

Formulation of *Ocimum Sanctum* transdermal patch and *in silico* exploration for mosquito repellent activity

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Abstract

Ocimum sanctum is known for its insecticide and mosquito repellent activity. The present study aimed to formulate and evaluate the mosquito repellent patch. The patch can be placed on the skin or the cloth to prevent mosquito bites. Tulsi being an herbal drug is safe and effective for use, it helps to protect from mosquitoes that spread malaria, dengue, and yellow fever. The transdermal mosquito repellent patch was prepared by incorporating the ingredients namely, Tulsi leaves (*Ocimum Sanctum*), polyvinyl alcohol, carbapol, lemon oil, methylparaben, and ethanol were envisaged. The solvent Evaporation Technique was used for the preparation of the patch. The *in silico* studies for mosquito repellent activity were carried out using AutoDock vina. The prepared mosquito repellent patch was evaluated for organoleptic characteristics and other physicochemical properties, such as thickness, weight uniformity, folding endurance, moisture content, drug content, and tolerability and acceptability of the patch. The presence of conventional hydrogen bond interactions of active constituents of the tulsi plant with amino acid residues of the binding cavity of general odorant binding protein 2 of *B. Mori* resulted in *in silico* mosquito repellent activity. The mosquito repellent patch is a small transdermal patch that is applied directly to the surface of the skin. On application, the patch transmits a steady dosage of thiamine, the active component of Vitamin B1, through the skin. The natural vitamin flows through the bloodstream and is released from the skin pores, protecting the entire body from painful mosquito bites.

Keywords: Transdermal drug delivery system, Tulsi, formulation, mosquito repellent, *in-silico* study.

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Introduction:

Transdermal delivery has been confirmed to be one of the most favorable methods among novel drug delivery systems. Since these drugs administered by transdermal delivery systems avoid the gastrointestinal tract and thus avoid conversion by the liver, the side effects of GI irritation and liver dysfunction effects seem to be low. Transdermal medication administration is performed utilizing patches that comprise distinct and particular layers (AMELIA-YAP *et al* 2022). The use of these systems can overcome the drawbacks linked with other routes of administration such as oral and parenteral.

Drug delivery system innovation is a fundamental method used to increase the bioavailability of active medicinal compounds (APIs). Due to the benefits given, such as dose form diversity, painless simplicity of administration, convenience, self-administration, high safety, and patient compliance, oral delivery systems remain the most preferred way for administering API to date.

The skin is used as the medication administration location in transdermal drug delivery systems. The delivered medicine enters the systemic circulation via blood vessels in the skin and circulates throughout the body.

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Transdermal drug delivery systems have some benefits for patients, like being less invasive (some methods are completely noninvasive), avoiding first-pass metabolism, being easy to apply and administer, not requiring expert personnel, and having the ability to reduce the frequency of administration (ASHTEKAR AND BHATIA 2022).

Herbal dhoop/tulsi -Transdermal drug delivery systems have some benefits for patients, such as being less invasive (a few techniques are completely noninvasive), avoiding first-pass metabolism, being easy to apply and administer, not requiring expert personnel, and having the ability to reduce the frequency of administration (EBERHARDT *et al* 2021).

India is having a history of using medicinal plants for millennia and the tulsi plant is one of these medications. It is prevalent across India. It is harvested in the vicinity of Hindu temples or other places of devotion. Ayurvedic medicine uses its leaves, seeds, and roots. Tulsi is high in nutrients and bioactive substances. Linalool, caryophyllene, oleanolic acid, and rosmalinic acid are the main chemical components of tulsi (HAIGH AND SMITH 1994).

Arboviruses' pervasiveness in causing havoc on healthcare has lasted on worldwide health agendas. A lack of mosquito-borne illness vaccines and treatments necessitates immediate attention, as billions of people globally are in danger of infection (KAUR *et al* 2022).

For a long time, natural plant chemicals have been employed effectively to combat insect pests. Plant-based bioinsecticides are currently seen to be a far safer and less hazardous alternative to synthetic pesticides (KUMAR *et al* 2022)

Insecticide-treated mosquito nets, textiles, and indoor sprays have all been successfully utilized to protect individuals from mosquito bites. Despite significant success in lowering mosquito-borne illnesses, widespread pesticide use has resulted in substantial health issues for humans and animals, pest management or lack of sensitivity in mosquitoes, and environmental degradation (KUMAR *et al* 2022)

Materials and Methods:

Materials

Fresh and natural leaves of Tulsi also known as - Sacred basil, Great basil, Kali – Tulsi, Veranda. Tulsi consists of fresh and dried leaves of *Ocimum* species like *Ocimum sanctum* Linn and *Ocimum basilicum*

Linn, etc. belonging to the Lamiaceae Family, which were extracted from the plant. The plant materials were identified, cleaned, and dried in shade at room temperature moreover, dried plant materials were finely pulverized using a grinder. Carbapol 934, Methylparaben, Ethanol, and Polyvinyl alcohol were used in the Laboratory. From the local market, lemon oil was extracted. In silico studies were carried out using Auto Dock vina (LEWIS *et al* 2006, National Library of Medicine 2022)

Methods

Preparation of Tulsi leaves extract:

Basil is an erect, much-branched, fragrant, and erected plant attaining a height of about 30-60 cm when mature. Its aromatic leaves are simple tulsi flowers that are small having purple to reddish color, present in small compact clusters on cylindrical spikes. Stalkless heart-shaped bracts are there at the base of each flower cluster. The sepal cup is not hairy within. Flowers are rarely longer than 5mm, and calyx tubes bearded outside near the base. The flower tube is hairy. The fruits are small and the seeds are yellow to reddish. Sweet basil (*Ocimum basilicum*) will grow to a size of 1-2 feet in height. Holy Basil will prolifically produce large green leaves, measuring around 2 inches in length, throughout the summer. Holy Basil flowers are white and are commonly removed to increase the yield of leaves. Cultivars of sweet basil include Lemon Basil, Italian or Curly Basil, and Lettuce-leaf Basil; the names of these cultivars give way to their variances. Tulsi can synthesize and convert phenylpropenes (important chemicals in determining the flavor of herbs which can also act as cultivator attractants or herbivore deterrents) (PATEL 2012).

Fresh and mature leaves of Tulsi were plucked from the Tulsi (*Ocimum sanctum*) plant. The leaves were washed properly under running tap water followed by distilled water. Leaves were dried in a hot air oven at a temperature of 40°C. Dried leaves were powdered using a mechanical grinder. 100 gm of plant powder was taken and soaked into a 200ml ethanol solvent. Shaken on a mechanical shaker at 150 rpm with a temperature of 25°C to obtain the extract. The soaking process was repeated three times. The extract was obtained then evaporated and concentrated under reduced pressure using rota vapor. The dried extract was collected in an airtight container and stored for further use.



Figure 1. Dried tulsi extract

Formulation of transdermal patch

The patch was prepared by solvent evaporation technique. The polymer PVA was dissolved into the water using a homogenized mixer with continuous stirring, the mixture was cooled. Carbapol 950 was dissolved in ethanol and these prepared mixtures were added to the PVA mixture. Methylparaben and perfuming agent were added to the above mixture. The solution was then poured into the Petri plate to prevent fast evaporation of the solvent. The films were dried at room temperature for 24 hrs. Then dried films were cut into 2 cm² - 2cm².

Evaluation of Prepared Patch

Organoleptic Characteristics: The prepared patch was physically inspected for its appearance, color, clarity, flexibility, and smoothness (Ramadon *et al* 2022).

Thickness: The thickness of the patch was measured by Vernier calipers. The thickness uniformity was measured at different sites and the average was calculated (Ramadon *et al* 2022)

Weight Uniformity: The patch was taken and folded at the same point till it breaks. The number of times the patch could be folded without breaking was noted.

Folding Endurance: The patch was taken and folded repeatedly at the same point till it breaks. The number of times the patch could be folded without breaking was noted (RCSB Protein Data Bank 2022)

Moisture Content: The prepared patch was weighed and kept in the desiccator containing fused calcium chloride for about 24 hrs. After that, it was taken out

and weighed again. The percentage of moisture content was calculated based on the following formula (Reddy *et al* 2003, Sengul Demirak and Canpolat 2022)

$$\text{Percentage of moisture content} = \frac{[\text{Initial weight} - \text{Final weight}]}{\text{Final Weight}} \times 100$$

Drug Content: The patch was dissolved in methanol and the remaining volume was made up of distilled water to 100 ml. Then the solution was filtered and the absorbance of the solution was taken at 304nm and concentration was calculated (Sengul Demirak and Canpolat 2022).

In silico analysis for mosquito repellent activity

The study was focused to check the affinity of formulation as mosquito repellent activity. Docking was carried out using AutoDock ver. 4.0, which utilizes the Lamarckian genetic algorithm (LGA) for examining the conformational space of ligands. The scoring of compounds uses the energy-based scoring function which includes interactions of hydrogen bonds, electrostatic, aromatic, or Pi and Van Der Waals. In this regard, the structures of major active constituents present in the tulsi plant were downloaded from PubChem data base (Shah *et al* 2010) which includes structures of carnosic acid (CID: 65126), eugenol (CID: 3314), isothymusin (CID: 630253), rosmarinic acid (CID: 5281792), sinapic acid (CID: 637775) and ursolic acid (CID: 64945). The receptors structure of general odorant binding protein 2 of B. Mori (PDB id: 2WC5) was downloaded from the RCSB protein data bank (Suryadevara 2010). The optimization of protein was performed using Biovia Discovery studio by removal of heteroatoms and addition of polar hydrogen atoms

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to the corresponding carbon atoms. The grid size of 50:50:50 for XYZ points, grid spacing of 0.3750 Å, and grid center dimensions at x, y, and z: 7.063, -4.029, 15.823, was used for 2WC5. All other parameters were set to their default settings (TROTT AND OLSON 2010)

Results

In this study, an aqueous extract of the *Ocimum Sanctum* leaves was screened for the presence of major phytochemical groups (Figure 1). The preliminary phytochemical screening revealed the

Table 1: Organoleptic characteristics of the prepared patches.

Sr. No.	Physical Characteristic	Result
1.	Appearance	Jelly like
2.	Colour	Colourless
3.	Clarity	Slightly Opaque
4.	Flexibility	Good
5.	Smoothness	Good

Thickness: The thickness of the patches was measured in triplicate and the mean thickness of prepared patches was observed as 0.6 mm (Table 2) indicating the formulated patch with appropriate thickness.

Table 2: Thickness of patch.

Sr. No.	Thickness (in mm)
1	0.67
2	0.59
3	0.58
Mean	0.6

presence of phenolic compounds, flavonoids, alkaloids, tannins, saponins, glycosides, protein, and phytosterols, whereas, carbohydrates and fats were not present in the extract. These phytochemical compounds are the key candidates for the medicinal value of this plant.

Organoleptic Characteristics of the prepared patches. The prepared patches were slightly opaque, pale-colored, and jellified preparations showing good flexibility and smoothness as given in, Table 1.

Weight Uniformity. The mean weight of prepared patches in triplicate was observed as 0.28 gm as mentioned in Table 3.

Table 3: Weight uniformity of patch.

Sr. No.	Weight
1	0.31 gm
2	0.28 gm
3	0.26 gm
Mean	0.28 gm

Folding Endurance. The mean folding endurance of prepared patches was 74.6 as given in Figure 3, Table 4.



Figure 2. Folding Patch

Table 4: Folding endurance of patch.

Sr. No.	Folding endurance
1	77
2	74
3	73
Mean	74.6

Moisture Content. The mean moisture content of prepared patches was 5.76 % as given in Table 5.

Table 5: Moisture content of patch.

Sr. No.	Moisture Content
1	6.12 %

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2	5.81 %
3	5.36 %
Mean	5.76 %

In-silico studies

In silico studies showed the binding affinity of active constituents of tulsi towards general odorant binding protein 2 of *B. Mori* (PDB id: 2WC5). The docking score and amino acid residues interactions are represented in Table 6. The binding score of the standard compound citronellal was found to be -6.4. The active constituents of the tulsi plant were observed to show docking scores in the range of -3

to -7.8. The ursolic acid showed the highest binding affinity with a score of -7.8 while eugenol showed the lowest of -5.3. The eugenol showed conventional hydrogen bond interaction with amino acid residue THR9 which was present with standard citronellal. All the active constituents from tulsi were observed to have interactions with amino acid residues of a binding cavity or fit in the vicinity of a binding cavity of general odorant protein 2. The rosmarinic acid and eugenol showed good affinity as well as best fit in the binding cavity indicating the developed patch will work for mosquito repellent activity (Figure 4).

Table 6: In silico analysis for mosquito-repellant activity.

Compound name	Docking score	Interactions
Carnosic acid	-6.3	ASP31 (Pi-anion), ASP28, ILE29 (H bond)
Eugenol	-5.3	PHE33 (H bond), THR9 (C-H bond), LYS14, TYR141, VAL134 (PI-alkyl), ILE138 (Pi-sigma)
Isothymusin	-6.3	ASP31 (H bond), HIS35, PHE41 (Pi-Pi), ASP40, ASP42 (C-H)
Rosmarinic acid	-6.1	ASP31 (H bond), GLU32 (Pi-anion), ILE138 (Pi-sigma)
Sinapic acid	-5.6	ILE138, LYS34 (H bond), VAL134, LEU16 (Pi-alkyl)
Ursolic acid	-7.8	ALA10 (H bond)
Citronellal	-6.4	THR9, TRP37 (H bond), PHE12, PHE118 (Pi-sigma), VAL114, ILE94 (Pi-alkyl), PHE36, ALA115, ILE52 (Alkyl)

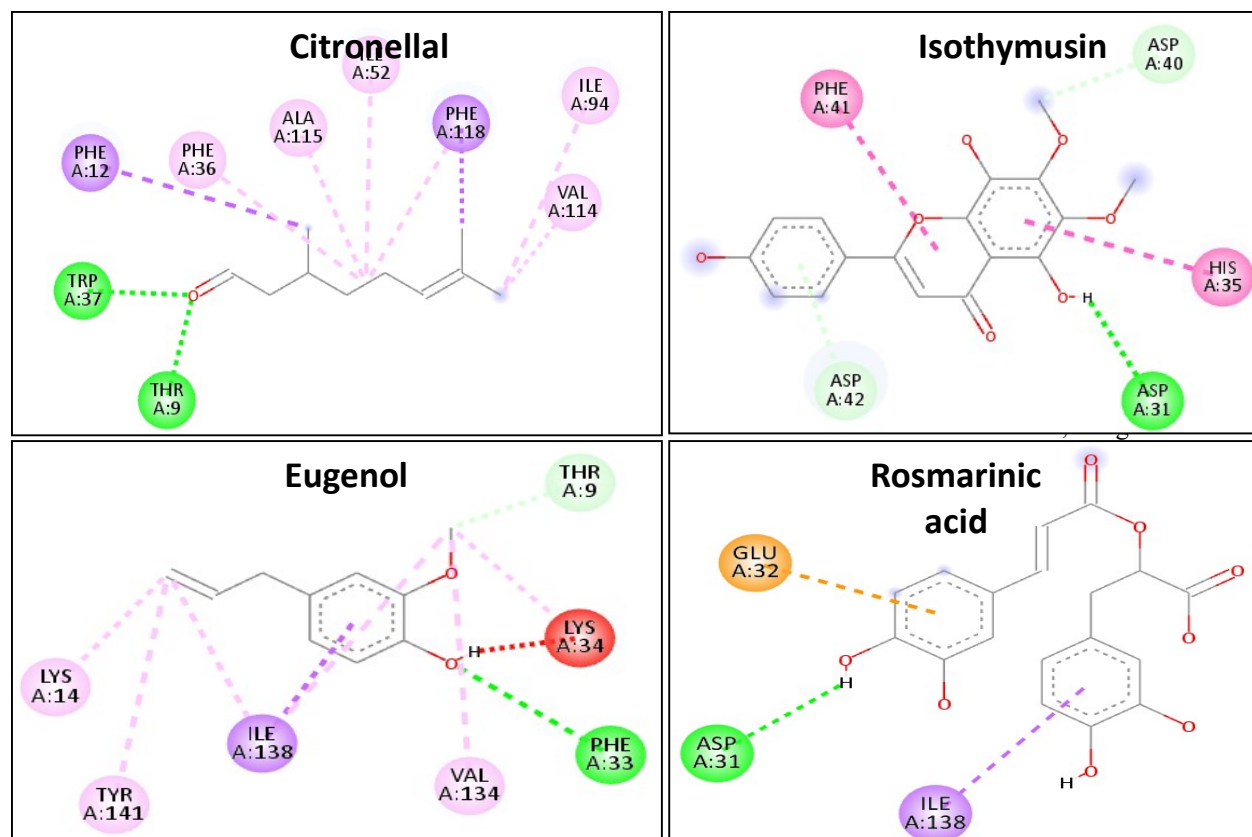


Figure 3. Interactions of active constituents of tulsi with 2WC5

Discussion

The use of synthetic products as mosquito repellants leads to several human health hazards. An alternative solution is to use natural products that possess good effectiveness. The natural product contains essential oils from plants that have been extensively tested to assess their insect-repellant properties. In the present study, *Ocimum Sanctum* used as the active herbal drug which shows mosquito repellent activity. It also has antibacterial and Anti-inflammatory activity. The herbal transdermal patch was evaluated for different parameters. It was found that the film was transparent, clear, and colorless with no liquid content. For optimal protection, apply the patch two hours before venturing outside, and apply two patches if the weight is more than 80 lbs. Tulsi contains carnosic acid, eugenol, isothymusin, rosmarinic acid, sinapic acid, and ursolic acid as active phytoconstituents which showed affinity like the standard citronellal compound indicating the mosquito repellent activity. Additionally, due to the presence of Vitamin B1, they penetrate our body and flow through the bloodstream and releases from skin pore, and protect the whole body from a mosquito bite.

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