.27±0.12	4.27±0.32	7.49±0.002	4.62±0.005	8.12±0.51	44.30±1.0
F2	3.31±0.27	4.31±0.17	7.37±0.001	4.53±0.002	8.5±0.98	44.00±1.0
F3	3.10±0.11	4.33±0.21	7.67±0.003	4.64±0.003	8.07±0.52	43.00±1.0
F4	3.27±0.16	4.27±0.26	7.54±0.006	4.61±0.001	10.00±0.76	48.40±1.0

In vitro study-

In vitro dissolution study was performed by using a united states pharmacopeia(USP) Type II (Paddle) apparatus at a rotational speed of 50 rpm. Exactly 900 ml of 0.1N HCl is used as the dissolution medium and the temperature was maintained at $37^{\circ}\text{C} + 0.5^{\circ}\text{C}$ a sample of the solution was withdrawn from the dissolution apparatus at a specified time interval for 6 hrs and the same volume acid with pre-warmed fresh dissolution media.

Agitation speed (rpm): 50

Medium : 0.1 N HCl, 900 ml

Temperature : $37 \pm 0.5^{\circ}\text{C}$

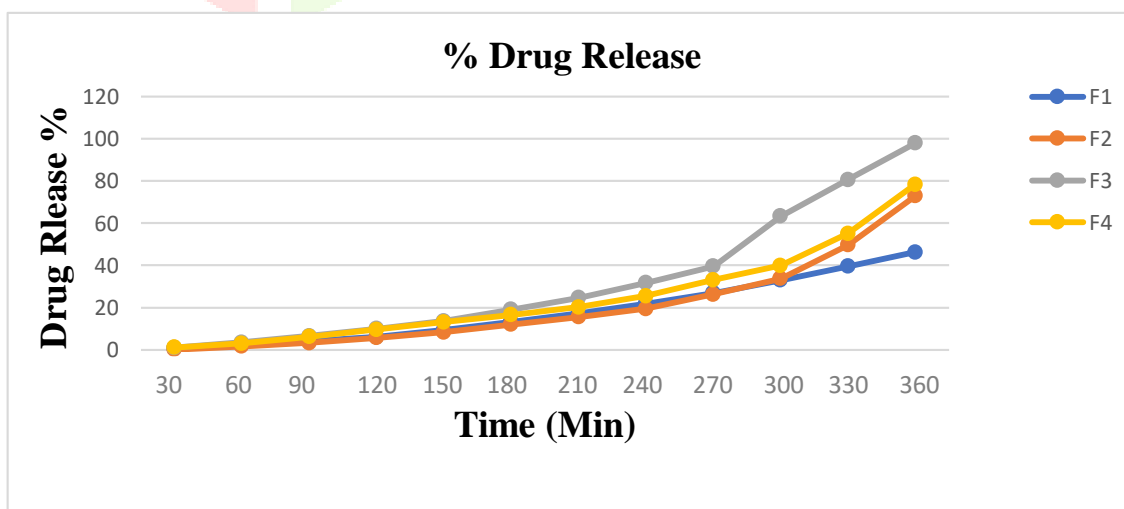
Time (hrs) : 30 min of interval

Wavelength : 396- 411 nm

The samples were withdrawn at predetermined time points, diluted 10 times, and were analyzed Spectrophotometrically at 396- 411 nm. In-Vitro drug release was performed.

Table no 7- The Percentage drug release record mentioned in the table as per particular minutes

Time (Min)	F1	F2	F3	F4
30	0.645708	0.099769	1.027226	1.087088
60	2.242018	1.572366	3.447233	2.952377
90	3.990777	3.257272	6.600745	6.205658
120	6.082742	5.674087	9.950603	9.632139
150	9.376729	8.359879	13.66123	13.06341
180	13.2837	11.93242	19.09427	16.54816
210	17.19306	15.51774	24.62708	20.17098
240	21.84711	19.45584	31.77217	25.55294
270	26.88507	26.28086	39.52465	33.05161
300	32.97659	33.72286	63.32804	39.93411
330	39.53184	49.68278	80.67036	55.1262
360	46.26109	72.99929	98.02944	78.43553



Graph no 5 - The Percentage drug release record mentioned in the graph

The graph shows that the formulation number F3-98.029% has the highest Drug release & Formulation number F1- 46.26% is the very less

RESULT:

The present study indicates that the *Ocimum sanctum* is a rich source of phytochemical constituents. The antibacterial efficacy of *Ocimum sanctum* leaves indicates that the plant has potent antibacterial properties, since *Ocimum*, which is very popular in India, can be touted as a readily available and regenerative source of antibacterial agent showing activity antibacterial instead of synthetic chemicals.

CONCLUSION:

Antibacterial activity of different *Ocimum sanctum* extracts against *Staphylococcus aureus* (Gram-negative bacteria) was studied. According to the results, all different types of extracts obtained from *Ocimum sanctum* leaves are shown to be with antibacterial activity against tested microbial pathogens. The herbal formulation of the tablet formulation number (F3) has the great activity of antimicrobial, and antifungal, compared to synthetic chemicals & this formulation we can use in various diseases without any side effects.

Conflicts of interest-

There are no conflicts of interest and disclosures regarding the manuscript

Acknowledgment-

The authors express their sincere gratitude to Swami Vivekanand Sanstha college of pharmacy, Mungase, University Libraries, sophisticated laboratories, and all other sources for their cooperation and advice in this research work.

REFERENCES-

1. Cohen MM. Tulsi - *Ocimum sanctum*: A herb for all reasons. Vol. 5, Journal of Ayurveda and Integrative Medicine. Medknow Publications; 2014. p. 251–9.
2. Bast F, Rani P, Meena D. Chloroplast DNA phylogeography of holy basil (*Ocimum tenuiflorum*) in Indian subcontinent. The Scientific World Journal. 2014;2014.
3. Sah AK, Vijayasimha M. The Tulsi, Queen of Green medicines Occurrence of Human parasitic infections in Uttarakhand View project. Int J Pharm Sci Rev Res [Internet]. 2018;50(2):106–14. Available from: <https://www.researchgate.net/publication/329705892>

4. Mahajan N, Rawal S, Verma M, Poddar M, Alok S. A phytopharmacological overview on *Ocimum* species with special emphasis on *Ocimum sanctum*. Vol. 3, Biomedicine and Preventive Nutrition. 2013. p. 185–92.
5. Pattanayak P, Behera P, Das D, Panda S. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Vol. 4, Pharmacognosy Reviews. 2010. p. 95–105.
6. Mondal S, Mirdha BR, Mahapatra SC. The science behind the sacredness of tulsi (*Ocimum sanctum* Linn). Indian J Physiol Pharmacol. 2009;53(4):1–16.
7. Mittal R, Kumar R, Chahal H. Antimicrobial activity of *Ocimum sanctum* leaves extracts and oil. Journal of Drug Delivery and Therapeutics. 2018 Nov 15;8(6):201–4.
8. S Amdeka, v Singh. *Ocimum Sanctum* (tulsi): Bio-pharmacological Activities. Webmed Central [Internet]. 2010;5(18):2–8. Available from: <https://www.researchgate.net/publication/48408293>
9. Yamani HA, Pang EC, Mantri N, Deighton MA. Antimicrobial activity of Tulsi (*Ocimum tenuiflorum*) essential oil and their major constituents against three species of bacteria. Frontiers in Microbiology. 2016;7(MAY).
10. Hammer KA, Carson CF, Riley T v. Antimicrobial activity of essential oils and other plant extracts. Vol. 86, Journal of Applied Microbiology. 1999.
11. Eswar P, Devaraj CG, Agarwal P. Anti-microbial activity of tulsi {*Ocimum Sanctum* (Linn)} extract on a periodontal pathogen in human dental plaque: An invitro study. Journal of Clinical and Diagnostic Research. 2016 Mar 1;10(3):53–6.
12. Mallikarjun S, Rao A, Rajesh G, Shenoy R, Pai M. Antimicrobial efficacy of Tulsi leaf (*Ocimum sanctum*) extract on periodontal pathogens: An in vitro study. J Indian Soc Periodontol. 2016 Mar 1;20(2):145–50.
13. Bharatkumar Khambholja D, Bharatbhai Mehta K. Efficacy of Tulsi (*Ocimum sanctum*) Extract Incorporated onto Guided Tissue Regeneration (GTR) Membrane against Periodontal Pathogens.

International Journal of Health Sciences & Research (www.ijhsr.org) [Internet]. 2019;9(2):68.

Available from: www.ijhsr.org

14. Jayanti I, Jalaluddin M, Avijeeta A, Ramanna PK, Rai PM, Nair RA. In vitro antimicrobial activity of ocimum sanctum (tulsi) extract on *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. *Journal of Contemporary Dental Practice*. 2018 Apr 1;19(4):415–9.
15. Jayanti I, Jalaluddin M, Avijeeta A, Ramanna PK, Rai PM, Nair RA. In vitro antimicrobial activity of ocimum sanctum (tulsi) extract on *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. *Journal of Contemporary Dental Practice*. 2018 Apr 1;19(4):415–9.
16. Parida A, Siddeeqh S, Jose M, Manju V. Antimicrobial effects of ocimum Sanctum on oral microbes. *Asian Journal of Pharmaceutical and Clinical Research*. 2018 May 1;11(5):316–8.
17. Sen G, Bera B. Mini Review Black tea as a part of daily diet: A boon for healthy living. Vol. 9, *International Journal of Tea Science*. 2013.
18. Mankar SD, Shaikh SB, Tamboli AA. Formulation of Herbal Tablet with the Help of Tulsi&Turmeric Extract which Showing Antimicrobial Activity. *Research J Science and Tech* [Internet]. 2020;12(1):1–6. Available from: www.anvpublication.orgwww.rjstonline.com
19. Magesh V, Lee JC, Ahn KS, Lee HJ, Lee HJ, Lee EO, et al. Ocimum sanctum Induces Apoptosis in A549 Lung Cancer Cells and Suppresses the In Vivo Growth of Lewis Lung Carcinoma Cells. *Phytother Res* [Internet]. 2009;23:1385–91. Available from: www.interscience.wiley.com
20. Nakamura CV, Ishida K, Faccin LC, Filho BPD, Cortez DAG, Rozental S, et al. In vitro activity of essential oil from *Ocimum gratissimum* L. against four *Candida* species. *Research in Microbiology*. 2004 Sep;155(7):579–86.
21. Nair VD, Jaleel CA, Gopi R, Gomathinayagam M, Panneerselvam R. Antioxidant potential of *Ocimum sanctum* under growth regulator treatments. *EurAsian Journal of Biosciences*. 2009;1–9.
22. Modak M, Dixit P, Londhe J, Ghaskadbi S, Paul T, Devasagayam A. Serial Review Indian Herbs and Herbal Drugs Used for the Treatment of Diabetes. Vol. 40, *J. Clin. Biochem. Nutr*. 2007.

23. Vyas A, Kumar Sonker A, Gidwani B. Carrier-based drug delivery system for treatment of acne. Vol. 2014, The Scientific World Journal. ScientificWorld Ltd.; 2014.
24. Zhou Q, Wang SS, Yang G, Zhao W, Li HL. Development and evaluation of a herbal formulation with anti-pathogenic activities and probiotics stimulatory effects. Journal of Integrative Agriculture. 2016 May 1;15(5):1103–11.
25. Vyas A, Kumar Sonker A, Gidwani B. Carrier-based drug delivery system for treatment of acne. Vol. 2014, The Scientific World Journal. ScientificWorld Ltd.; 2014.
26. Gong LH, Chen XX, Wang H, Jiang QW, Pan SS, Qiu JG, et al. Piperlongumine induces apoptosis and synergizes with cisplatin or paclitaxel in human ovarian cancer cells. Oxidative Medicine and Cellular Longevity. 2014;2014.
27. Gupta R. Herbal antibiotics: A Review. Bulletin of Environment, Pharmacology and Life Sciences [Internet]. 2020;9(11):1–8. Available from: <https://www.researchgate.net/publication/349088594>
28. Gupta P, Wright SE, Srivastava SK. PEITC treatment suppresses myeloid derived tumor suppressor cells to inhibit breast tumor growth. OncoImmunology. 2015;4(2):1–8.
29. Slobodníková L, Košťálová D, Labudová D, Kotulová D, Kettmann V. Antimicrobial activity of Mahonia aquifolium crude extract and its major isolated alkaloids. Phytotherapy Research. 2004 Aug;18(8):674–6.
30. Kaur N, Puri R, Jain SK. Drug-cyclodextrin-vesicles dual carrier approach for skin targeting of the anti-acne agent. AAPS PharmSciTech. 2010 Jun;11(2):528–37.
31. Bagade VB, Jadhav VM, Kadam VJ. International journal of pharmacy & life sciences Study on Antimicrobial activity of Herbal Formulation. Int J of Pharm & Life Sci (IJPLS). 2013;4(11):3099–104.
32. Isaac VLB, Chiari-Andréo BG, Marto JM, Moraes JDD, Leone BA, Corrêa MA, et al. Rheology as a tool to predict the release of alpha-lipoic acid from emulsions used for the prevention of skin aging. BioMed Research International. 2015;2015.

33. Fofaria NM, Srivastava SK. STAT3 induces anoikis resistance, and promotes cell invasion and metastatic potential in pancreatic cancer cells. *Carcinogenesis*. 2015 Jan 1;36(1):142–50.
34. Nasri H, Bahmani M, Shahinfard N, Nafchi AM, Saberianpour S, Kopaei MR. Medicinal plants for the treatment of acne vulgaris: A review of recent evidence. Vol. 8, *Jundishapur Journal of Microbiology*. Kowsar Medical Publishing Company; 2015.
35. Slobodníková L, Košťálová D, Labudová D, Kotulová D, Kettmann V. Antimicrobial activity of *Mahonia aquifolium* crude extract and its major isolated alkaloids. *Phytotherapy Research*. 2004 Aug;18(8):674–6.
36. Borges GSM, Prazeres PHDM, de Souza ÂM, Yoshida MI, Vilela JMC, E Silva ATM, et al. Nanostructured lipid carriers as a novel tool to deliver sclareol: Physicochemical characterisation and evaluation in human cancer cell lines. *Brazilian Journal of Pharmaceutical Sciences*. 2021 Oct 1;57.
37. Borges GSM, Prazeres PHDM, de Souza ÂM, Yoshida MI, Vilela JMC, E Silva ATM, et al. Nanostructured lipid carriers as a novel tool to deliver sclareol: Physicochemical characterisation and evaluation in human cancer cell lines. *Brazilian Journal of Pharmaceutical Sciences*. 2021 Oct 1;57.
38. Tajbakhsh A, Hasanzadeh M, Rezaee M, Khedri M, Khazaei M, ShahidSales S, et al. Therapeutic potential of novel formulated forms of curcumin in the treatment of breast cancer by the targeting of cellular and physiological dysregulated pathways. Vol. 233, *Journal of Cellular Physiology*. Wiley-Liss Inc.; 2018. p. 2183–92.
39. Guorgui J, Wang R, Mattheolabakis G, Mackenzie GG. Curcumin formulated in solid lipid nanoparticles has enhanced efficacy in Hodgkin's lymphoma in mice. *Archives of Biochemistry and Biophysics*. 2018 Jun 15;648:12–9.
40. Lima AM, Pizzol CD, Monteiro FBF, Creczynski-Pasa TB, Andrade GP, Ribeiro AO, et al. Hypericin encapsulated in solid lipid nanoparticles: Phototoxicity and photodynamic efficiency. *Journal of Photochemistry and Photobiology B: Biology*. 2013;125:146–54.

41. Clemente N, Ferrara B, Gigliotti CL, Boggio E, Capucchio MT, Biasibetti E, et al. Solid lipid nanoparticles carrying temozolomide for melanoma treatment. Preliminary in vitro and in vivo studies. *International Journal of Molecular Sciences*. 2018 Feb 1;19(2).
42. Clemente N, Ferrara B, Gigliotti CL, Boggio E, Capucchio MT, Biasibetti E, et al. Solid lipid nanoparticles carrying temozolomide for melanoma treatment. Preliminary in vitro and in vivo studies. *International Journal of Molecular Sciences*. 2018 Feb 1;19(2).
43. Jiao Z, Shi XJ, Li ZD, Zhong MK. Population pharmacokinetics of sirolimus in de novo Chinese adult renal transplant patients. *British Journal of Clinical Pharmacology*. 2009 Jul;68(1):47–60.
44. Ramos J, Taylor D, Rege K. Gold nanoparticle mediated photo-Chemotherapy. Vol. 3, *Journal of Nanomedicine and Nanotechnology*. 2012. p. 9.
45. Desgrosellier JS, Cheresch DA. Integrins in cancer: Biological implications and therapeutic opportunities. Vol. 10, *Nature Reviews Cancer*. 2010. p. 9–22.
46. Khan VR, Brown IR. The effect of hyperthermia on the induction of cell death in brain, testis, and thymus of the adult and developing rat. Vol. 7, *Cell Stress & Chaperones*. Cell Stress Society International; 2002.
47. Rand RW, Bristol Ave N. United States Patent (19) Rand et al. (54) induction heating method for use in causing necrosis of neoplasm.
48. Tra VN, Dube DH. Glycans in pathogenic bacteria-potential for targeted covalent therapeutics and imaging agents [Internet]. Vol. 00, *Chem. Commun.* 2014. Available from: www.rsc.org/chemcomm
49. Tra VN, Dube DH. Glycans in pathogenic bacteria-potential for targeted covalent therapeutics and imaging agents [Internet]. Vol. 00, *Chem. Commun.* 2014. Available from: www.rsc.org/chemcomm
50. Matteis V, Cascione M, Brunetti V, Toma CC, Rinaldi R. Toxicity assessment of anatase and rutile titanium dioxide nanoparticles: The role of degradation in different pH conditions and light exposure. *Toxicology in Vitro*. 2016 Dec 1;37:201–10.