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Research Article

Formulation And Evaluation Of Herbal Dhoop From Temple Waste For Mosquito Repellent Activity

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ABSTRACT

Mosquito borne diseases is a major health issue nowadays. Numerous diseases including dengue, malaria and yellow fever are mostly transmitted by mosquitoes. This research work focuses on the three factors Recycle, Reuse, and Recover. This 3 Rs are crucial to any waste management procedure. This study identifies the use of marigold waste to prepare Herbal Dhoop. There are a lot of chemical-based mosquito repellents in the market that are bad for the environment and people. This attempt was made just by using natural components & Temple flowers. Various evaluation parameters have been examined on the prepared dhoop such as Mosquito repellent activity, Microbiological evaluation, Moisture content, Consistency, Irritability, Burning time, Ash value, Colour & Odour. The creation and assessment of a natural and herbal dhoop formulation & evaluation for environmental purification is the main focus of the current work. The aim is to suggest that dhoop production could become a possible market in the future if a focused approach is adopted and natural ingredients are used. It was also determined that the herbal dhoop stick formulation preserves a healthy, mosquito-free environment for society and is more cost-efficient, non-toxic, and effective than the commercially available insect repellents based on chemicals. This formulation can be used as a substitute for other commercially available chemical-based mosquito repellents because it is less harmful, environmentally friendly, and prevents insects from developing resistance.

INTRODUCTION

Mosquito borne diseases

The World Health Organization (WHO) estimates that malaria, one of the common mosquito-transmitted diseases, kills about 3 million people

worldwide each year [1]. This disease, which are only transmitted to humans by mosquito bites, do not yet have an effective vaccination for either prevention or treatment. Avoiding mosquito bites is one of the most important ways to prevent or

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lessen the spread of these diseases [2]. Treatments for malaria and other diseases carried by mosquitoes are numerous, but preventing illness altogether is always preferable. Consequently, the word "mosquito repellent" was created. Compounds known as repellents work by making surfaces uncomfortable for mosquitoes. To prevent mosquitoes from landing on skin or other surfaces, apply it on [3]. The biggest concern in the modern era is environmental change. The population's attention has been drawn by the extent of ongoing pollution. A clean environment is essential to human survival on this planet. A clean environment includes fresh air to breathe, potable water, fertile land, and a suitable energy supply [4,5]. These days, a variety of methods are used to purify the air so that people can breathe it. Although there are a lot of chemical alternatives for the same purpose on the market, they do have a lot of unfavorable side effects that can be harmful to living things [6,7]. Herbal products are a viable substitute for chemicals to prevent their undesirable effects. Herbal products not only purify the air, but they also contribute properties like aroma and positivity to the space in which they are utilized. The current effort primarily focuses on developing the technique of creating dhoop sticks, which can be used to reduce the number of microorganisms in the air without the need for chemicals. All of the essential ingredients needed to prepare the dhoop sticks are natural. Clarified butter, some herbs, cow manure, and cow milk are among the ingredients. Since ancient times, cow dung has been utilized as a disinfectant [8]. Several religions utilize materials like cow dung, camphor, cow urine, and cow ghee to perform rituals like "home" or "havens," which help to purify the surroundings and create a nice atmosphere [9]. Through the use of traditional knowledge, a technique for making dhoop sticks

from readily available components has been created. The three Rs—Recycling, Reuse, and Recovery—are particularly crucial in any waste management process. Concerns for human society include destruction of the environment, climate change, waste management and disposal, and sustainable development.

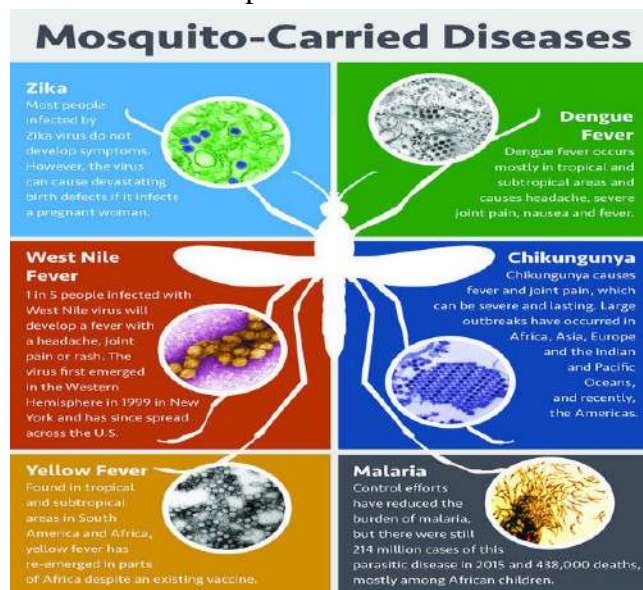


Figure No 1: Mosquito borne diseases

India is home to a great number of temples that draw a substantial number of believers. The pilgrims present the God with flower garlands, veggies, coconuts, chocolates, and other foodstuffs. Typically, the tempting treats are removed and given to the devotees as prasada, after which priests, temple employees, and other staff members eat them. Whenever not edible items, like flower garlands, are offered to God, they are discarded. The majority of items in municipal solid waste (MSW) are typically more biodegradable (>70%) and may contribute to greenhouse gas emissions in the environment [10]. The country's rapidly increasing urbanization has made trash management a major problem. India has 7,935 cities and towns home to more than 377 million urban residents, who generate 62 million tones (Mt) of MSW annually. 31 Mt of garbage is dumped in landfills, 11.9 Mt is handled, and only

43 Mt of waste is collected [11] . 1.5 Mt of the 62 Mt of waste produced is e-waste, 7.90 Mt is hazardous waste, 0.17 Mt is hospital/biomedical waste, and 5.6 Mt is plastic waste. Only 22–28% of the municipal waste that is collected gets handled and processed, out of a total of 75–80% collected [12]. Over 90% of MSW in India is disposed of inappropriately and unscientifically on land [13] . India is the home of a wide variety of civilizations and faiths. India is home to many distinct religions, but despite this diversity, each location has a place of worship. The country is distinguished by its various philosophies, traditions, and rituals. Temples are seen as a representation of worship and prayer. Every region of the nation is home to a large number of temples, which generate holy waste and temple rubbish. Floral trash, commonly known as flower waste, is included in MSW. Thus, management is also very important. An estimated 800 million tons of flowers are presented annually in India's temples, mosques, gurudwaras, and other places of worship [12]. The deterioration of flower waste proceeds much more slowly than that of kitchen garbage [14]. Therefore, a suitable and eco-friendly method of treating flower debris is required. The flower offerings can be turned into useful goods in order to avoid the bad effects of throwing them out. One way to make herbal incense sticks is by gathering flowers from temples. Rose water is manufactured from roses, and burning sticks are made from flowers like marigold.



Figure No.2: Collection of Floral Waste from Temples

Problems due to mismanagement of flower waste

Temple offerings are regarded as sacred hence it is not advised to dispose of them in landfills. The majority of temples dump trash into nearby lakes, ponds, and rivers. Currently, the least environmentally beneficial way to dispose of flowers is to throw them into water bodies in different parts of India. Every year, about 8 Mt of floral waste are disposed of in Indian rivers [15]. The sheer volume of pollutants produced by the flowers is astounding. Since the majority of flower debris is dumped straight into lakes, the organic substance in the flowers breaks down and causes eutrophication and algae blooms, which can further drop oxygen levels in the water and kill marine life. These withered blossoms can potentially pollute the earth. The environment is eventually altered by these kinds of actions. Numerous temples across the nation, especially those situated in the Ganga basin, dispose of their daily waste into the river without distinguishing between non-biodegradable and biodegradable materials. While there are other factors contributing to the pollution in the Ganga, flower waste is not only to blame. 16 percent of the river's overall pollution is caused by floral waste [12].

Phytochemical Investigation of Marigold :[16]



Biological name:

Calendula officinalis, Tagetes erecta

Family:

Asteraceae

Chemical Constituents:

Alkaloids, flavonoids, steroids, terpenoids

Uses:

Antifungal, Antioxidant, Antibacterial



Figure No.3: Calendula officinalis (Marigold)

Herbal mosquito repellent Dhoop sticks

The relaxing aromas of dhoop sticks help to calm the mind and create a peaceful atmosphere around them. Mosquito-repelling qualities are found in many plants, which is important for illness prevention. They usually have no negative effects on the environment and are environmentally beneficial because they are constructed of plants. They offer mosquito protection in this way without causing any unfavorable side effects. While synthetic coils have negative consequences and can be harmful, natural coils are effective and useful. Herbal dhoop sticks can be an ideal replacement to avoid the negative consequences of the chemicals added to commercialized insect repellents, which have an influence on both people and the environment. The Dhoop tree, which is native to eastern India, is the source of the word Dhoop. When these trees are burned, the fragments release a nice scent. Agarbatti or incense sticks are not comparable to dhoop or dhoopbatti. Even in terms of look, they differ. While dhoop is available in a paste type with a

small amount of moisture in it, incense is dry and found as a stick [7].

Types of incense [7]

Different varieties of incense exhibit varying medicinal qualities. A few different kinds of incense sticks are listed below.

1. Dragon's Blood Incense:

This incense relieves symptoms of stomach viruses, diarrhea, intense fevers, and ulcer pain, among other medical conditions.

2. Lavender Incense:

It offers a relaxing and sedative effect. Getting some relief after a demanding stressful day is essential.

3. Sandalwood Incense:

It eases anxiety and gives one a sense of spirituality.

4. Indian Cedar Incense:

Aids in the healing of sadness and mood problems

5. Amber Incense

The body's numerous systems are in balance

6. Patchouli incense:

it strengthens and calms the nerves

Different types of dhoopbatti [7]

a. Charcoal Type:

To make this type of dhoopbatti, an unscented stick is dipped in an oil and perfume combination. In addition, charcoal is added, which will serve as fuel for burning the dhoop. Black resins, which give the property of binding, are used to attach the entire mixture to the stick.

b. Masala Dhoop:

As the name implies, a solid Dhoopbatti is prepared by combining a number of aromatic components. Then, it is transformed into a sticky paste by adding water or other adhesives. For the creation of masala Dhoop, a variety of natural components are used, including sandalwood, rubber resins, natural oils, root extracts, the leaves and stems of several medicinal plants.

Benefits of Using Dhoop [17]

- a. Dhoop has positive effects for the body, mind, and spirit. Dhoop incense is well recognized for enhancing focus, which is beneficial for learning and meditation. It also fights depression, prevents infections, eases headaches, and eases tension and anxiety.
- b. Dhoop sticks, with their calming scents, contribute to a peaceful environment and assist to relax the mind.
- c. People with bronchitis, colds, and asthma are benefiting from the resins and herbs used to make Dhoop sticks.

Uses of Dhoop

- a. **Antimicrobial: It is well known that dhoop has antibacterial properties.**

Agents that possess the capacity to eradicate or inhibit the growth of microorganisms are known as antimicrobials [4]. Based on the bacteria they target, antimicrobial medications are categorized. Antibiotics are used to treat bacteria, while antifungals are used to treat fungus. The classification of antimicrobials also takes into account their function. Agents that have the ability to eradicate microorganisms are referred to as microbicidal, whereas those that halt their growth are called biostatic. Since numerous herbs with microbial properties are used to make incense, the preparation process imparts anti-microbial action [18].

Mosquito Repellent:

One of the main transmitters of many fatal diseases is the mosquito. A mosquito feeds on human blood, which leads to illness in the victim. Numerous mosquito

- b. species from the genera Anopheles, Culex, and Aedes act as carriers of pathogens that cause a number of illnesses, including dengue fever, malaria, yellow fever, and other. Dhoop's ability to repel mosquitoes can aid in

combating this vector and providing relief to people [19].

Limitations of Dhoop

While dhoop has numerous benefits, there is a drawback as well. In certain people, dhoop or incense increases the risk factor for elevated IgE in blood, which can lead to allergic diseases like dermatitis. However, more research needs to be done in order to have a clear picture. Meanwhile while using incense or dhoop, ventilation of the room should be taken into consideration so that the unpleasant effect of the incense or dhoop can be under control [20].

MATERIALS & METHODS:

Collection of Plant Material:

The first step is to collect flowers from different temples around Butibori & Segregation of flowers is carried out on separate papers, plastics, and other unwanted materials. The petals were separated from the collected flowers and then sun dried. Dried Cow dung was acquired from a local dairy milk supplier from thane district. The cow dung was then pulverized in a domestic grinder and sieved to obtain the fine powder. Cow's ghee was also procured from local market after checking its quality [6].



(a)



(b)



(c)

Figure No.4: Preliminary process a) Collection b) Drying petals c) Making powder

Identification & Authentication of Herbs:

The Herbs collected for the research project is authenticated by Dr. Jagdish Baheti (Pharmacognosist) and it confirm that the collected herbs for the study was of the same plant.

Extraction of Plant Material:

Extraction of plant material was done by maceration method of extraction in this method the plant material was dipped into hydro alcoholic solvent like mixture of ethanol & water for 72 hours. The ration of ethanol & water was 70:30 respectively.

The plant material like

- a. Marigold powder

- b. Neem powder
- c. Tulsi powder
- d. Pudina powder



Figure No.5: Extracted Herbs

These 4 extractions of herbs are taken by maceration process which was used for the phytochemical investigation of the Herbs.

Phytochemical Investigation:

Identification Test:

For Marigold [21]:

- a. Test for Terpenoids: To 0.5ml of sample, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown colour at the interface indicated the presence of terpenoids.
- b. Test for Phenol: To 1ml of the sample, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green colour indicated the presence of phenols.

For Neem:

- a. Shinoda Test: To test solution of extract(neem) was dissolved in 95% of ethanol to this, a small piece of magnesium foil metal was added in test solution after that 3 to 5 drops conc HCL was added. The intense cherry red colour indicated the presence of Flavonoids
- b. Sodium Hydroxide Test: Take 1 to 2g sample in titration flask and dilute up to 50 ml. add 2-

3 drops of 1% Phenolphthalein indicator fill the burette with 1N HCL solution. titrate the said solution against 0.1N HCL solution until colour disappear .

For Tulsi [22]:

a. Test for Steroids:

5 mg extract of osmium sanctum was mixed with 1 ml of chloroform then few drops of concentrated Sulfuric acid and acetic acid were added into it. The greenish colour was indicate the presence of steroids.

b. Test for Carbohydrates:

Benedict's reagent was taken for the analysis of carbohydrate. the 5 mg extract was mixed with few drops of benedict's reagent, then allowed to boiled, the reddish-brown precipitate is found with the presence of the carbohydrates (absent).

For Spearmint [23]:

a. Test for Terpenoids:

Mixed, 5 ml of leaves extracts with 2 ml of chloroform and then, 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish-brown coloration at the interface indicates the presence of the terpenoids.

b. Test for Tannin:

For tannins, 2 ml leaves extracts of pudina were heated by adding concentrated HNO₃ with excess ammonia. The formation of white precipitation indicates the presence of tannins.

Formulation of Dhoop:

Preparation of mix blend of all the herbs and ingredients:

- Powder form of Marigold, Neem, Tulsi & Pudina was passed through mesh no. 60
- All the powders were weighed according to the formula
- Then were mixed in a mortar pestle
- The fine powder of camphor is mixed into it

- The other ingredients like cow dung, guggul, loban sandalwood powder was also weighed and mixed
- Starch was mixed into water
- Then Ghee was added my continuous mixing
- Rose water & distilled water was added to prepare dough



Figure No. 6: Dough of mosquito repellent Dhoop Formulation of Mosquito repellent Dhoop stick

The ingredients were mixed Homogeneously so that all the ingredients should bind each other. Then the mixture was added to the mould for the proper shape of dhoop. The fragrance material or perfume is added. Then by the help of mould method incense sticks are prepared. A plastic syringe was cut from the apical side so as to open the mouth of the syringe completely. Dhoop sticks were made using the opened syringe and a plunger. These dhoop sticks were dried for 4 days in an oven at 40 degree C and then stored in an air tight container. After storage for a month, the sticks were used for evaluation of cleansing activity. Dhoop sticks were further tested for their antimicrobial effect.



Figure No.7: Formulated Herbal Mosquito repellent Dhoop



The base material (sandalwood powder, charcoal powder, jigit & rose powder) was mixed with water



Pure ghee was added after getting a wet mass volatile oil (orange oil & marigold oil) was added



After that mixed with rose water



Then mixture put into mound

Figure No.8: Method of preparation of incense Dhoop

Table 1: -The details of ingredients of Herbal Dhoop

Sr. no.	Ingredients	Quantity taken
1	Marigold powder	1.5gm
2	Neem powder	1.5gm
3	Tulsi powder	1gm
4	Pudina powder	1gm
5	Sandalwood powder	1gm
6	Starch	2gm
7	Camphor	5g
8	Guggul	5g
9	Orange peel oil	1ml
10	Rose water	2ml
11	Cow Dung	4gm
12	Cow Ghee	5ml
13	Raad	5gm
14	Kapurkachari	5gm
15	Loban	5gm
16	Distilled water	As per requirement

Evaluation of Mosquito repellent Dhoop:**Physical Analysis:**

The prepared scrub was evaluated for its odour and colour. The colour was found to be brown in colour and odour was found to be characteristics.

Moisture Content:

The prepared dhoop's initial weight was lighted, and the dried dhoop's ultimate weight was recorded. The moisture content was found to be 17.7%

Flammability & Burning Time:

The flammability and the burning time of the dhoop was checked by burning the dhoop. It was observed that the dhoop was burnt completely creating low smoke and the burning time was 20 minutes.

**Figure No.9: Flammability & Burning Time****Ash value:**

The dhoop stick was burnt completely and the ash was collected and weighed. The weight of ash 0.140 gm.

Mosquito Repellent Activity:

The dhoop was burnt in the mosquito prone areas in the evening and night period. For investigating mosquito repellent activity, the prepared incense sticks are checked for causal effect such as irritation, coughing, and tears was observed and recorded.





Figure No.10: Mosquito Cage

Microbiological Evaluation: [24]

Microbiological evaluation was carried out to assess the efficacy of the formulation with respect to its antagonistic effect against respiratory pathogens. Firstly, Nutrient agar powder was dissolved in water & kept in autoclave for 30-45min. Then transferred to petri plates & allow it to settle for 20 min. A sterile corn borer was used to make wells of equidistance in each plate. 2 wells were made in which one was standard which was Ofloxacin , & second well was Test Sample. At last, the E. coli culture was spread evenly and kept in incubator for 24 hours at 37 degrees Celsius.

Irritability:

The dhoop was burnt and its irritancy was checked whether its irritating to our nose or leads to coughing or sneezing.

RESULT & DISCUSSION:

Identification and Authentication of leaves:

The herbs collected for research project is authenticated by Dr. Jadish Baheti and it conform that collected herbs for study was of the same plant.



Figure No.4: Marigold









Figure No.11: Neem



Percentage yield of extraction:

Percentage yield of extraction after completion of extraction process the extraction was subjected to evaporation by recovering the ethanol using distillation process. Then pour the concentrated extract in to Petri dishes for complete evaporation then collect the ethanol free extract into a separate bottle. The percentage yield was calculated using initial weight of all four-extract used and final weight of extract.

$$\begin{aligned} \text{The percentage yield} &= \left[\frac{\text{weight of powdered drug}}{\text{weight of dried drug extract}} \right] \\ &= \frac{22.5}{5} \\ &= 4.4\% \end{aligned}$$

Phytochemical Investigation Result:**Table 2: Phytochemical Investigation of Plant extract**

Sr.no.	Test	Observation	Result
Marigold	a. Terpenoids		Brown colour formed pass the test
	b. Phenol		Yellowish green colour formed pass the test
Neem	a. Shinoda test		Cherry red colour formed pass the test
	b. Sodium hydroxide test		Colorless solution formed pass the test
Tulsi	a. Steroids		Greenish colour formed pass the test
	b. Carbohydrates		Reddish brown ppt formed failed the test

spear mint	a. Terpenoids		Brown colour formed at interface pass the test
	b. Tannins		Yellow colour formed fail the test

Evaluation of Dhoop:

Table 3: Evaluation parameter of Dhoop

Sr.no	Parameter	Observation
1	Colour	Brown
2	Oduor	Characteristics
3	Burning time	20min
4	Ash value	0.140gm
5	Mosquito repellent activity	positive
6	Microbiological evaluation	pass
7	Moisture content	17.7%
8	Consistency	Solid
9	Irritability	Non irritant

Table 4: Mosquito Repellent Activity

Sr .no	Report	Result
1.	No. of mosquitoes	5
2.	No. of mosquitoes escaped	2
3.	No. of mosquitoes dead	3
4.	Time (in minutes)	25
5.	Temperature	21°C
6.	Burning time	20

Microbiological evaluation result

Sr. no	Zone of Inhibition	Result
1.	Std Ofloxacin	65mm
2.	Product tested	61mm



Figure No.12: Microbiological Evaluation

CONCLUSION

The components of cow dung and the phytochemical elements of plant extract are what keep mosquitoes away. The market's mosquito coils produce a lot of smoke, which can cause respiratory issues, particularly for those who suffer from COPD, asthma, and other respiratory conditions. As an alternative to synthetic chemical repellents, cow dung offers a herbal repellent that is long-lasting, safe for human life, human and domestic animal skin, and does not have any negative effects on the environment. The mixture offers maximum mosquito repellent power and is safe, inexpensive, eco-friendly, and simple to use. Making these natural repellents from cow dung & Marigold could increase the average person's income. The current research aims to suggest that dhoop production could become a possible market in the future if a focused approach is adopted and natural ingredients are used. It was determined that the herbal dhoop stick formulation preserves a healthy, mosquito-free environment for society and is more cost-efficient, non-toxic, and effective than the commercially available insect repellents based on chemicals. This formulation can be used as a substitute for other commercially available chemical-based mosquito repellents because it is less harmful, environmentally friendly, and prevents insects from developing resistance.

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