DEVELOPMENT OF POLYHERBAL ANTIMALARIAL FORMULATION

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ABSTRACT

The objective of present study was to formulate and evaluate the polyherbal tablet formulation (PHF) for the treatment of malaria disease. Plants like Aegle marmelos L., sida acuta B., and Ocimum basilicum L. give various pharmacological properties like anti-malarial, anti-oxidant, anti-diabetic and anti-inflammatory activities. Literature survey revealed that many phytoconstituents like flavonoids, saponins, alkaloids, tannins, glycosides were extracted from leaves of Aegle marmelos L.(Bael), Sida acuta B.(Common wireweed), and Ocimum basilicum L.(Sweet basil). Preliminary phytochemical screening of the Methanolic extract of leaves of Bael, Ethanolic extract of leaves of common wireweed and sweet basil revealed the presence of alkaloids, carbohydrates, flavonoids. Saponins were absent in methanolic/ethanolic extracts except common wireweed. Glycosides were absent in methanolic/ethanolic extracts except sweet basil. TLC studies were carried out for extracts. In this study, the PHF contained three different drugs viz., Aegle marmelos L., Sida acuta B., and Ocimum basilicum L. in variable amount along with Starch/PVP as binder, MCC as disintegrant, dibasic calcium phosphate as diluent, PEG 4000 as a polymer and magnesium stearate (1%) as lubricant, methyl paraben (0.1%) as preservative by wet granulation method. Tablet properties of the formulation (F5) were found to be satisfactory and stable when tested for stability studies at 30 ± 2°C / 65 ± 5% RH.

Key words: Anti-malarial; Polyherbal formulation (PHF); Aegle marmelos L.; sida acuta B.; and Ocimum basilicum L.
INTRODUCTION

Malaria is a parasitic disease caused by a protozoan of the genus *Plasmodium*. Most of the lethal cases are caused by *Plasmodium falciparum*, the most virulent of the four *Plasmodia* species that infect humans [1]. The disease is confined to tropical and sub-tropical regions of the world and is transmitted by the female Anopheles mosquito [2]. *Plasmodium falciparum* the most widespread etiological agent for human malaria has become increasingly resistant to standard antimalarials e.g. chloroquine and antifolates [3]. The history of anti-malarial chemotherapy is intimately linked with the history of herbal medicinal products [4]. Herbal Medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history [5]. Traditional methods of malaria treatment could be a promising source of new antimalarial compounds [6]. The use of medicinal plants for the treatment of parasitic diseases is well known and documented since ancient times [7]. Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international marketplace [8]. Chemotherapeutic agents will continue to be in demand for the complete management of malaria [9].

The need for new compounds active against malaria parasites is made more urgent by the rapid spread of drug-resistance to available antimalarial drugs. Nowadays, anti-malarial drug resistance has become one of the most important challenges to malaria control efforts [10]. So, the objective of present work is to develop a polyherbal formulation for malaria disease. The intended polyherbal formulation contains a mixture of three different herbal drugs namely; using the leaves of Bael (*Aegle marmelos L.*) [11, 12, 13], leaves of Common wireweed (*Sida acuta B.*) [14, 15, 16], leaves of Sweet basil (*Ocimum basilicum L.*) [17, 18] which are having a potent anti-malarial activity and these herbal drugs are used individually in ancient literature of therapeutics in malaria. To improve therapeutic efficacy and delay the development of resistance, the World Health Organization has since 2001 recommended for antimalarial treatment, the use of combination therapy based on the synergistic or additive potential of two or more drugs [19].

MATERIALS AND METHODS

Plant material

a) The leaves of *Aegle marmelos* were collected from University of Agricultural Sciences, Bangalore, Karnataka in the month of June 2013. The plant material was identified and
authenticated taxonomically at Department of Horticulture, UAS, GKVK, Bangalore-560065, Karnataka, India (Ref no- 16/M&A/2013, dated- 25.06.2013).

b) The leaves of *Sida acuta* were collected from Institute of Ayurveda and Integrative Medicine, Bangalore, Karnataka in the month of August 2013. The plant material was identified and authenticated taxonomically at FRLHT, 74/2, JB Kaval, Attur post, Via Yelahanka, Bangalore-560106, Karnataka, India (Ref no- FRLH 53737, dated- 19.08.2013).

c) The leaves of *Ocimum basilicum* were collected from GKVK, Bangalore, Karnataka in the month of June 2013. The plant material was identified and authenticated taxonomically at Department of Horticulture, UAS, GKVK, Bangalore-560065, Karnataka, India (Ref no- 16/M&A/2013, dated- 25.06.2013).

**Preparation of Extracts**[^20, 21]

1. **Preparation of *Aegle marmelos* (AM) leaves extract**
   The collected plant leaves were cleaned, air-dried under shade and powdered by a mechanical grinder. Fifty grams of the pulverized leaves was extracted with petroleum ether and methanol successively in a soxhlet apparatus at 40±5°C. Petroleum ether was used in initial step of extraction for defatting the plant materials. The successive extracts were separately filtered and concentrated at reduced temperature on a rotary evaporator and the residue obtained was collected and stored in desiccator. Extracts obtained were weighed and calculated for percentage yield.

2. **Preparation of *Sida acuta* (SA) leaves extract**
   The collected plant leaves were cleaned, air-dried under shade and powdered by a mechanical grinder. Fifty grams of the pulverized leaves was extracted with petroleum ether and ethanol successively in a soxhlet apparatus at 40±5°C. Petroleum ether was used in initial step of extraction for defatting the plant materials. The successive extracts were separately filtered and concentrated at reduced temperature on a rotary evaporator and the residue obtained was collected and stored in desiccator. Extracts obtained were weighed and calculated for percentage yield.

3. **Preparation of *Ocimum basilicum* (OB) leaves extract**
   The collected plant leaves were cleaned, air-dried under shade and powdered by a mechanical grinder. Fifty grams of the pulverized leaves was extracted with petroleum ether and ethanol
successively in a soxhlet apparatus at 40±5°C. Petroleum ether was used in initial step of extraction for defatting the plant materials. The successive extracts were separately filtered and concentrated at reduced temperature on a rotary evaporator and the residue obtained was collected and stored in desiccator. Extracts obtained were weighed and calculated for percentage yield.

**Determination of loss on drying**

About 2g of extract were accurately weighed and transferred to a tarred china dish which was already known for its weight and kept in vacuum dryer at 50°C for an hour. Then the sample was weighed along with china dish to deduct the actual weight of empty tarred china dish. The procedure was continued till the weight of extract remained constant.

\[
\% \text{Loss on drying} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100
\]

**Preliminary Phytochemical Screening (Qualitative Analysis)** \(^{[22]}\)

The preliminary photochemical studies were performed for testing different chemical groups present in Ethanolic extract. The chemical group tests were performed and are presented in results.

a) Alkaloids

i. **Dragendorff’s test**

To 2 mg of the Methanolic/Ethanolic extract 5 ml of distilled water was added, 2M Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff’s reagent was added. Formation of orange or orange red precipitate indicated the presence of alkaloids.

ii. **Hager’s test**

To 2 mg of the Methanolic/Ethanolic extract taken in a test tube, a few drops of Hager’s reagent were added. Formation of yellow ppt confirmed the presence of alkaloids.

iii. **Wagner’s test**

To 2 mg of Methanolic/Ethanolic extract was acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner’s reagent was added. A yellow or brown ppt. indicated the presence of alkaloids.
iv. Mayer’s test

To a few drops of the Mayer’s reagent, 2 mg of Methanolic/Ethanolic extract was added. Formation of white or pale yellow ppt. indicated the presence of alkaloids.

v. Tannic acid test

To the test solution add few drops of tannic acid solution. Formation of buff colour ppt. indicates the presence of alkaloids.

b) Carbohydrates

i. Fehling’s test

To 2 ml of extract, 1 ml mixture of equal parts of Fehling’s solution A and B were added and boiled for few minutes. Formation of red or brick red colour precipitate indicated the presence of reducing sugar.

ii. Molisch’s test

To the test solution add few drops of alcoholic a-naphthol, then add few drops of concentrated sulphuric acid through the sides of test tube, purple colour ring appears at the junction indicates the presence of carbohydrates.

iii. Barfoed’s test

1 ml of test solution is heated with 1ml of Barfoed’s reagent on water bath, if red cupric oxide is formed, monosaccharide is present.

c) Fats and fixed oils

Treat 5 drops of sample with 1 ml of 1 % copper sulphate, then add 10 % sodium hydroxide solution. A clear blue solution shows glycerine present in the sample.

d) Flavonoids

i. Shinoda test

To the test solution add few magnesium turnings and concentrated hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes indicates the presence of flavonoids.

ii. Zinc hydrochloride test

To the test solution add a mixture of zinc dust and conc. hydrochloric acid. It gives red colour after few minutes indicates the presence of flavonoids.
e) Glycosides

i. General test

TEST A: Extract 200 mg of drug with 5 ml of dilute sulphuric acid by warming on a water bath. Filter it. Then neutralize the acid extract with 5 % solution of sodium hydroxide. Add 0.1 ml of Fehling’s solution A & B until it becomes alkaline (test with pH paper) and heat on a water bath for 2 minutes. Note the quantity of red ppt. formed and compare with that of formed in TEST B.

TEST B: Extract 200 mg of the drug using 5 ml of water instead of sulphuric acid. After boiling add equal amount of water as used for sodium hydroxide in the above test. Add 0.1 ml Fehling’s solution A and B until alkaline (test with pH paper) and heat on water bath for 2 min. Note the quantity of red ppt. formed. Compare the quantity of ppt. formed in TEST B with that of formed in TEST A. If the ppt. in TEST A is greater than in TEST B indicates the presence of glycosides.

f) Saponins

In a test tube containing about 5 ml of an Ethanolic extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicated the presence of saponins.

g) Tannins

i. Ferric chloride test

Treat the extract with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

ii. Gelatin test

To the test solution add 1 % gelatine solution containing 10 % sodium chloride. Precipitate is formed.

h) Proteins

i. Warming test

Heat the test solution in a boiling water bath, proteins gets coagulated.

ii. Biuret test

To the test solution add 2 ml biuret reagent, violet colour indicates the presence of proteins.
Identification Studies for Extract

TLC Studies

Materials used
1. Silica gel G 60 F\(_{254}\) precoated plates
2. Solvents
3. Chromatographic chamber
4. Capillary tubes
5. Fusion tubes
6. UV chamber

Standard: Raw material
Test: Extract

Constituents of Herbs for Identification in Extracts

Table 1: Constituents of herbs

<table>
<thead>
<tr>
<th>S.No</th>
<th>Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Aegle marmelos</em> L. – Alkaloids</td>
</tr>
<tr>
<td>2.</td>
<td><em>Sida acuta</em> B. – Alkaloids</td>
</tr>
<tr>
<td>3.</td>
<td><em>Ocimum basilicum</em> L. – Alkaloids</td>
</tr>
</tbody>
</table>

TLC studies of extract obtained by comparing with raw material which acts as reference standard. For each extract and standard of fine dried powder has taken into fusion tube for solubility checking in different solvents and a soluble mixture was used for sampling and the mobile phase was chosen by changing the different solvent or solvent system polarities are developed. Spots were observed under UV chamber of short and long wavelengths (\(\lambda_{max}\)). Rf values were calculated.

Development of Polyherbal Tablet Formulation \(^{[23, 24]}\)

Six polyherbal formulation have been made with different ratio of previously mentioned herbal drugs as per the literature. In the preparation of formulation, leaves of *Aegle marmelos* L., *Sida acuta* B., *Ocimum basilicum* L. were used. All the above raw materials were first cleaned and rinsed in water to get rid of dirt and then dried. The ingredients numbered 1 to 3 (Table 2) (Soxhlet extraction) of formulation composition was crushed individually and tablets were prepared by wet granulation method.
• Extracts of Aegle marmelos (AM) leaves, Sida acuta (SD) leaves, Ocimum basilicum (OB) leaves, Starch/ PVP (5%,10% & 15%) , PEG 4000, MCC (half quantity) made into granules using water as granulating agent.
• Granules were passed through sieve no 22/ 44. The remaining amount of disintegrant, calcium phosphate, magnesium stearate (1%) and methyl paraben (0.1%) were added to granules and compressed into tablets.

Table 2: Formulation chart

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aegle marmelos extract</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Sida acuta extract</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ocimum basilicum extract</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Starch</td>
<td>5%</td>
<td>10%</td>
<td>15%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PVP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5%</td>
<td>10%</td>
<td>15%</td>
</tr>
<tr>
<td>MCC</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Dibasic calcium phosphate</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>30</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>PEG 4000</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

Preformulation Study

Powder Characteristics

Herbal powders are of wide range with varied physical properties and micrometric properties. Powdered solids are heterogeneous because they are composed of individual particles of widely differing sizes and shapes randomly interspersed with air spaces. It is more complicated in case of herbal powders to convert into tablet.

Evaluation of Preformulation Parameters

1) Micrometric properties
a) Angle of repose
The frictional force in powder/granules can be measured by angle of repose by fixed funnel method. An accurately weighed quantity of powder/granules was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap of the powder/granules. The powder/granules were allowed to flow through funnel freely onto the surface and the diameter of the powder/granules cone was measured and angle of repose was calculated using the following equation.
Tan \( \theta = h/r \)
Where, \( \theta \) is angle of repose; \( h \) is height of the cone; \( r \) is radius of the cone base.

b) Bulk density & Tapped density
Both loose bulk density (LBD) and tapped bulk density (TBD) were determined. Powder/granules from each formulation, previously lightly shaken to break any agglomerates formed were introduced into a 10ml measuring cylinder. After the initial volume was observed, the sample is then tapped on a tap density apparatus. Bulk density and tapped density is calculated by the following formula.

- **Bulk density** = Weight of the powder/granules / Bulk volume of the powder/ granules

- **Tapped density** = Weight of the powder/granules / Tapped volume of the powder/ granules

c) Carr’s index
The Carr’s index of the powder/granules was determined by using formula

\[
\text{Carr's index (\%)} = \left( \frac{TBD - LBD}{TBD} \right) \times 100
\]
Where, LBD = Weight of the powder (or granules)/Volume of the packing
TBD = Weight of the powder (or granules)/Tapped volume of the packing

d) Hausner’s ratio
The hausner’s ratio of the powder/granules was determined by the formula

\[
\text{Hausner’s ratio} = \frac{TBD}{LBD}
\]
Where, LBD = Weight of the powder (or granules)/Volume of the packing
TBD = Weight of the powder (or granules)/Tapped volume of the packing

**Evaluation of Herbal Tablets**

**Tablet hardness**
The hardness of tablet of each formulation was measured by using Pfizer hardness tester.

**Tablet thickness**
Thickness of the tablet was measured by using screw guage on 5 randomly selected tablets.

**Friability**
Friability is the measure of tablet strength. Roche friabilator was used for testing the friability using the following procedure. Ten tablets were weighed accurately and placed in the plastic
chamber that revolves at 25 rpm for 4 min dropping the tablets through a distance of six inches with each revolution. After 100 revolutions the tablets were re-weighed and the percentage loss in tablet weight was determined using the formula,

\[
\text{\% Loss} = \frac{\text{Initial wt of tablets} - \text{final wt of tablets}}{\text{initial wt of tablets} \times 100}
\]

**Disintegration time**

Disintegration time is the time taken by a tablet for its complete disintegration after administration and this process has been carried out in distilled water in the Electrolab disintegration apparatus, the process has to be carried out after attaining the bath temperature up to 37°C three tablets has taken and placed individually in cells and process has to be started and initial time is noted and after complete disintegration of tablet the final time has noted this indicates the total time for complete disintegration of tablet.

**Stability Studies**\(^{25}\)

Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, stability studies were carried out.

The stability studies were carried out of the most satisfactory formulation. The most satisfactory formulation sealed in aluminum packaging and kept in humidity chamber maintained at 30 ± 2°C / 65 ± 5 % RH for three months. At the end of studies, samples were analyzed for hardness, friability and disintegration time.

**RESULTS AND DISCUSSION**

Table 3: Materials & Method of Extraction

<table>
<thead>
<tr>
<th>BOTANICAL NAME</th>
<th>PART OF PLANT</th>
<th>METHOD OF EXTRACTION</th>
<th>%YIELD</th>
<th>% LOSS ON DRYING</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aegle marmelos L.</em></td>
<td>Leaves</td>
<td>Soxhlet extraction at 40±5°C</td>
<td>17.4±1.11</td>
<td>0.15</td>
</tr>
<tr>
<td><em>Sida acuta B.</em></td>
<td>Leaves</td>
<td>Soxhlet extraction at 40±5°C</td>
<td>24.5±0.44</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Ocimum basilicum L.</em></td>
<td>Leaves</td>
<td>Soxhlet extraction at 40±5°C</td>
<td>28.04±0.07</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*All the values are expressed as mean ±Standard deviation; \( n=3 \)
Percentage Yield of Extracts
Extraction of leaves of *Aegle marmelos* was carried out using methanol as a solvent. The total yield of the extract was found to be 17.4% respectively. Extraction of leaves of *Sida acuta* was carried out using ethanol as a solvent. The total yield of the extract was found to be 24.5% respectively. Extraction of leaves of *Ocimum basilicum* was carried out using ethanol as a solvent. The total yield of the extract was found to be 28.04% respectively (Table 3).

Preliminary Phytochemical Screening (Qualitative Analysis)
Alkaloids, carbohydrates, flavonoids, glycosides, saponins, tannins and proteins were present in *Aegle marmelos* leaves extract whereas fats and fixed oils were absent. Alkaloids, carbohydrates, flavonoids, glycosides, tannins and proteins were present in *Sida acuta* leaves extract whereas saponins, fats and fixed oils were absent. Alkaloids, carbohydrates, flavonoids, saponins, tannins and proteins were present in *Ocimum basilicum* leaves extract whereas glycosides, fats and fixed oils were absent (Table 4).

Table 4: Preliminary Phytochemical Screening

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Test</th>
<th>Aegle marmelos leaves</th>
<th>Sida acuta leaves</th>
<th>Ocimum basilicum leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>For alkaloids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Dragandroffs test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b.</td>
<td>Hagers test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>c.</td>
<td>Wagners test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d.</td>
<td>Mayers test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>e.</td>
<td>Tannic acid test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>For carbohydrates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Fehlings test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b.</td>
<td>Molisch test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>c.</td>
<td>Barfoeds test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>For fats and fixed oils</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>For flavonoids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Shinoda test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b.</td>
<td>Zinc hydrochloride test</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>5.</td>
<td>For glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>For saponins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>For tannins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Gelatine test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>For proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warming test</td>
<td>+</td>
<td>+</td>
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<td></td>
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<tr>
<td>Biuret test</td>
<td>+</td>
<td>+</td>
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</table>
Identification Studies for Extracts

Identification studies for individual extracts have been performed and Rf values were determined and these values are comparable with standard Rf values and it is concluded that extracts obtained are identified (Table 5).

### Table 5: TLC Studies

<table>
<thead>
<tr>
<th>EXTRACT</th>
<th>SOLUBILITY</th>
<th>SOLVENT SYSTEM</th>
<th>SOLVENT RATIO</th>
<th>SPOTS APPEARED AT WAVELENGTH</th>
<th>RF VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aegle marmelos</em> L.</td>
<td>Test(Methanol)</td>
<td>Chloroform: diethyl amine</td>
<td>9:1</td>
<td>Long wavelength</td>
<td>Rf test =0.82 Rf std =0.81</td>
</tr>
<tr>
<td><em>Sida acuta</em> B.</td>
<td>Test(Ethanol)</td>
<td>Chloroform: diethyl amine</td>
<td>9:1</td>
<td>Long wavelength</td>
<td>Rf test =0.79 Rf std =0.79</td>
</tr>
<tr>
<td><em>Ocimum basilicum</em> L.</td>
<td>Test(Ethanol)</td>
<td>Chloroform: diethyl amine</td>
<td>9:1</td>
<td>Long wavelength</td>
<td>Rf test =0.73 Rf std =0.71</td>
</tr>
</tbody>
</table>

### Table 6: Preformulation Parameters

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Angle of repose (°)* Mean ± SD</th>
<th>Bulk density (g/ml)* Mean ± SD</th>
<th>Tapped density (g/ml)* Mean ± SD</th>
<th>Carr’s index (%)* Mean ± SD</th>
<th>Hausner’s ratio* Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>28.33 ± 0.569</td>
<td>0.60 ± 0.010</td>
<td>0.70 ± 0.010</td>
<td>14.22 ± 0.145</td>
<td>1.16 ± 0.001</td>
</tr>
<tr>
<td>F2</td>
<td>28.10 ± 0.484</td>
<td>0.61 ± 0.015</td>
<td>0.69 ± 0.014</td>
<td>11.85 ± 1.872</td>
<td>1.13 ± 0.024</td>
</tr>
<tr>
<td>F3</td>
<td>27.99 ± 0.310</td>
<td>0.61 ± 0.010</td>
<td>0.68 ± 0.010</td>
<td>11.02 ± 1.513</td>
<td>1.12 ± 0.019</td>
</tr>
<tr>
<td>F4</td>
<td>28.38 ± 0.137</td>
<td>0.60 ± 0.012</td>
<td>0.70 ± 0.012</td>
<td>13.61 ± 3.518</td>
<td>1.15 ± 0.042</td>
</tr>
<tr>
<td>F5</td>
<td>28.30 ± 0.310</td>
<td>0.60 ± 0.011</td>
<td>0.70 ± 0.015</td>
<td>14.40 ± 0.276</td>
<td>1.16 ± 0.003</td>
</tr>
<tr>
<td>F6</td>
<td>28.53 ± 0.209</td>
<td>0.61 ± 0.010</td>
<td>0.70 ± 0.010</td>
<td>12.62 ± 1.331</td>
<td>1.14 ± 0.017</td>
</tr>
</tbody>
</table>

*All the values are expressed as mean ±Standard deviation; n=3*

Evaluation Parameters of Tablets

### Tablet Hardness

The resistance of tablet for shipping or breakage, under conditions of storage, transportation and handling, before usage, depends on its hardness. Hardness of the developed formulation F5 varied from 5.03±0.047 kg/cm² (Table 7) in all the formulation indicating good mechanical strength with an ability to withstand physical and mechanical stress condition while handling.
Tablet Thickness

Thickness of tablets was important for uniformity of tablet size. Thickness of the developed formulations F5 varied from 5.40±0.023 mm (Table 7) in all the formulation and the average thickness are within the range of ± 5%. Each sample was analyzed in triplicate.

Friability

The loss in total weight of the tablets due to friability was in the range of 0.083±0.015 in the formulation F5 and the friability value is less than 1% which ensures that formulated tablets were mechanically stable (Table 7).

Disintegration time

Disintegration process has been carried out in distilled water in the electrolab disintegration apparatus, three tablets has been carried out and results found to be 8.48±0.028 min in the formulation F5 which ensures that slow release of drug (Table 7).

Table 7: Evaluation parameters

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Thickness (mm)*</th>
<th>Hardness (kg/cm²)*</th>
<th>Friability (% loss)*</th>
<th>% Weight variation</th>
<th>Disintegration time (min)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>5.35 ± 0.054</td>
<td>4.67 ± 0.078</td>
<td>0.078 ± 0.011</td>
<td>0.02</td>
<td>12.06 ± 0.036</td>
</tr>
<tr>
<td>F2</td>
<td>5.37 ± 0.050</td>
<td>4.74 ± 0.024</td>
<td>0.076 ± 0.011</td>
<td>0.05</td>
<td>10.15 ± 0.075</td>
</tr>
<tr>
<td>F3</td>
<td>5.38 ± 0.042</td>
<td>4.80 ± 0.033</td>
<td>0.082 ± 0.021</td>
<td>0.05</td>
<td>10.00 ± 0.011</td>
</tr>
<tr>
<td>F4</td>
<td>5.37 ± 0.025</td>
<td>5.01 ± 0.086</td>
<td>0.088 ± 0.018</td>
<td>0.04</td>
<td>10.01 ± 0.010</td>
</tr>
<tr>
<td>F5</td>
<td>5.40 ± 0.023</td>
<td>5.03 ± 0.047</td>
<td>0.083 ± 0.015</td>
<td>0.01</td>
<td>8.48 ± 0.028</td>
</tr>
<tr>
<td>F6</td>
<td>5.39 ± 0.008</td>
<td>5.26 ± 0.082</td>
<td>0.080 ± 0.012</td>
<td>0.01</td>
<td>17.43 ± 0.115</td>
</tr>
</tbody>
</table>

*All the values are expressed as mean ±Standard deviation; n=3

Stability Studies

Stability studies were carried out of the most satisfactory formulation F5, at 30 ± 2°C / 65 ± 5% RH for three months to assess their stability as per ICH guidelines. At various time intervals of 30 to 90 days, samples were evaluated. There was no major change in the various physicochemical parameters evaluated like hardness, friability and disintegration. There was no significant difference between the initial values and the results obtained during stability studies (Table 8).
Table 8: Stability studies

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Hardness (kg/cm²)*</th>
<th>Friability (% loss)*</th>
<th>Disintegration time (min)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td></td>
<td>F5</td>
<td>F5</td>
<td>F5</td>
</tr>
<tr>
<td>0</td>
<td>5.03 ± 0.047</td>
<td>0.083 ± 0.015</td>
<td>8.48 ± 0.028</td>
</tr>
<tr>
<td><strong>30</strong></td>
<td><strong>4.93 ± 0.115</strong></td>
<td><strong>0.082 ± 0.010</strong></td>
<td><strong>8.40 ± 0.107</strong></td>
</tr>
<tr>
<td>At 30 ± 2°C</td>
<td>At 65 ± 5 % RH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>4.90 ± 0.100</td>
<td>0.082 ± 0.009</td>
<td>8.39 ± 0.097</td>
</tr>
<tr>
<td>At 30 ± 2°C</td>
<td>At 65 ± 5 % RH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>4.86 ± 0.060</td>
<td>0.081 ± 0.097</td>
<td>8.39 ± 0.125</td>
</tr>
<tr>
<td>At 30 ± 2°C</td>
<td>At 65 ± 5 % RH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All the values are expressed as mean ± Standard deviation; n=3

CONCLUSION

In the present study, an attempt was made to prepare polyherbal anti-malarial tablet formulation with the combination of extracts from *Aegle marmelos* leaves, *Sida acuta* leaves and *Ocimum basilicum* leaves. Preliminary phytochemical screening of the methanolic extracts of leaves of *Aegle marmelos* (Bael), ethanolic extracts of leaves of *Sida acuta* (Common wireweed) and ethanolic extracts of leaves of *Ocimum basilicum* (Sweet basil) revealed the presence of alkaloids, carbohydrates, flavonoids, tannins and proteins. Saponins were found to be absent except in *Sida acuta* leaves. Glycosides were found to be absent except in *Ocimum basilicum* leaves. TLC studies were also carried out for extracts. Formulations of anti-malarial tablet dosage forms were developed by wet granulation method. Evaluation parameters for tablets have performed and shows the satisfactory results even after stability studies, so it can be concluded from the present study that the polyherbal tablet formulation have proved useful in the treatment of malaria.

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