# Design and Evaluation of Polyherbal Formulation for Treatment of Malaria

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## ABSTRACT

Herbal products have been employed by humans as a medicine for various ailments. The main goal of this research is to design a polyherbal dosage form to treat malaria effectively as well as to modulate the immune response. The basic behind choosing herbs for formulation development was a better treatment option with minimal side effects. Three herbs were taken for the development of formulation; these are *Nyctanthes arbor*, *Tinospora cordifolia*, *Ocimum sanctum*. The formulation was developed in the form of vati as per the convenience of administration to the patient. Results of research conclude that the minimum inhibitory concentration of designed formulation was found 0.98 µg/ml, where chloroquine and quinine were used as standard drugs and the minimum inhibitory concentrations were found to be 0.020 µg/ml and 0.268 µg / ml respectively. All the experimental work suggested that the polyherbal formulation developed can be another effective treatment option for malaria.

**Key Words:** *Polyherbal, Malaria, Nyctanthes-arbor, Tinospora cordifolia, Ocimum sanctum*

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## INTRODUCTION

Herbal products obtained from natural sources including plants, animals, and minerals have been employed by humans as a medicine for treatment, prevention, mitigation, and cure of human ailments, disorders, and diseases [1, 2]. Decades ago, a raw crude drug from natural origin and semi-pure extracts of natural and herbal sources of medicine are the only available medications for the prevention, mitigation, and restoration of health. In the 20th century, a revolution occurs, which develops the idea that the effect of any drug in the human body is mediated by a specific interaction of the administered drug entity with macromolecule which is abundantly present in the human body. This idea gives a leads to scientists that the individual chemical entity present in the extract is only responsible for the desired effect or biological and pharmacological activity of the drug. This was the starting of a new epoch in the field of pharmacology and pharmacognosy. Isolated chemical compounds have become a standard treatment option for various diseases [3].

Malaria is an infectious disease that occurs due to protozoa parasites that belong to the genus *plasmodium* which are transmitted to human beings after the female anopheles bites the human. Male anopheles feed only on plants juices and nectar, cannot transmit malaria. This disease is a serious global public health issue. Approximately 100 species of parasite *plasmodium* exist but only four of them can spread the infection to human beings [4].

As available in ayurvedic literature, leaves of *N. arbor* (Harsingar) treat malaria effectively and reduce symptoms like increased body temperature, chills immediately after administration [5-9]. Whereas *Tinospora cordiflia* (Guduchi), which is termed as Amrita means nectar, which gives strength to the human body by modulating immunity. Guduchi is also known as Indian quinine [10-13]. *Ocimum sanctum* (Tulsi) is termed as a divine herb in Ayurveda that averts and treats various bacterial infections. It is used to cure malaria, as stated in literature; infusion of leaves is administered to the patient [14-18].

Modern dosage form tablet is a modified form of Ayurvedic dosage form vati. Vati Kalpana plays a prominent role in the pharmaceutics of Ayurveda, has many advantages like easy administration, palatability, ease for dispensing, and transportation. Vati Kalpana is a pharmaceutical manufacturing procedure in which the powder of crude drugs is triturated together with certain

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liquids or even honey and the medicines are prepared in the form of pills or tablets [19-21].

The main aim of this research work deals with better as well as more effective eradication of malaria parasite from the human body with lesser side effects and strengthening of the human immune system.

## MATERIALS AND METHODS

*Collection and identification of crude material*

Leaves of *N. arbor* stems of *T. cordifolia* & leaves of *O. sanctum* were collected from village Barlai Jageer, Indore, Madhya Pradesh. The crude plant materials were identified by Dr. Chitralekha Soni Kadel, Department of Botany, Vikram University, Ujjain (MP), and voucher specimens *O. sanctum* voucher no. MIPS/O/01/2017, *T. cordifolia* voucher no. MIPS/T/02/2017, and *N. arbor* voucher no. MIPS/N/03/2017 was deposited in the herbarium of the Department of Pharmacognosy, MIPS, Ujjain (MP).

*Processing of collected crude drug*

All the collected crude materials were dried separately in shade at normal, room temperature by spreading them uniformly. Dried crude materials were pulverized individually in the grinder to obtain a powder. They were then subjected to extraction using soxhlet apparatus, ethanol was used as solvent [22, 23].

*Phytochemical screening*

Phytochemical screening of all three extracts was performed as per the procedure prescribed [24-26].

*Qualitative analysis*

Qualitative analysis of all three crude drugs was performed as per the procedures prescribed [27-30].

*Formulation of vati*

Fine crude powder of *N. arbor*, extract of *T. cordifolia*, and extract of *O. sanctum* were mixed well together by blending according to formula **(Table 1)**. Jaggery was taken and a little amount of water was added to it to make paka. Paka was added to the above mixture to make vati. Manually formulated vati was then allowed to dry [31-34].

**Table 1.** Formula for vati

| S. No. | Ingredient | Quantity (mg) |
|---|---|---|
| 01 | Crude powder of *N. arbor* | 1500 |
| 02 | Extract of *T. cordifolia* | 500 |
| 03 | Extract of *O. sanctum* | 100 |
| 04 | Jaggerypaka | q.s. |

*In-vitro* antimalarial evaluation

The designed formulation was screened for anti-malarial activity in the Micro-care laboratory & TRC, Surat, Gujarat. The *in-vitro* antimalarial assay was carried out in 96 well micro-titre plates according to the microassay protocol of Rieckmann and co-workers with minor modifications. The *P.falciparum* strain cultures were maintained in medium RPMI 1640 supplemented with 25 mMHEPES, 10% heat-inactivated human serum, 0.23% sodium bicarbonate, and 1%D-glucose. To obtain only the ring stage parasitized cells, the asynchronous parasites of *P.falciparum* were synchronized after 5% D-sorbitol treatment. For carrying out the assay, an initial ring stage parasitemia of 0.8 to 1.5% at 3% haematocritina total volume of 200 µl of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya staining to assess the percent parasitemia (rings) and uniformly maintained with 50% RBCs ($O^+$). A stock solution of 5mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with a culture medium. The diluted samples in 20 µl volume were added to the test wells to obtain final concentrations (at five-fold dilutions) ranging between 0.4µg/mlto100 µg/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37˚C in a candle jar. Thin blood smears from each well were prepared and stained with Jaswant Singh Bhattacharya stain after 36 to 40 h incubation. To record the ring-stage parasites maturation into schizonts and trophozoites when different concentrations of the test agents present, the slides were microscopically observed. The concentration of test that inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC) [35-42]. The reference drug that was used was Chloroquine.

## RESULTS AND DISCUSSION

In this research work, three medicinal plants were selected for formulation development for malaria infection. The basic motive of this formulation development is to provide a treatment option to the patient with better results in terms of cure with minimal side effects. *N. arbor tristis*, *T. cordifolia*, and *O. sanctum* were selected and analyzed for this purpose.

Extraction of crude drugs was done individually by using soxhlet apparatus and ethanol as solvent, percent yield was calculated and was found to be 32% for *N. arbor*, 21% for *T. cordifolia*, and 17% for *O. sanctum.*

*Phytochemical screening*

Qualitative phytochemical analysis was performed and results show that *N. arbor* contains phytoconstituents such as carbohydrates, alkaloids, phenolics, tannins, flavonoids, fixed oils, and glycosides. *T. cordifolia* contains alkaloids, glycosides, phenolics, tannins, and

steroids, whereas *O. sanctum* contains alkaloids, flavonoids, glycosides, phenolics, tannins, steroids, and volatile oils **(Table 2)**.

**Table 2.** Phytochemical screening of extracts of *N. arbor*, *T. cordifolia*, and *O. sanctum*

| S. No. | Phytoconstituent | *N. arbor* extract | *T. cordifolia* extract | *O. sanctum* extract |
|---|---|---|---|---|
| 01 | **Carbohydrate** | | | |
| I | Molish's test | - | - | - |
| II | Fehling's test | + | - | - |
| III | Benedict's test | + | + | - |
| 02 | **Alkaloids** | | | |
| I | Dragendorff's test | +++ | +++ | ++ |
| II | Mayer's test | - | - | - |
| III | Hager's test | - | ++ | - |
| IV | Wagner's test | ++ | ++ | + |
| 03 | **Phenolics and tannins** | ++ | ++ | + |
| 04 | **Flavonoids** | | | |
| | Shinoda test | ++ | - | + |
| 05 | **Fixed oil and fats** | + | - | - |
| 06 | **Glycosides** | | | |
| I | Modified Borntrager's test | +++ | ++ | + |
| II | Foam test | ++ | + | + |
| 07 | **Steroids** | - | + | + |
| 08 | **Volatile oil** | - | - | +++ |

+ present, ++ moderately present, +++ strongly present, - absent

*Qualitative studies*

Foreign matter was determined and results show that all three drugs have no adulteration. A morphological evaluation of three drugs was performed for identification purposes. *N. arbor*, *T. cordifolia*, and *O. sanctum* successfully identified. *N. arbor* leaves are green in color with a pungent odor and bitter taste. The size is approximately 8-10 cm in length. *T. cordifolia* stems are observed grayish-green with a bitter taste. *O. sanctum* leaves are green with an aromatic odor and slightly pungent taste. Moisture content was determined to know the presence of moisture in crude material which may initiate microbial growth during storage. Moisture content in *N. arbor* and *T. cordifolia* was found nil whereas *O. sanctum* contains 0.25 mg/g moisture. Ash value analysis was performed to determine the quality of the crude drug, total ash gives an idea about extraneous matter adhering to the plant surface, Acid-insoluble ash measures the amount of silica present, especially sand and siliceous earth, and Water-soluble ash is the difference in weight between the total ash and the residue after-treatment of the total ash with water. For *N. arbortristis* total ash was 185 mg/g, acid-insoluble ash was 140 mg/g and water-soluble ash was found to be 35 mg/g, for *T. cordifolia* total ash was 125 mg/g, acid-insoluble ash was 105 mg/g and water-soluble ash was 20 mg/g, for *O. sanctum* total ash was 250 mg/g, acid-insoluble ash was 90 mg/g and water-soluble ash was 45 mg/g. Extractive values were calculated to determine the number of phytoconstituents extracted with different solvents. Water-soluble and alcohol-soluble extractive values were calculated for three experimental crude drugs and were found for *N. arbor* 190 mg/g and 290 mg/g respectively. For *T. cordifolia* was found to be 90 mg/g and 140 mg/g respectively. For *O. sanctum* it was found to be 110 mg/g and 120 mg/ g respectively. The swelling index confirms the presence of mucilage in crude drugs. In the present study, the swelling index was zero, it suggests that there is no presence of mucilage in crude drugs. Foaming index indicates the presence of saponins in crude drugs. The foaming index was found to be less than 100 in *N. arbor*, *T. cordifolia*, and *O. sanctum* as well.

*Thin layer chromatography*

Thin-layer chromatography was performed by using extracts and the Rf value for *N. arbor* extract was found to be 0.48, for *T. cordifolia* extract it was found 0.5 and for *O. sanctum* extract it was 0.3.

*UV spectrophotometry*

UV spectrophotometric analysis was performed for extracts, *N. arbor* extract shows a peak at 220 nm which confirms the presence of iridoid glycoside, which is hypothetically responsible for the antimalarial effect of *N. arbor*. *T. cordifolia* extract shows a peak at 394 nm, 328 nm 218 nm, and 214 nm which indicates the presence of phytoconstituents responsible for the activity. *O. sanctum* extract shows a peak at 287 nm which confirms the presence of eugenol in the extract which is responsible for antimalarial potential.

*Evaluation of formulation*

Vati, an ayurvedic dosage form was selected for polyherbal formulation. Jaggery is used as a binder in formulation, whereas to meet the particular dose 3-4 vati should be taken once a day. Evaluation of vati was performed and results indicate that it meets all the required limits. Weight variation was found to be less than 5%, hardness was 0.5 kg/cm square, friability was less than 1%, and disintegration was 3-4 minutes. Dissolution test confirms release of active principle from the formulation.

*In-vitro studies*

*In-vitro* antimalarial evaluation of formulation was performed in Microcare laboratory & TRC, Surat, Gujarat, and the minimum inhibitory concentration (MIC) value was found 0.98µg/ml **(Table 3)**, where chloroquine and quinine were used as a standard drug and the minimum inhibitory concentration was found to be 0.020 µg/ml and 0.268 µg / ml respectively.

**Table 3.** In vitro antimalarial activity using Plasmodium falciparum (Minimal inhibition concentration)

| S. No | Compound ID | Mean IC50 values (a) |
|---|---|---|
| 1 | AMV 01 | 0.98 µg/ml |

## CONCLUSION

In the present study, pharmacognostic and phytochemical evaluation of *N. arbor*, *T. cordifolia*, and *O. sanctum* was performed which confirms the presence of active phyto molecules which are possibly responsible for antimalarial potential.

Polyherbal formulation, which is vati were evaluated for dosage form parameters and it passes all the tests. The *in-vitro* evaluation showed that the formulation posses a significant anti-malarial effect. All the outcomes suggest that the present formulation can be developed as a better treatment option for malaria with minimal side effects.

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