



**DESIGN, DEVELOPMENT AND EVALUATION OF TRADITIONAL POLYHERBAL
FORMULATION TO CURE DENGUE AND CHIKUNGUNYA**

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Article Received on 03/08/2022

Article Revised on 23/08/2022

Article Accepted on 13/09/2022

ABSTRACT

Herbal medicine is the most traditional method of treatment that has been used throughout history and is utilised in all cultures. The present study was focused on development and evaluation of polyherbal syrup to cure dengue and chikungunya. Dengue and chikungunya viruses are caused by an arbovirus. Both viruses are arthropod-borne viruses sharing a common vector. These two diseases are transmitted from infected person to the healthy person through bite of virus carrying female mosquito. There is no specific treatment for dengue and chikungunya. Generally, the doctors prescribe papaya leaves extract and NSAID drugs to treat the symptoms of dengue and chikungunya. Keeping our hopes up, we took steps to locate a remedy using all natural herbs that have been documented centuries ago and came up with a comparatively safer solution for this ailment. In present research some natural herbs are used to develop safe cost-effective syrup for dengue and chikungunya. The aqueous extracts of selected herbs were formulated in particular ratio to form herbal syrup. The plants chosen were *carica papaya*, *aloe vera*, *tinospora cordifolia* and *ocimum tenuiflorum*. The raw materials were collected, authenticated accordance with WHO guidelines. Several experimental batches were created by adjusting the percentage of simple syrup. These batches were tested for a variety of assessment factors. This formulation's accelerated stability was also studied. The formulation complies with all the phytochemical and physicochemical parameters, therefore it is concluded that polyherbal syrup was found to be safe.

KEYWORDS: Dengue, Chikungunya, Polyherbal syrup, Phytochemical screening, Accelerated stability studies.

INTRODUCTION

Dengue and chikungunya are two mosquito-borne viral diseases of great public health concern in India. Dengue virus (DENV) and chikungunya virus (CHIKV) are transmitted by the same species of mosquito, *Aedes aegypti* and share spatiotemporal territories. DENV belongs to the Flaviviridae family and CHIKV belongs to the genus Alphavirus of Togaviridae.^[1] DENV and CHIKV typically incubate for 4-7 days and 3-7 days, respectively. Patients infected with either virus often have an initial onset of fever, myalgia, and headache, with some developing a maculopapular rash and/or gastrointestinal symptoms.^[2] Mild dengue fever to severe dengue hemorrhagic fever and/or dengue shock syndrome are the clinical symptoms.^[3] The symptoms of dengue are thrombocytopenia, high fever, severe headache, muscle and joint pains, nausea and vomiting, mild pain in throat and extreme weakness.^[4] Chikungunya virus (CHIKV) is an arbovirus spread by mosquitoes. When symptomatic (85 to 95% of cases), CHIKV infection causes an acute fever–arthralgia syndrome that can evolve into chronic inflammatory rheumatism.^[3] The symptoms of chikungunya are polyarthralgia, sudden high fever, joint pains and muscle

pains, diarrhoea, abdominal pain and fatigue.^[5]

Till date, there are no specific globally accepted treatments for dengue fever and chikungunya fever in any system of medicine. DENV and CHIKV does not cause very high mortality. Traditionally large numbers of plants are reported for their use against contagious diseases, including infection caused by viruses.^[6] During the critical phase of dengue, malaria, chikungunya that thrombocytopenia is characterized by a decrease in platelet count below 100000 perm³ from the baseline.^[7]

Herbal formulation

Herbal medicine are treated as traditional medicines since they were extensively used in traditional system of medicine like Ayurveda, siddha, Unani.^[8] The majority of herbal syrup was obtained from plants, and herbal medicine refers to the use of extract for therapeutic purposes. Herbal medications are also available in syrup form, in addition to conventional dosage forms.^[9]

Following are the ingredients used in formulation

1. Papaya leaves: *Carica papaya* (papaya, paptia, paw paw) is an herbaceous plant belonging to the family

Caricaceae. Traditionally the *Carica papaya* leaf extract used in dengue and chikungunya fever patients with thrombocytopenia; it accelerates the increase in the platelet count.^[10,11,12]

2. **Aloe vera:** It consist of fresh leaves of *Aloe barbadensis* belonging to family *Liliaceae*. Aloe vera reduces inflammation and arthritis caused by adjuvants.^[13,14,15]
3. **Gulvel:** *Tinospora cordifolia*, also known as "Guduchi" in Sanskrit. It is a significant medicinal herb used in Ayurvedic medicine to treat polyarthralgia, osteoporosis, colds, fevers, diabetes, and even rheumatoid arthritis.^[16,17,18,19,20]
4. **Tulsi:** Tulsi is an aromatic plant belonging to the family *Lamiaceae*. It is useful in dengue, chikungunya and malarial fever.^[21,22,23,24]

MATERIALS AND METHODS

Collection and Authentication of plant material

Leaves of *Carica papaya*, *Aloe barbadensis*, *Tinospora cordifolia*, *Ocimum tenuiflorum* were collected from the herbal supplier. All the plant material were authenticated by Y. C. College, Department of Botany, Satara.

Preparation and Phytochemical evaluation of extracts

The plant material was washed thoroughly with running tap water, more than five times. The main stems of the

Formulation table

Composition of polyherbal syrup

| Ingredients | Quantity | | | Activity |
|-------------------|----------|--------|--------|---------------------------|
| | A | B | C | |
| Papaya Leaf Juice | 25 ml | 25 ml | 25 ml | Platelet increasing agent |
| Aloe vera Juice | 18 ml | 18 ml | 18 ml | Anti-inflammatory agent |
| Gulvel Juice | 8 ml | 8 ml | 8 ml | Anti-arthritis agent |
| Tulsi Juice | 10 ml | 10 ml | 10 ml | Analgesic agent |
| Simple Syrup | 46.66% | 56.66% | 66.66% | Base, Viscosity modifies |

Evaluation parameters

a. Colour, Odour, Taste

The syrup's colour, odour and taste were examined.^[25]

b. Determination of pH

The 10ml of final syrup was taken in to the volumetric flask and filled the volume upto 100ml with distilled water. The pH paper was used to measure the Ph.^[25,26]

c. Determination of viscosity

The viscosity of syrup was measured using an ostwald

Formula for viscosity

$$\text{Viscosity} = \frac{\text{Density of test liquid} \times \text{Time required to flow test liquid} \times \text{Viscosity of water}}{\text{Density of water} \times \text{time required to flow water}}$$

d. Determination of density

The density of syrup was calculated using the beaker. The beaker was cleaned with chromic acid or nitric acid. The beaker was rinsed two to three times with distilled water. The weight of the empty dry beaker was noted.

leaves was removed using a scissor. The material was cut in to pieces and washed it well with boiled cool water. Chopped into even smaller pieces. The pieces were grinded well for about 15 minutes with 50mL boiled cold water till a uniform pulp is formed. The pulp was placed into the juice extractor and squeezed it till get the pure extract. The phytochemical evaluation of extracts was done individually.

Preparation of simple syrup

The 66.67gm of sugar was weighed and added to purified water and heated until it dissolve with occasional stirring. Sufficient boiling water was added upto 100ml.

Preparation and Phytochemical evaluation of polyherbal syrup

The simple syrup (66.67%w/v) was prepared as per Indian pharmacopoeia. The extracts were added into simple syrup I.P. and the volume was made upto 100ml. The 3 trial batches were prepared by varying the concentration of simple syrup.

viscometer. Firstly the ostwald viscometer was cleaned with warm chromic acid or acetone. The water was filled up to the mark "G" in the dry viscometer and placed the viscometer vertically on a suitable platform. The time was noted while water was flowing from mark A to mark B. This operation was done at least three times and recorded the time to acquire reliable readings. Then the viscometer was rinsed and filled it with test liquid (syrup) till mark A, then the time was calculated to takes for the liquid to flow up to mark B.^[26]

The beaker was filled with test liquid, and the excess liquid out of the beaker was wiped. Finally, calculated the weight in grams of a liquid.^[19]

Formula for density

$$\text{Density of liquid under test (syrup)} = \frac{\text{Weight of liquid under test}}{\text{Volume of liquid under test}}$$

e. Determination of specific gravity

After cleaning with chromic acid or nitric acid, the bottle was rinsed with filtered water two to three times. The weight of an empty dry bottle was taken with a capillary tube stopper (w1). The bottle was filled with distilled water, screw on the stopper, and the liquid from the

outside of the bottle was wiped. And using an analytical balance, weighed the bottle with distilled water (w2). After emptying and drying, the procedure was repeated by replacing water with the liquid under test (syrup). The container was weighed with the stopper and the liquid under test on an analytical balance (w3).^[9,27]

Formula for specific gravity

$$\text{Specific gravity of liquid} = \frac{\text{Weight of liquid under test}}{\text{Weight of water.}} \text{ under test (syrup)}$$

Accelerated stability study

Based on the results, the trial batch C was chosen as the most acceptable normal range of parameters. The produced polyherbal syrup was evaluated to an accelerated stability investigation for three months. The

syrup was maintained at room temperature and was kept in an amber-colored container. Every month, pH, viscosity, density, and specific gravity were measured. The phytochemical analysis was done at the end of every month.^[28]

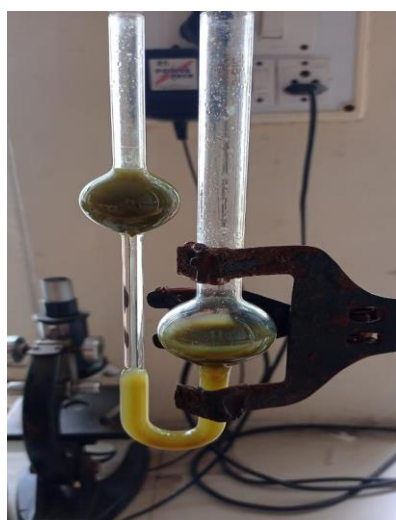


Fig. 1: Viscosity.

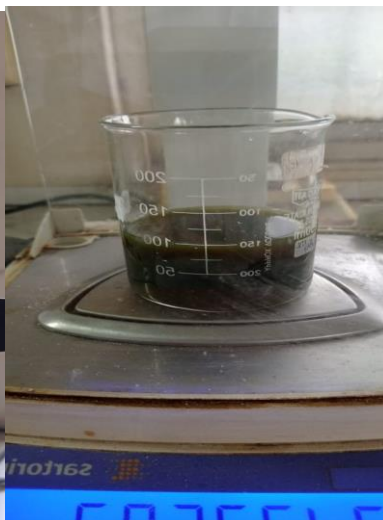


Fig. 2: Density.



Fig. 3: Specific gravity.

RESULT AND DISCUSSION**Raw material analysis**

The phytochemical evaluation of individual herbs are given in table:

| Components | Papaya | Aloe vera | Gulvel | Tulsi |
|--------------|--------|-----------|--------|-------|
| Alkaloid | + | + | + | + |
| Flavonoid | + | + | + | + |
| Tannin | + | + | + | + |
| Saponin | + | + | - | + |
| Phenol | + | - | - | + |
| Carbohydrate | - | + | + | - |
| Glycoside | + | - | + | - |
| Terpenoid | - | - | - | + |
| Protein | + | + | + | + |
| Steroid | + | - | + | + |

Evaluation of trial batches

The trial batches were evaluated for physical parameters such as colour, odour, taste, pH, viscosity, density and specific

gravity etc. The results are given in table:

| Sr. No. | Parameters | Batch A | Batch B | Batch C |
|---------|------------------|----------------|----------------|----------------|
| 1 | Colour | Greenish Brown | Greenish Brown | Greenish Brown |
| 2 | Odour | Characteristic | Characteristic | Characteristic |
| 3 | Taste | Bitter | Bitter | Bitter |
| 4 | pH | 6 | 6.1 | 6.2 |
| 5 | Viscosity | 0.0153 | 0.01625 | 0.018 |
| 6 | Density | 1.05 | 1.06 | 1.07 |
| 7 | Specific gravity | 1.432 | 1.424 | 1.424 |

Based on the results, the trial batch C was chosen as the most acceptable normal range of parameters.

Phytochemical analysis of polyherbal syrup for batch C

| Chemical constituents | Results |
|-----------------------|---------|
| Alkaloid | + |
| Flavonoid | + |
| Tannin | + |
| Saponin | + |
| Phenol | + |
| Carbohydrate | + |
| Glycoside | + |
| Terpenoid | + |
| Protein | + |
| Steroid | + |

Accelerated stability study for batch C

Physical Parameters of Polyherbal syrup:

| Sr. No. | Parameters | Initial study | First month | Second month | Third month |
|---------|------------------|----------------|----------------|----------------|----------------|
| 1. | Colour | Greenish Brown | Greenish Brown | Greenish Brown | Greenish Brown |
| 2. | Odour | Characteristic | Characteristic | Characteristic | Characteristic |
| 3. | Taste | Bitter | Bitter | Bitter | Bitter |
| 4. | pH | 6.2±0.03 | 6.0±0.02 | 6.1±0.02 | 6.1±0.04 |
| 5. | Viscosity | 0.018±0.03 | 0.018±0.03 | 0.017±0.04 | 0.016±0.05 |
| 6. | Density | 1.06±0.05 | 1.07±0.04 | 1.07±0.03 | 1.06±0.06 |
| 7. | Specific gravity | 1.424±0.01 | 1.432±0.02 | 1.424±0.02 | 1.433±0.03 |

Phytochemical analysis of polyherbal syrup for batch C

| Chemical constituents | Initial study | First month | Second month | Third month |
|-----------------------|---------------|-------------|--------------|-------------|
| Alkaloid | + | + | + | + |
| Flavonoid | + | + | + | + |
| Tannin | + | + | + | + |
| Saponin | + | + | + | + |
| Phenol | + | + | + | + |
| Carbohydrate | + | + | + | + |
| Glycoside | + | + | + | + |
| Terpenoid | + | + | + | + |
| Protein | + | + | + | + |
| Steroid | + | + | + | + |

CONCLUSION

The polyherbal syrup consisting of four herbs which folklore claim of being used in dengue and chikungunya and these were evaluated and standardized. Also the physicochemical properties of prepared syrup like colour, odour, taste, pH, viscosity, density and specific gravity were satisfactory and the formulation was within the all specification. The phytochemical analysis was

done of prepared syrup for three months. The accelerated stability study for three months indicates that the formulation is stable under room temperature.

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