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

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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.
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. antibacterial agents instead of synthetic chemicals.

**KEY 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. Ocimumtenuiflorum (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 Ocimumgratissimum 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 Ocimumtenuiflorum having six times less DNA than Ocimumgratissimum is traditionally used in the same way to treat similar ailments. For consistency, this review uses the term Tulsi to refer to both Ocimumtenuiflorum and Ocimumgratissimum [5].

EXPERIMENTAL WORK-

Material & methods-

Tulsi leaves were obtained from the medicinal garden of Swami VivekanandSansthaCollege 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 gOcimum 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 extractobtained 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 theOcimum 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 stoke 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]

![Extraction assembly & Extract photograph](image)

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 μm 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 flack, 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].

![Image](image_url)

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

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

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

<table>
<thead>
<tr>
<th>Extract</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>Dil5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform extract</td>
<td>9</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Haxen Extract</td>
<td>9</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Alcoholic Extract</td>
<td>20</td>
<td>21</td>
<td>21</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>18</td>
<td>17</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

![Zone of inhibition (in mm) against Staphylococcus aureus](image)

**Graph No1-** The Zone of inhibition of various concertation is seen in the graph
Table no 3- Solubility of Tulsi Extract

<table>
<thead>
<tr>
<th>Sr. NO</th>
<th>Solvent</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alcohol</td>
<td>0.984</td>
</tr>
<tr>
<td>2</td>
<td>Dimethyl ether</td>
<td>0.544</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>0.095</td>
</tr>
<tr>
<td>4</td>
<td>Water</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Graph no 2- In this graph shows the solubility of Tulsi extract in various solvents & various concentrations.
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

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>PPM solution</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.4548</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.9916</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>1.4901</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>1.9361</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>2.4674</td>
</tr>
</tbody>
</table>

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

Tulsi extract - 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

<table>
<thead>
<tr>
<th>Formula</th>
<th>Angle of repose</th>
<th>Bulk Density</th>
<th>Tapped Density</th>
<th>Compressibility Index (%)</th>
<th>Hausner”s Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>23.56±0.695</td>
<td>0.740±0.002</td>
<td>0.881±0.001</td>
<td>13.521±0.008</td>
<td>1.182±0.002</td>
</tr>
<tr>
<td>F2</td>
<td>21.96±1.211</td>
<td>0.729±0.003</td>
<td>0.879±0.003</td>
<td>15.781±0.003</td>
<td>1.198±0.003</td>
</tr>
<tr>
<td>F3</td>
<td>23.78±0.473</td>
<td>0.702±0.009</td>
<td>0.809±0.009</td>
<td>13.928±0.007</td>
<td>1.189±0.002</td>
</tr>
<tr>
<td>F4</td>
<td>22.84±0.512</td>
<td>0.719±0.001</td>
<td>0.821±0.003</td>
<td>15.127±0.002</td>
<td>1.151±0.004</td>
</tr>
</tbody>
</table>
Table no 6- Post compression evaluation of herbal antimicrobial tablets.

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

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°C ± 0.5°C. A sample of the solution was withdrawn from the dissolution apparatus at a specified time interval for 6 hrs and the same volume added with pre-warmed fresh dissolution media.

Agitation speed (rpm): 50

Medium : 0.1 N HCl, 900 ml

Temperature : 37 ± 0.5°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

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.645708</td>
<td>0.099769</td>
<td>1.027226</td>
<td>1.087088</td>
</tr>
<tr>
<td>60</td>
<td>2.242018</td>
<td>1.572366</td>
<td>3.447233</td>
<td>2.952377</td>
</tr>
<tr>
<td>90</td>
<td>3.990777</td>
<td>3.257272</td>
<td>6.600745</td>
<td>6.205658</td>
</tr>
<tr>
<td>120</td>
<td>6.082742</td>
<td>5.674087</td>
<td>9.950603</td>
<td>9.632139</td>
</tr>
<tr>
<td>180</td>
<td>13.2837</td>
<td>11.93242</td>
<td>19.09427</td>
<td>16.54816</td>
</tr>
<tr>
<td>210</td>
<td>17.19306</td>
<td>15.51774</td>
<td>24.62708</td>
<td>20.17098</td>
</tr>
<tr>
<td>270</td>
<td>26.88507</td>
<td>26.28086</td>
<td>39.52465</td>
<td>33.05161</td>
</tr>
<tr>
<td>300</td>
<td>32.97659</td>
<td>33.72286</td>
<td>63.32804</td>
<td>39.93411</td>
</tr>
<tr>
<td>330</td>
<td>39.53184</td>
<td>49.68278</td>
<td>80.67036</td>
<td>55.1262</td>
</tr>
<tr>
<td>360</td>
<td>46.26109</td>
<td>72.99929</td>
<td>98.02944</td>
<td>78.43553</td>
</tr>
</tbody>
</table>

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.

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