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Standardization of a Traditional Polyherbal Malaria Prophylactic Formulation

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Abstract

Polyherbal preparations are well known in certain Indian communities for malaria prophylaxis. However, quality control and quality assurance of these formulations still remain as challenges because of the high variability in combination of herbs and their phytochemical compositions. Quality control is an essential operation of the pharmaceutical process to ensure the quality herbal formulation with safety and therapeutic activity. The present study consists of preparation and standardization of a traditional polyherbal malaria prophylactic formulation (in decoction form) for parameters like organoleptic, physico-chemical, phytochemical, microbial analyses, heavy metal and pesticide residue analysis. The polyherbal formulation revealed the presence of carbohydrates, saponins, phenolics and tannins. High Performance Thin Layer Chromatography (HPTLC) carried out for comparing the decoction and the individual plants, revealed the presence of common bands. It provided unique fingerprints for the plants and the formulation that can be used as appropriate parameters for quality control.

Keywords: Quality control, Standardization, Malaria prophylaxis, Traditional medicine, Herbal medicine.

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INTRODUCTION

Traditional medicine is the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses. [1] Medicinal plants have played a key role in world health and are the major integral part of traditional medicine. Around 80% of the population depends on traditional medicine for primary health care in some Asian and African countries [2]. In spite of the great advances observed in modern medicine in recent decades, medicinal plants still make an important contribution to health care [3].

The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing countries and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy [4]. Traditional medicines have contributed to a great extent in the treatment of both communicable and non-communicable disorders. Malaria is one such communicable, vector-borne disease, causing at least 225 million infections and around one million deaths every year in developing countries [5]. Two of the most effective drugs for malaria originated from traditional medicine: quinine from bark of the Peruvian *Cinchona* tree, and artemisinin from the Chinese antipyretic *Artemisia annua*. Earlier studies have revealed the use of over 1200 plants species throughout the world to treat malaria [6].

In India, several medicinal plants are traditionally used for treatment and prophylactic purpose among tribal population as these are locally available and easily accessible to the population of malaria-endemic areas [7, 8]. Ethnobotanical study conducted by our team in three districts of Odisha state, India, has revealed 16 traditional plant species used by local traditional healers for prevention of malaria [9]. Majority of the remedies described in this study are combination of at least three plants that are locally available. It was observed that one polyherbal formulation (decoction form) having five medicinal plants were frequently recommended by many traditional healers as malaria prophylaxis. We selected this traditional polyherbal malaria prophylactic (TPMP) formulation along with the five medicinal plant ingredients for standardization and quality control tests as a preliminary step before studying their potential usefulness including evaluation, safety and efficacy. Quality control in traditional medicine emphasizes the need to ensure the quality of medicinal plant products by using modern

control techniques and applying suitable standards to ensure the safety and therapeutic activity.

MATERIALS AND METHODS

Collection and authentication:

The plants used in TPMP formulation [Table 1] were collected from Koraput district of Odisha state, India, by a taxonomist who was conversant with the flora of the area. The plants were verified and authenticated by a botanist and voucher specimens deposited at our institute. The physical impurities were removed and the drugs were washed with water, sorted, shade dried and crushed to a coarse powder. The crude drugs with their botanical identities, regional names, parts used and quantity are given in Table 1.

Plant species (Voucher specimen number)	Regional name	Family	Part used	Quantity used (g/l)
<i>Azadirachta indica</i> (L/09/01/003)	Neem	Meliaceae	Leaf	156
<i>Andrographis paniculata</i> (L/09/01/001)	Bhuin-neem	Acanthaceae	Whole plant	156
<i>Nyctanthus arbortristis</i> (L/09/01/006)	Gangaseuli	Oleaceae	Leaf	156
<i>Piper nigrum</i> (L/09/01/008)	Golmarica	Piperaceae	Fruit	16
<i>Zingiber officinalis</i> (L/09/01/009)	Sunthi	Zingiberaceae	Rhizome	16

Table 1: Composition of the formulation: Regional, botanical and family names with part and quantity used

Method of preparation of decoction:

A total of five hundred grams of identified crude, coarse powders were mixed thoroughly with four liters of water in a stainless steel container with continuous stirring under medium heat until the water was reduced to one liter. Later, the decoction was filtered through single folded cotton cloth. The decoction was stored at -20°C.

Standardization parameters:

The individual plant species and polyherbal decoction were subjected to various analytical parameters as follows:

Organoleptic description:

Organoleptic evaluation was carried out to assess the color, odor and taste of the individual ingredients and formulation [10].

Physicochemical evaluation:

Physicochemical analysis of the Individual drugs and formulation has been done to evaluate the quality and purity of the formulation. In physicochemical evaluation, foreign organic matters, ash value such as total ash, acid insoluble ash was evaluated. The ash value indicates the presence of inorganic salts present

in the drug. The alcohol soluble and water soluble extractive values of *A. indica* [11], *A. paniculata* [12], *N. arbortritis* [13], *P. nigrum* [14], *Z. officinale* [15] and physical characteristics like pH, specific gravity, refractive index, total solids, brix value of formulation were determined. The information collected from this evaluation was useful for standardization and obtaining the quality control for crude drugs as well as for formulation.

Phytochemical evaluation:

The qualitative chemical tests were carried out for the identification of nature of phyto-constituents present in the formulation [16-18].

Toxicological study:

The toxicological evaluations were carried out for the presence of heavy metal and pesticide residues in the formulation [19].

Microbial analysis:

Microbial tests were carried out to assess the total bacterial and fungal counts [20].

Chromatographic analysis

HPTLC finger printing profile of methanolic extracts of the individual ingredients, *A. indica* [21] *A. paniculata* [22], *N. arbortritis* [23], *P. nigrum* [24], *Z. officinale* [25] and formulation were carried out along with the different marker compounds corresponding to the active ingredients to ensure the presence of active ingredients in the formulation. For HPTLC, 5gm of each sample was extracted with 100ml of methanol in a soxhlet apparatus for 6 hours, filtered and concentrated. The chromatograph was performed by spotting standards and extracted samples on pre-coated silica gel aluminium plate 60F-254 (10cm×10cm with 250µm thickness) using Camag Linomat IV sample applicator (CAMAG, Muttenz, Switzerland) and 100µl Hamilton syringe. The length of the chromatogram run was 8 cm. Plates were developed using specific mobile

phases. Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the reflectance mode at 254 nm and 366 nm using win CATS software.

RESULTS

The organoleptic evaluation of crude plants and formulation (decoction) were as shown in the Table 2. The results of physicochemical parameters of crude drugs were reported in Table 3. There was no foreign organic matter in the crude drugs. The result of moisture showed, 4% minimum in all the crude ingredients having least of 4.2% in *A. paniculata* and highest of 9.8% in *Z. officinalis*. The result of total ash value indicated the purity of drug that is the presence or absence of foreign matter such as metallic salt or silica present in the crude drug.

The percentage values of water soluble extractive of crude drugs were comparatively more than the alcohol soluble extractive, which signifies that the large amount of drug was soluble in water than alcohol. Reducing sugars and Hydrolysable sugars in water soluble extract were positive for all the crude ingredients.

Sl. No.	Name of plant	Color	Odour	Taste
1	<i>Azadirachta indica</i>	Green	Characteristic	Bitter
2	<i>Andrographis paniculata</i>	Green	Bitter	Bitter
3	<i>Nyctanthus arbortritis</i>	Green	Characteristic	Bitter
4	<i>Piper nigrum</i>	Black	Aromatic	Pungent
5	<i>Zingiber officinalis</i>	Yellow	Aromatic	Sweet
6	Formulation (Decoction)	Light blackish	Pleasant	Bitter

Table 2: Organoleptic descriptions of individual ingredients and formulation

Plant species	F.O.M.%	Total Ash %	Acid Insoluble Ash %	Alcohol Soluble Ext %	Water Soluble Ext %	Tannins for ASE	Reducing sugars for ASE	Hydrolysable sugars for ASE	Tannins for WSE	Reducing sugars for WSE	Hydrolysable sugars for WSE
<i>A. indica</i>	Nil	9.89 ± 0.21	0.95 ± 0.02	13.47 ± 0.07	25.31 ± 0.60	-	-	+	Traces	+	+
<i>A. paniculata</i>	Nil	10.64 ± 0.41	0.90 ± 0.07	8.22 ± 0.13	21.51 ± 0.05	-	-	-	-	+	+
<i>N. arbortritis</i>	Nil	10.35 ± 0.05	1.96 ± 0.05	10.17 ± 0.10	28.14 ± 0.20	-	Traces	Traces	-	+	+
<i>P. nigrum</i>	Nil	5.82 ± 0.03	0.6 ± 0.01	10.25 ± 0.05	10.71 ± 0.05	-	-	-	-	+	+
<i>Z. officinalis</i>	Nil	4.6 ± 0.11	0.88 ± 0.03	8.93 ± 0.11	38.49 ± 0.14	-	Traces	+	-	+	+

Table 3: Physicochemical evaluation of individual ingredient of formulation.

The result of physical parameter of TPPM formulation (decoction) was tabulated in Table 4. The pH of decoction was 6.19 shows slight acidic in nature. Brix value, refractive index total solids (%w/v) and specific gravity were determined. The Phytochemical analysis of formulation is tabulated in Table 5, which showed presence of carbohydrates, saponins and phenolics & tannins. Table 6 shows the values of heavy metals presence in the crude drugs which was within the permissible limits. The microbial analysis showed the absence of microbes in the formulation (Table 7) and there were no any detectable pesticide residues in the crude drugs.

Parameters	Decoction (n=3)
pH	6.19 ± 0.05
Brix Value	12 ± 0
Refractive Index	1.36 ± 0.01
Total solids (%w/v)	16.32 ± 0.03
Specific gravity	1.05 ± 0.01

Table 4: Physical characteristics of formulation (decoction)

Chemical constituents	Decoction
Alkaloids	-
Carbohydrates	+
Glycosides	-
Saponins	+
Phytosterols	-
Fixed oils & Fats	-
Resins	-
Phenolics & Tannins	+
Proteins & Amino acids	-
Flavonoids	-
Gums & Mucilage	-

+ Present, - absent

Table 5: Phytochemical analysis of formulation (decoction)

Heavy metal	Results (ppm)	Limit (ppm)
Lead (Pb)	3.8	10
Cadmium as (Cd)	0.26	0.3
Arsenic (As)	<0.01	3
Mercury (Hg)	<0.01	1.0

Table 6: Heavy metal analysis of crude drugs

Parameter	Result	Limit
Total microbial count	Absent	10 ⁵ CFU/mL
Total fungal count	Absent	10 ³ CFU/mL
Escherichia coli	Absent	10 CFU/mL
Enterobacteriaceae	Absent	10 ³ CFU/mL
Salmonella spp.	Absent	None

Table 7: Microbial analysis of formulation (decoction)

HPTLC finger print profile of methanol extracts of individual plants with their standard markers namely Andrographaloid, Azadirachtin, Quercetin, Piperine, and 6-gingerol were shown in Figure 1. A comparative HPTLC fingerprint profile of the decoction and aqueous extract of each plant is shown in Figure 2. Rf values of individual ingredient and formulation were given in Table 8. Densitograms of the decoction at 250nm and 350nm were given in Figure 3 and 4 respectively.

Plant	Rf
<i>Azadirachta indica</i> [Az]	0.68 (light pink), 0.50 (blue), 0.31(yellow)
<i>Andrographis paniculata</i> [An]	0.71 (faint yellow), 0.64 (dark pink), 0.45(light brown), 0.36 (dark pink/red), 0.31 (faint yellow)
<i>Nyctanthus arbortristis</i> [N]	0.50 (light brown), 0.31 (faint yellow)
<i>Zingiber officinalis</i> [Z]	0.76 (dark brownish), 0.31(dark yellow)
<i>Piper nigrum</i> [P]	0.31(light yellow)
Decoction [D]	0.71(faint yellow), 0.65 (dark pink/red), 0.61 (faint yellow), 0.55 (faint brown), 0.50 (light blue), 0.44 (dark blue), 0.36 (dark pink/red), 0.31 (faint yellow)

Table 8: Rf spots of crude drugs with formulation (decoction)

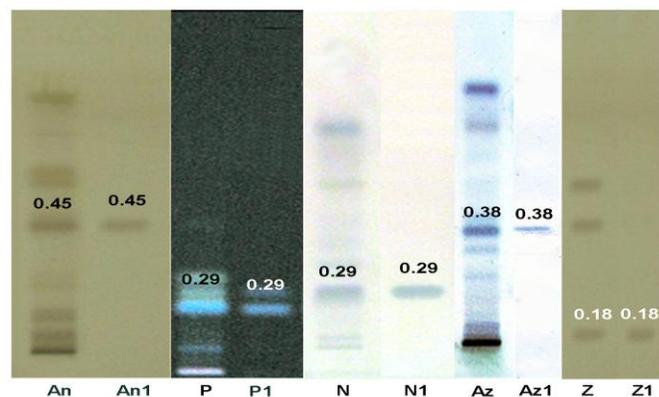


Figure 1: Fingerprint profile of individual plants observed under 366nm

Figure 1 continued:

An: *Andrographis paniculata*, Az: *Azadirachta indica*, N: *Nyctanthus arbortristis*, P: *Piper nigrum*, Z: *Zingiber officinalis*; An1: Andrographaloide; Az1: Azadirachtin; N1: Quercetin; P1: Piperine; Z1: 6-gingerol

Mobile Phase: An: Chloroform: Methanol (7:1); P: Benzene: Ethyl acetate (2:1); N: Toluene: Ethyl acetate (8.0:2.0); Az: Toluene: Ethyl acetate (8:2); Z: n-Hexane: ether (4:6)

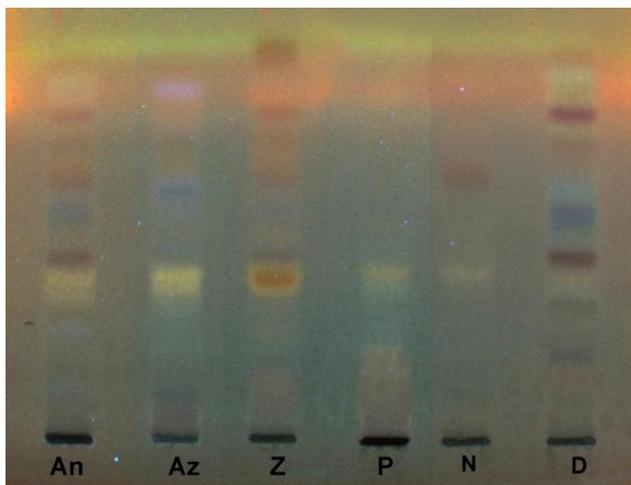


Figure 2: Comparative fingerprint profile of the decoction (D) with the individual plants (An, Az, Z, P, N) observed under 366nm after derivatization.

Mobile Phase: Butanol:Acetic acid:Water (7:2:1)

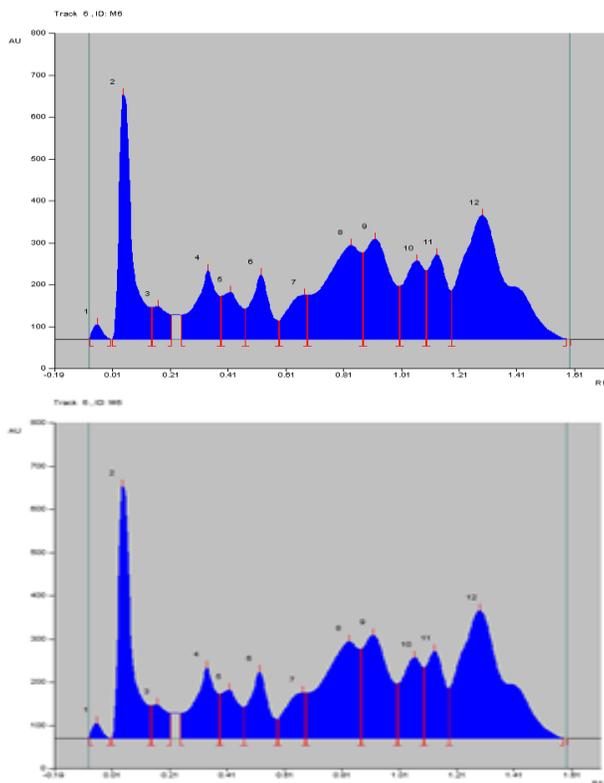


Figure 3: Densitogram of decoction at 250nm

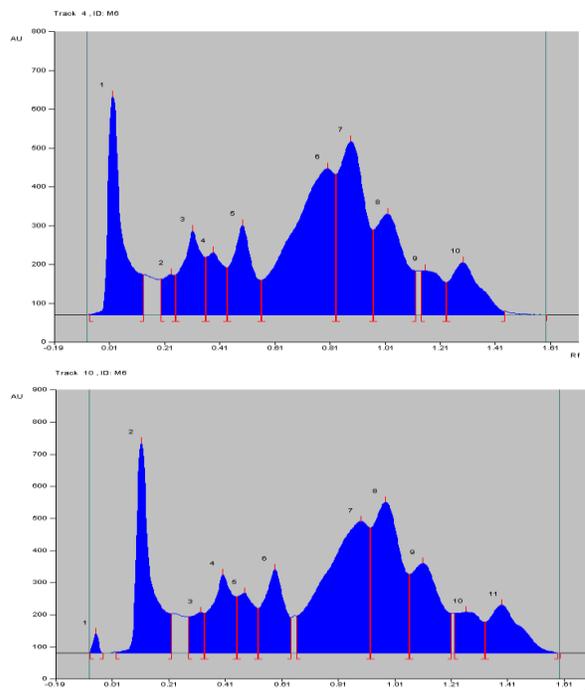


Figure 4: Densitogram of decoction at 350nm

DISCUSSION

Standardization of drugs aids in confirmation of identity, quality and purity. The quality of herbal drugs is the sum of several factors, which contribute directly or indirectly to the safety, effectiveness and acceptability of the product. Traditional medicines are usually prepared as a combination of several herbs which have high medicinal values.

Earlier studies have shown that the plants which are widely used as anti-malarials by traditional healers are significantly more active *in vitro* and/or *in vivo* against *Plasmodium* sp than plants which are not widely used, or not used at all, for the treatment of malaria [26-28]. This highlights the importance of ethnobotanical survey conducted to select traditional polyherbal malaria prophylactic (TPMP) formulation. The five plants of TPPM are mentioned in Ayurvedic texts for their action against fever (as *jvarahara* / *vishama jvarahara* - anti-pyretic) [29]. These five plants are also known for their anti-malarial activity [30-34]. In malaria-endemic villages of Odisha, traditional healers advise the use of TPMP formulation during high malaria transmission season (June to October) for the purpose of prevention of malaria infection. Even though these plants are known for their anti-malarial activity and used as prophylaxis by tribal communities, the potential use of these plants for their malaria preventive activity is scientifically unexplored. As a first step, we aimed to standardize the selected TPMP formulation using standard quality control parameters.

The five individual plants were subjected to organoleptic evaluation which provides the simplest and quickest means to establish the identity and ensure the quality of formulation. The bitter taste of the decoction was a characteristic parameter due to the specific properties of *A. indica* and *A. paniculata*. According to Ayurvedic pharmacology, bitter taste herbs have the action of *jvarahara* (anti-pyretic) [35]. In the physicochemical analysis, the ash value and solvent extractive values of these five plants were matched with values given in Ayurvedic Pharmacopoeia of India or Indian Herbal Pharmacopoeia which signifies their good quality and purity. Total ash usually consists of both physiological ash, which is derived from the plant tissue itself, and non-physiological ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface [4]. As the ash values of the crude drugs used for formulation lies within the fair limit which signify its quality and purity and gives idea about the total inorganic content.

The pH conventionally represents the acidity and alkalinity; pH of formulation was showing slightly acidic nature which may be because of acidic salts present with in raw materials. Brix value, refractive index, total solids and specific gravity of the decoction were determined in three independent analyses which ensure the quality of the formulation. Carbohydrates, saponins, phenols and tannins were expressed in the phytochemical analysis of decoction. Microbial analysis confirms the absence of bacteria, fungus, *E. coli*, enterobacteriaceae and salmonella species. Heavy metals analysis of the crude plants was within the standard limit which ensures the safety of the formulation. Crude plants were also subjected to the analysis of pesticide residues like organochlorine, organophosphorous and synthetic pyrethroids. None of the pesticides were detected, thus establishes the purity of the raw drugs.

Figure 1 shows the fingerprint profile of individual plant with their standard markers. The common bands observed in the plant and marker confirms the botanical identity of plants which are used for the preparation of decoction. Figure 2 demonstrates the comparative fingerprint of the decoction and the individual plants. The common bands observed in the plants and decoction explains the presence of several compounds which are extracted through the process of preparation of decoction, which may be responsible for the activity of the drug.

CONCLUSION

Traditional polyherbal malaria prophylactic formulation has certain advantages as these plants are locally available, easily accessible and affordable to the rural malaria-endemic population.

The present study with various standardization parameters such as physicochemical standards, phytochemical profiles and safety evaluation, it can be concluded that the quality control parameters for TPMP formulation are presents within the permissible limits as per standard guidelines. This quality control mechanism supports in development of new prophylactic drug for malaria which are safe and therapeutically effective.

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