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Évaluation of the anti-malarial properties of the combined aqueous and alcoholic extract of *Heliotropium indicum* and *Parquentina nigrescencs*

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Abstract

This research investigates the potential antimalarial efficacy of combined extracts derived from *Heliotropium indicum* and *Parquetina nigrescens*, considering their traditional applications in addressing various ailments, including malaria.

The primary objective is to assess the suppressive antimalarial activity of the combined extracts and compare it with individual extracts, utilizing toxicity assessments and syrup formulations.

The study includes LD_{50} assessments to confirm the safety of the extracts, employing doses beyond 5000mg/kg. Suppressive assays are conducted on mice, evaluating both individual and combined extracts, with varying doses.

White mice from a reputable source are used for the experiments. The extracts are prepared as syrups, and different doses are administered to the mice for toxicity and antimalarial assessment. Parasitemia is evaluated through blood films, and statistical analysis is performed.

The obtained data are analyzed using one-way ANOVA and Student-Newman Keul's test to determine statistical significance. Results are expressed as mean ± SEM.

LD_{50} assessments reveal the safety of the extracts, and suppressive assays demonstrate significant antimalarial effects for both individual extracts at distinct doses. The combined aqueous extract shows promising outcomes, indicating potential synergistic interactions.

Traditional plant-based remedies, such as *Heliotropium indicum* and *Parquetina nigrescens*, hold promise for developing innovative antimalarial strategies. The combined extract exhibits potential as a complementary antimalarial intervention, warranting further exploration and research in the field of malaria management.

Keywords: LD_{50}; Suppressive assay ; Syrups; Antimalarial; ANOVA.

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1. Introduction

1.1. Malaria

Malaria is a severe disease caused by parasites of the genus Plasmodium, which is transmitted to humans by a bite of an infected female mosquito of the species Anopheles. Malaria remains the leading cause of mortality around the world, and early diagnosis and fast-acting treatment prevent unwanted outcomes (Jasminka et al., 2019). It is the most common disease in Africa and some countries of Asia, while in the developed world malaria occurs as imported from endemic areas.

Between 2010 and 2015, malaria incidence among populations at risk (the rate of new cases) fell by 21% globally. In 2015 more than half of the population of sub-Saharan Africa slept under insecticide-treated mosquito nets compared to just 2% in 2000. Increased availability of rapid diagnostic tests and antimalarial medicines has allowed many more people to access timely and appropriate treatment. Malaria incidence rates have decreased by 37% globally and mortality rates by 60% since 2000. It is estimated that 70 % of the reductions in numbers of cases in sub-Saharan African can be attributed to malaria interventions (Richard et al., 2016). In that same period, malaria mortality rates among populations at risk fell by 29% globally among all age groups, and by 35% among children under five years of age (WHO, 2016).Sub-Saharan Africa carries a disproportionately high share of the global malaria burden. In 2015, the region was home to 90% of malaria cases and 92% of malaria deaths(Wilfred et al., 2019)

Malaria is an acute febrile illness. In a non-immune individual, symptoms appear 7 days or more (usually 10–15 days) after the infective mosquito bite(WHO, 2014). The first symptoms fever, headache, chills and vomiting may be mild and difficult to recognize as malaria. If not treated within 24 hours, P. falciparum malaria can progress to severe illness, often leading to death.

Children with severe malaria frequently develop one or more of the following symptoms; severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria. In adults, multi-organ involvement is also frequent.Individuals from malaria-endemic regions often acquire partial immunity after multiple repeated infections throughout their lives. This partial immunity prevents them from developing severe complications and they often remain asymptomatic with a persistent, low parasite density in the blood, and therefore the necessity for treatment is neglected. These patients with chronic, asymptomatic malaria serve as a reservoir for *Plasmodium* parasite transmission, becoming a major obstacle for eradication efforts(Michelle and Cevayir 2018)

According to the latest WHO estimates, released in December 2016, there were 212 million cases of malaria in 2015 and 429 000 deaths.

Drug resistance poses a growing problem in the treatment of malaria, since resistance is now common against all classes of antimalarial drugs. The global adoption of artemisinin-based combination therapies (ACTs) in the early 2000s heralded a new era in effectively treating drug-resistant Plasmodium falciparum malaria (Benjamin et al., 2017).

This situation has resulted in the treatment of resistant strains becoming increasingly dependent on this class of drugs. However, the artemisinins are expensive, which limits their use in the developing world. Worrisome evidence is now emerging of malaria on the Cambodia-Thailand border that are resistant to combination therapies that include artemisinins, which raises the possibility that strains of malaria may have evolved that are untreatable with currently-available drugs. Exposure of the parasite population to artemisinin monotherapies in sub therapeutic doses for over 30 years, and the availability of substandard artemisinins, have probably been the main driving force in the selection of the resistant phenotype in the region (WHO, 2014).

Herbal plants play an important role in preventing and treating of human diseases. People have been using plants as a traditional medicine for thousand years ago. Plants have been associated with the development of human civilization around the whole world. However, plants are considered as rich sources of phytochemical ingredients which enable to have medicinal value. Medicinal plants are a potential source for the development of new herbal drugs. In the 21st century, the pharmacological effects of medicinal plants have been considered as a promising future drug/medicine for the management of health care. In recent years, there has been a resurgence of interest to rediscover medicinal plants as a source of potential drug candidate (Arvind, 2016).

Medicinal plants play an energetic role in the discovery of new therapeutic agents, thus growing interest in the use of pharmaceutical consumption [Sharifi et al., 2020]. Medicinal plants contain many constituents such as alkaloids, flavonoid, tannin, phenol, saponin, and glycosides, with notable biological activities such as antimicrobial, analgesic,

antipyretic, anti-tumor, wound healing, and cardio-protective, among others that can be useful against diverse human diseases [Salehi et al., 2020].

Heliotropium indicum, commonly called as Indian Turn-sole, is a herb with slightly woody at base (Mukesh et al., 2016), *Heliotropium indicum* L. belongs to the family Boraginaceae. The plant has been used as a folk medicine because it contains substances of various biological activities. It is also identified as a common weed which grows wildly in crop fields in tropical and subtropical regions of the world (Sirinapa et al., 2018)

Heliotropium indicum Linn. (Family- Boraginaceae) an annual herbaceous medicinal weed . It is not only a common weed but also it is an important medicinal herb, too. These medicinal herbs are found in tropical and temperate parts of the world along with India, Bangladesh, and some other African countries. Many pyrrolizidine categories of alkaloids have been separated from this particular medicinal plants (Pranabesh et al., 2018)

1.2. Phytochemical Constituents

Based on the history of traditional and folk medicinal uses of H. indicum, many researchers have been investigating its phyto-chemical and pharmacological properties to identify the compounds responsible for its wide use as herbal medicines. The plant contains many important phyto-components, including alkaloids (e.g., acetyl indicine, cynoglossine, echinitine, heleurine, heliotrine, helindicine, europine N-oxide, heleurine N-oxide, heliotridine N-oxide, heliotrine N-oxide, indicine, indicinine, indicine N-oxide, lasiocarpine, lycopsamine, trachelanthamidine, retronecine, and supinine), triterpenes (e.g., β-amyrin, lupeol, rapone, and rapanone), sterols (e.g., β-sitosterol, estradiol, chalinasterol, campesterol, hexacosane-1-ol, and stigmasterol), amines (e.g., putrescine, spermidine, and spermine), and volatile oils (e.g., 1-dodecanol, β-linalool, and phytol) [Sivagnanam et al., 2014].

Scientific reports revealed that the herb showed antioxidant, analgesic, antimicrobial, anticancer, anti-tuberculosis, anti-plasmodial, anti-cataract, anti-fertility, wound healing, anti-inflammatory, antinociceptive, antihyperglycemic, anti-helmintic, diuretic, anti-tussive, anti-glaucoma, anti-allergic, and larvicidal activity(Chandan et al., 2021)

Figure 1 Picture of herbs of *Heliotropium Indicum*

Figure 2 Picture of *Parquentina nigrescens*

Parquetina is a mono-typic genus with *Parquetina nigrescens* being the only species in it and from the Apocynaceae family, widely used in Africa for the treatment of many diseases. It is commonly found in secondary forests and around villages in Senegal, Nigeria and Ghana. It is a perennial evergreen woody climber with twining stems; often herbaceous but becoming woody with age. It has relatively large and coriaceous leaves 10-15cm long, 6- 8cm broad, and fleshy coriaceous corolla with inside pink, maroon or deep crimson to black-violet, and pubescent or hirsute stamens with pollen in tetrads. The leaf is normally odourless with a light green abaxial colour, a dark green adaxial colour, and an acuminate apex (Sopeyin and Ajayi; 2016). The local names for the plant vary from country to country. For instance, in Nigeria, the plant is called Ewe Ogbo, Mgbidim gbe, Kwankwanin, Inuwu elepe, and Ovie ukpakoma in tribes such as Yoruba, Igbo, Hausa, Yoruba (Ife), and Avianwu (Etsako), respectively (Kayode and Yakubu; 2017).

It was observed that P. nigrescens is rich in phytochemical constituents and possesses pharmacological properties. Hence, it's diverse positive effects on different diseases (Adase et al; 2022). The phytochemical screening of ethanolic extract of the leaf and stem showed the presence of reducing sugars, tannins, terpenoids, saponins, flavonoids, alkaloids, phlobatannins, cardiac glycosides, steroids, and coumarin (Sopeyin and Ajayi; 2016, and Airaodion et al; 2019). Studies have revealed antioxidant, anti-inflammatory, anti-sickling, haematological, cytotoxic, antimicrobial, antipyretic, sympathomimetic, uterotonic, hematopoietic, analgesic, and antiulcerogenic properties of the plant [1, 3, 9]. Nafiu and colleagues [8] reported an in-vivo study of the antimalarial potential of aqueous leaf extract of P. nigrecsens and a combination of aqueous leaf extract of P. nigrescens and Tithonia diversifolia in albino mice. The result showed that the aqueous leaf extract of P. nigrescens administered at a dose of 150 mg/kg body weight showed good inhibition with an 86% reduction in the parasitaemia by chemo-suppression for 18 days against the malaria parasite (Plasmodium berghei) in albino mice. The combined extracts (P. nigrescens + T. diversifolia) demonstrated significant antimalarial activity and 90% parasite inhibition at administered doses of 150 mg/kg body weight. The mean survival time for mice recorded was 19 days for the combined extracts, and the aqueous leaf extract of P. nigrescens was 18 days when compared to 7 days for the control (0.3 ml of distilled water). *Parquetina nigrescens* has also shown to have antityphoid activities (Akinyemi and Dada; 2014) amongst many other antimicrobial and pharmacological benefits (Adase et al; 2022).

2. Materials and methods

2.1. Materials

2.1.1. Animals

White mice of both sexes, weighing between 18 g and 21 g obtained from Animal House, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria were used as experimental animals for this study.

2.1.2. Drug

Chloroquine (Phosphate salt – Batch No. 533), obtained from May and Baker Nigeria Plc., PMB 21049, 3/5 Sapara Street, Industrial Estate, Ikeja, Lagos was employed as standard reference for the antimalarial screening in this study.

2.1.3. Parasite

Sample of *Plasmodium berghe*i (NK65) obtained from Institute of Medical Research and Training (IMRAT) at the University College Hospital, Ibadan, Nigeria was used for the research to evaluate the antimalarial activity of the plant material used in this study. Parasitized blood from donor with known parasitaemia was got by first anesthetizing the mice with chloroform, and through the cardiac puncture, using sterile syringe, blood was collected into sterile disposable syringes. The number of parasitized red blood cells in a volume of blood was then calculated by multiplying the percentage parasitaemia by the number of red blood cells.

The desired volume of blood then obtained from the donor mouse was suitably diluted with sterile normal saline. Thus, the volume of blood collected from the donor mouse should be diluted with normal saline so that 0.2 ml of the standard inoculum will contain 1×10^7 parasitized red blood cells. This is the standard inoculum for the infection of a single mouse.

2.1.4. Extraction of plant

The leaves of the two plant were dried separately at 60°C in the oven and powdered in the grinding machine. The powdered samples (103 g)were then dissolved in distilled water in a round-bottomed flask. The mixture was macerated for 48 hours and then filtered. The filtrate was concentrated to dryness in-vacuo with the rotary evaporator. The residue was collected and stored at 4 °C for onward use. On drying, the leaf extract weighed 15.4 g.

2.2. Syrup preparation

Simple syrup was prepared by dissolving 66.7g of sucrose in 100 g of solution and heating the mixture until the syrup is formed. The extracts were dissolved in simple syrup to make a preparation of their syrup dosage form and the combined syrup was made by dissolving the extracts of both plants in 1:1 ratio

2.3. Methodology

2.3.1. Bioassay

Evaluation of acute toxicity

Mice were divided into 3 groups of 3 mice each and doses of 10 mg/kg, 100 mg/kg and 100 0mg/kg of the individual syrup were administered to the groups respectively and mortality was observed for 24 hours. In the absence of mortality, another set of mice were divided into 3 groups of 3 mice each and 1600mg/kg, 2900 mg/kg and 5000 mg/kg of the test syrups were administered respectively and again checked for mortality within 24 hours .

- Suppressive assay

Groups of mice (n=5) were pretreated with $0.2\text{x}10^7$ plasmodium parasites from the diluted blood of the donor. Treatment with graded doses of the test agents commenced 2 hours after innoculation for 4 days. Doses of the extracts given were 50, 100 and 200 mg/kg, 10mg/kg of chloroquine (positive control) and 0.2ml of normal saline (negative control). Thin smear were taken from the tail on the fifth day, stained with giemsa and observed under the light microscope

- Preparation of thin and thick films and staining techniques

Small drop of blood from the tail of the mouse was collected on clear non-greasy slide. Thin and thick films were made according to requirements. The films were air-dried. The thin film only was then fixed using a few drops of methanol and left to air-dry. The slides were then covered with Giemsa stain. The Giemsa stock solution was first diluted with sodium phosphate buffer pH 7.2 in the ratio (4:10). The stain was applied on the slide and allowed to stand for between 10-15 minutes. The stain was poured off and the slide rinsed with the water and allowed to air-dry.

- Evaluation of Parasitaemia

Each of the blood films prepared was mounted on a microscope and a drop of immersion oil applied on the slide. The observation was made using X100 objective lens to locate the best fields of view for counting both parasitized and non-parasitized red blood cells. In a particular field, the total numbers of red blood cells (TRBC) as well as the total number of parasitized red blood cells (PRBC) were counted. Percentage parasitaemia in each field was calculated as follows:

$$\% \text{ parasitaemia } = \frac{\text{total number of PRBC} \times 100}{Total\ number\ of\ RBC}$$

Ten fields were counted on each slide and mean percentage parasitaemia was recorded for each mouse.

2.4. Statistical Analysis

The parasitaemia was determined to find qualitatively the presence and degree of activity at the screening doses. One-way ANOVA with Student-Newman Keul's test was used. Statistical significance was set at $P < 0.05$. The results were expressed as mean ± SEM (Standard error of mean).

3. Results

3.1. Results of LD_{50} assessment of *Heliotropium indicum* and *Parquentina nigrescens* leaf extract (syrup)

No mortality was recorded in all the doses administered. Thus, the extract can be considered safe.According to OECD guidelines, any LD_{50} beyond 5000mg/kg is of no practical interest.

3.2. Results of LD_{50} assessment of *Heliotropium indicum* extract (syrup)

No mortality was recorded in all the doses administered. Thus, the extract can be considered safe.According to OECD guidelines, any LD_{50} beyond 5000mg/kg is of no practical interest.

3.3. Results of LD_{50} assessment of *Parquentina nigrescens* extract (syrup)

No mortality was recorded in all the doses administered. Thus, the extract can be considered safe.According to OECD guidelines, any LD_{50} beyond 5000mg/kg is of no practical interest.

Results for the suppressive antimalarial extracts of *Heliotropium indicum* and *Parquentina nigrescens* extracts.

Table 1 The suppressive antimalarial activity of the *Heliotropium indicum* extract (syrup)

Dose(mg/kg)	Mean % Parasitemia
50	23.04±2.9*
100	25.3±3.2*
200	21.89±2.2*

Key: * indicates statistical significance (P<0.05)

Table 2 The suppressive antimalarial activity of *Parquentina nigrescens* extract (syrup)

Dose(mg/kg)	Mean% Parasitemia
50	47.46±26.3*
100	20.29±3.6*
200	26.26±10.1*

Key: * indicates statistical significance (P<0.05)

Table 3 The suppressive antimalarial activity of the combination of *Heliotropium indicum* and *Parquentina nigrescens* extract (syrup)

Dose(mg/kg) Normal saline	Mean % Parasitemia 3.24±0.9	%Chemosupression 0
50	14.81±5.1*	67.23
100	9.28±4.2*	65.09
200	9.88±2.0*	67.21
Chloroquine(10mg/kg)	7.8±1.3	58.46*

Key: * indicates statistical significance (P<0.05)

Table 4 The suppressive antimalarial activity of the combination of *Heliotropium indicum* and *Parquentina nigrescens* extract (aqueous)

Dose(mg/kg)	Mean%Parasitemia	%Suppression
Normal saline	3.30±2.3	0
50	22.36±8.7*	85.24
100	6.94±1.7*	52.45
200	4.58±1.0*	38.78
Chloroquine10mg/kg)	6.24±1.7	47.12

Key: * indicates statistical significance (P<0.05).

4. Discussion

The traditional use of plants for the treatment of human malaria and fevers all over the world has been widely documented, the number of investigations into their effects in vitro and in vivo is increasing. The validation of plants that are traditional treatments for malaria is currently stimulating the interest of researchers across the world (Bashir et al; 2015). A documentation by Bashir et al (2015) on Potential antimalarials from African natural products revealed that over 650 plant taxa from 146 families, with 134 isolated antimalarial compounds from 39 plants species were found to be reported in literature from 1996 to 2015. Also, a review from Boris et al. on the pharmacological evaluations of potential anti-malarial compounds from African Medicinal plants from 2013 to 2019 revealed 187 compounds classified in their various natural products from 23 plant families to have anti-malarial properties. The most active compounds belong to the same compound classes as the malarial drugs of natural origin, e.g. the alkaloid class for quinine and the terpenoid class for artemisinin (Boris et al; 2020).

Heliotropium indicum has been used in different traditional and folklore systems of medicine for curing various diseases. An ethnopharma-cological survey revealed that, the traditional healers in Kancheepuram district of Tamil Nadu, India use H. indicum to cure skin diseases, poison bites, stomachache and nervous disorders. In some African countries,

another ethnopharma-cological survey reports that H. indicum is believed to be useful in treating malaria, abdominal pain and dermatitis. The highest number of usages (22%) was reported for the treatment of malaria. In Jamaica, the decoction of the entire plant is taken orally for treatment of intractable fever, ulcers, venereal diseases and sore throat and used externally in vaginal cavity to induce abortion in pregnant females and administered rectally to treat local sores in the rectum while in Philippines and Senegal, used orally as diuretic and for the treatment of kidney stone. The infusion of the flower is taken orally by females for the treatment of menorrhagia in Jamaica. In Rodrigues, the decoction of the entire plant is used externally for treating herpes and the paste of fresh plant is used externally for cleansing and dressing of wounds and ulcers. The sap of the stem is used orally by females for treating dysmenorrhea. The hot water extract of the flower is taken orally by the females as an emmenagouge in small dose and abortive in large dose while a paste of fresh entire plant is used externally for treatment of head lice in the West Indies. In Thailand, the dried inflorescence is believed to produce permanent sterilization when taken orally in females. One gram of the dried and powdered inflorescence mixed with milk or water is used for three days beginning with the fourth day of menses to achieve the desired result. Other folk remedies include use of decoction of the leaves for treatment of fever, insect bites, stings, diarrhoea, skin rashes, menstrual disorder and urticaria15. The decoction of the leaves is also credited to be useful dose and emmenagouge in small dose. The leaf paste is applied externally to cure rheumatism in RayalSeema in Andhra Pradesh, India and skin infection in Nicaragua. The decoction of both leaf and root together is also used for treating whooping cough in children in Eastern Nicaragua. In Amazon, the paste of both leaf and root together is applied externally in scorpion stings, bug bites while the paste is recommended for treating sores and warts in Taiwan. In Malaysia, a paste made from the plant is applied to counteract putrefaction, to treat pyoderma and ringworm infection. In Burma, a decoction of the whole plant is used to treat gonorrhea while in Indonesia, an infusion of the leaves is used to soothe mouth spruce. A decoction of the dried roots is drunk in the Philippines to promote menses, while the seeds are used to treat cholera, malaria, and for wound-healing.

Aerial parts contain pyrrolizidine alkaloids, indicine(Principal), echinitine, supinine, heleurine, heliotrine, lasiocarpine, its N-oxide, acetyl indicine, indicinine and antitumour alkaloid, indicinen-oxide. The plant also contains rapone and lupeol and an ester of retronecine. Roots contain high amount of estradiol. Helindicine, a new pyrrolizidinealkaloid together with the known lycopsamine were isolated from the roots of *Heliotropium indicum*. Presence of cynoglossine, europine-N-oxide, heleurine-N Oxide, heliotridine-N-Oxide, heleotrine-N-Oxide and heliotrine have been identified from the seeds.

From the experiments, the plant extract has no lethal toxicity since the lethal dose of the plant is greater than 5000 mg/kg of which has no significance toxicity and hence the plant is relatively safe for use (Enegide et al; 2013).

4.1. Antiplasmodial Assay

The ethanolic extracts of the individual plant prepared as a syrup were tested for their suppressive antimalarial activites and it was found out that the mean % parasitemia for *Heliotropium indicum* extract at 50, 100, and 200mg/kg were 23.04±2.9, 25.3±3.2 and 21.89±2.2 respectively and a relatively higher activity was found at a dose of 200mg as shown on the table3.1.

Also, the mean% parasitemia for *Parquentina nigrescens* extract was found out at 50,100,and 200mg/kg to be 47.46±26.3, 20.29±3.6 and 26.26±10.1 respectively and here a better activity was found at the 100mg dose also as shown on table 3.2.

For the combined extracts of both plants first prepared as a syrup, the %parasitemia were 14.81±5, 9.28±2.0 and 22.36±8.7 as compared to the aqueous formulations of the combined extracts which were 22.36±8.7, 6.94±1.7 and 4.58±1.0, here the aqueous extracts gave a better %parasitemia value and a bettter %suppressive value.

5. Conclusion

In conclusion, the present experimental study has shown in animal model of malaria, the comparative efficacy of the combined extracts of *Heliotropium indicum* and *Parquentina nigrescens* used in the management of malarial attack. It was also found out that combination of chloroquine with the extract dose only result in slight improvement in activity.

Recommendation

The study recommends that further investigation should focus on the identification, separation, purification, and quantification of the most bioactive compounds present in the plants; *Heliotropium indicum* and *Parquentina nigrescens*

to ascertain their usefulness in the pharmaceutical industry. In addition, more studies are needed on practical doses in animals and humans. Clinical observation and trials may help to determine effective dosage regimes of the plants.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The present research has received ethical approval from Department of Pharmacology, Obafemi Awolowo University and as such the study adheres to the ethial guildelines and principles outlined by the department.

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