ANTI-MALARIA, NOCICEPTIVE AND ANTI-INFLAMMATORY POTENTIAL OF BI-HERBAL EXTRACT OF *Guiera senegalensis* AND *Psidium guajava*

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E. O. Oshomoh[1]* and G. O. Benjamin[2]
[1]Department of Science Laboratory Technology, University of Benin PMB 1154, Edo State, Nigeria
[2]Phytomedicine Unit, Department of Plant Biology & Biotechnology, University of Benin, PMB 1154, Edo State, Nigeria
*Corresponding author: emmanuel.oshomoh@uniben.edu

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Abstract: Malaria being universal worries largely with appearance of several drug resistivity strains in *Plasmodium falciparum*, requiring of novel and efficient anti-malarial agents. Bi-herbal leaves extract of *Guiera senegalensis* and *Psidium guajava* play a vital role in treatment of malaria in traditional forms of medicine. This study investigates anti-malarial, nociceptive and anti-inflammatory potential of the extract. The treated animal groups received graded doses of the plant extracts daily for three consecutive days. *In vivo* anti-plasmodial property was evaluated using Rane's curative protocol in *P. berghei* infected mice. Nociceptive and anti-inflammatory properties were evaluated on Swiss mice and rats via standard procedure. Acute toxicity evaluation was understudied with a modified lorke down techniques. The extract established its prophylactic and curative activity at (50 and 100 mg/kg). The result showed a significant increase in percentage inhibition of parasitemia clearance was attained (74 and 79%) (for 4 post-management days) which is significant than standard drug (chloroquine) at 76% for prophyllatic and curative 80 and 46 percentage for the bi-herbal and 52 percentage for chloroquine. Significant reduction in acetic acid induced pain $p< 0.05$ was obtained with an increase in percentage inhibition (60% for bi-herbal) and 76.5% for reference drug when compared with the control group. Also, the bi-herbal extracts exhibited a significant reduction in edema caused by formalin induced inflammation enhanced reduced the paw volume. The extract is safe given that the lethal dose was over 5000 mg/kg with paw volume of 3.57 mm of the bi-herbal when compared with acetylsalicyclic acid (3.50 mm) compared to the control group that showed inflammation at 5.27 mm. Also, the LD_{50} showed no lethality after 14 days of exposure to bi-herbal extract. This study showed that methanolic bi-herbal extracts displayed *a*n efficient anti-malaria, nociceptive and anti-inflammatory activity. This validates the ethnomedicinal uses of the two plants as an anti-malarial, analgesic and anti-inflammatory agent.

Keywords: Malaria, bi-herbal, nociceptive, anti-inflammation, *Guiera senegalensis*, *Psidium guajava*

Introduction

Since times of antiquity, plant materials are used in the curative and prophylactic measure in diseases conditions. Medicinal plants enhance healthy life, prevent diseases and treat disorders (Lakshman, 2012). World Health Organization approved the use of traditional medicine for its efficacy and safety. Medicinal value in plants depends on its bioactive phyto-constituent to promote therapeutic activity (Akinmoladun *et al*., 2007).

Malaria being a foremost general health problem associated with sub-tropical and tropical districts globlly. More than half a million (627,000) populaces, majorly children less than five die due to the occurrence of malaria yearly. There are approximately 207 million malaria cases annually and enormous majority (90%) take place in sub-Saharan Africa (Mayers *et al*., 2017). Severally,pregnant women are prone to malaria which causes sicknesses, miscarriages and death. Although artemisinin established mixture therapy vestiges the choice of malaria treatment. Artemisinins efficacy is endangered by several advent of *Plasmodium spp* resistivity (Bassat, 2011) and artemisinins resistances prompt research reported in selected Southern Asia. Similar to artemisinin established combination therapy, several other inexpensive and readily accessible antimalarial drugs includes; sulfadoxine, mefloquine, chloroquine, pyrimethamine, quinine are employed in malaria treatment, they are parasites resistance dependent recorded against these drugs (White, 2004; Zongo *et al*., 2005). Increasing prevalence of resistivity has caused the need to exploit for novel anti-malarial therapy.

Guiera senegalensis is a shrub found in tropical area, in the family Combrataceae. It is usually known as Sabara by the Hausas (Fiot *et al*., 2006). Its stem has several knots that propel branches. It is broadly dispersed in savannah zone of central and West Africa such as Nigeria, Mali, Niger, Senegal, Gambia, Ghana and Burkina Faso (Zeljan *et al*., 1998; Shettima *et al*., 2012; Tijjani *et al*., 2011). It is effective against respiratory congestion, fever and cough (Adedapo *et al*., 2009; Ali *et al*., 2011), and mostly given as antitussive, hypotension, venereal and hypertension diseases (Adedapo *et al*., 2009), to relieve and treat breathing, bronchial and lung diseases. It uses also involved malaria fever (Ancolio *et al*., 2002).

Psidium guajava L (Myrtaceae), usually known as guava, it is broadly establish in the globe. Teas gotten from the leaves are frequently used as diarrhea, cough, colic, uterine bleedings, gingivitis, scurvy, bronchitis, some intestinal parasitosis and arterial hypertension (Yamashiro *et al*., 2003). The subsequent *P. guajava* extracts effects were reported: depression, slowed locomotion, retroviral exchange transcriptase inhibition, antimalarial, cytotoxic, antimutagenic effect, antidiabetic, anti-inflammatory, antibiotic, antipyretic amoebicide, antiallergic (Olajide *et al*., 1999), antioxidant and myocardial defense against ischemia-reperfusion promoting injury maneuvers (Yamashiro *et al*., 2003).

Materials and Methods

Plant materials

Fresh leaves of *Guiera senegalensis* and *Psidium guajava* was purchased from the Northern part of Nigeria. The plant was identified and authenticated by Dr. Akinibosun, in the Department of Plant Biology and Biotechnology, Life Sciences, University of Benin. The leaves were rinsed with distilled water and shade dried. It was pulverized using a mechanical grinder and stored in an airtight container.

Preparation of extracts

The powdered materials were subjected to successive extraction by cold Maceration Method using methanol solvent with ratio 1:1 for 72 h. The various extracts were evaporated at 45°C to get a dried extract. The percentage yield of the extract was 109.7% w/w and stored for advance studies (Mukherjee, 2002).

Experimental animals

Male and female matured albino rats weighing 180-200 g and Swiss albino mice weighing 30-35 g were collected from the Department of Animal and Environmental Biology animal house, Faculty of Life Sciences, University of Benin. They were housed in conducive cages with free access to pelleted grower marsh and water; the animals were acclimatized to laboratory conditions for 2 weeks. The animals were handled according to standard procedures for the use of Laboratory animals.

Animals and parasites

Swiss albino mice weighing between 15 and 21 g of both sexes were used for this study. They were obtained from the animal house of pharmacology, University of Benin, Edo state, Nigeria. Housed under standard environmental conditions of temperature (22–29^{0}C) and 12 h dark– light cycle, and allowed free access to clean water and standard pellet diet. *P. berghei* (NK 65) was gotten from NNIMA, Lagos, Nigeria. The experimental procedures imbibed in this study were standardized by the Ethics Committee of Life Sciences, University of Benin, Edo state, Nigeria.

Experimental design

Parasite inoculation

Animals were quarantined prior to infection for 7 days. Mice were infected with *Plasmodium berghei* to further infect the whole animals for the study. Standard inoculums of 1×10^7 *P. berghei* infected erythrocytes in 0.2 mL were prepared via diluting infected blood with 0.9% normal saline. The mice were inoculated through intra-peritoneal injection of blood suspension (Moll *et al*., 2008). Parasites were maintained using serial channel of blood from infected to non-infected mice on weekly basis.

Effect of extract on curative test

The evaluation of the curative potential of the extract in Swiss albino mice was done using the methods described by Ryley and Peters (1970). Thirty mice were used for the experiments. Infected animals were divided into 5 groups (n = 5) when the level of parasitemia was observed to be > 4%. The extract was tested at 3 dose levels (25, 50, and 100 mg/kg). Three control groups (n = 5) includes; uninfected and untreated, infected and treated with 10 mg/kg chloroquine, infected and treated with distilled water. All treatments lasted for 3 consecutive days. Blood samples were collected from the tip of the tails of the animals on day 4 and day 7 post-treatment.

Parasitemia monitoring

Parasitemia was regulated via earlier method described by (Arrey *et al*., 2014). Blood samples were collected from the tip of the tails of the animals on day 4. Thin, blood films were dried, and fixed (for 15 min) using methanol, and stained with 10% Giemsa for 25 min. Stained film was washed off using phosphate buffer, pH 7.2 and allowed to dry. The film was deep in oil and viewed with X100 magnification. The parasitaemia level was resoluted by counting numbers of parasitized erythrocytes out of 100 erythrocytes in random fields of the microscope (Toma *et al*., 2015).

Average percentage parasitemia was calculated using the formula:

% Parasitemia =

$$\frac{total\ No.\ of\ parasitized\ erythrocytes\ parasitemia}{total\ No.\ of\ erythrocytes counted} x\,100$$

Acetic acid- induced mouse writhing test

This was done based on the modified method described by Oliveira *et al*., 2012 and de Sousa *et al*. (2010). Male and female mice were selected randomly into five (5) groups with 5 animals each. The control group received 0.2 ml/kg distilled water orally. The reference group received aspirin (100 mg/kg p.o) and group III, IV and V were orally pre-treated with 30, 50 and 100 mg/kg bi-herbal extracts, respectively. All drugs were administered 30 min before ip injection of 0.6% (v/v), 0.1 ml/kg glacial acetic acid solution. The number of writhes (hind limb extension due to contractile muscles of the abdomen) was taken 5 min after the injection of acetic acid. Reduction in the number of writhes is an indication of analgesic effect.

Formalin - induced paw oedema

Both sexes' rats were selected into five (5) groups with 5 animals each. The control group received 0.5 ml/kg distilled water orally. Standard group received aspirin 300 mg/kg p.o and group III, IV and V were orally pre-treated with 30, 50 and 100 mg/kg bi-herbal leaves extracts, respectively. All drugs were administered 30 min before inducing acute inflammation with a single sub-plantar injection of 0.1 ml of freshly prepared 2% (w/v) formalin suspension in distilled water using modified method of Agbaje *et al*. (2008). Formalin solution suspension in distilled water was injected into the sub-plantar tissue of the left hind paw of the rat served as the tested while the right hind paw served as the control. Modified method Adopted from Joseph *et al*. (2009).

Percentage inhibition =

$$\frac{(C_t - C_o)\ control - (C_t - C_o)\ treated}{(C_t - C_o)\ control} x\,100$$

Statistical analysis

Data were presented as Mean ± SEM of the respective replicates. Means of different groups were compared using ANOVA using graph pad prism 6 software packages.

Results and Discussion

Prophylactic study of the bi-herbal (*Guiera senegalensis* and *psidium guajava*) extractagainst *Plasmodium berghei* induced malarial showed a decrease in the level of significance across the graded doses (25, 50 and 100 mg/kg) of prevention when compared to negative control with no protection (Mustofa *et al*., 2007). The extract exhibited anti-malarial properties as showed in Table 1, with 50 and 100 mg/kg more effective than the control by reduction in *Plasmodium berghei*. The results obtained from the percentage inhibition of 10 mg/kg Chloroquine, 25, 50 and 100 mg/kg of the extracts at (76, 74, 79 and 66%). This report concurred with the study of Mustofa *et al*. (2007). The surviving days was significantly more at 50 mg/kg with the longest surviving day when compared with 10 mg/kg Chloroquine sensitivity against *Plasmodium berghei* and untreated group (20.67±1.76, 20.33 and 7.67±0.89). This explained the level of decrease of parasitemia level of the effectiveness of the extracts (Molta *et al*., 1992).

Curative study of the bi-herbal (*Guiera senegalensis* and *Psidium guyava*) extract against *Plasmodium berghei* induced malarial showed slight curative measure across the graded doses (25, 50 and 100 mg/kg) when compared with untreated control having increased parasitemia count, with no effect against *P. berghei*. The extract exhibited curative anti-malarial properties as showed in Table 2 specifically at 50 mg/kg by significant reduction in *P. berghei* as recorded from percentage inhibition in reference drug and treated groups (52 %, 46%, 80% and 47%) (World Health Organization, 2012). The surviving days showed an average survival in the treated groups compared to untreated group as shown in Table 2. This report showed a similar study of Mustofa *et al*. (2007).

Table 1: Effect of bi-herbal mixture of *Guiera senegalensis* and *Psidium guajava* extracts on prophyllatic treatment of *Plasmodium berghei* induced malarial

Drugs	Dose (mg/kg)	Mean±SEM of Parasitemia	% inhibition	Mean±SEM of survival days
DW	0.2 ml	1.00±0.00	0	7.67±0.89
Chloroquine	10	0.24±0.04[b]	76	14.33±0.33
Bi-herbal	25	0.26±0.12[b]	74	14.0±0.58
Bi-herbal	50	0.21±0.06[b]	79	13.0±1.16
Bi-herbal	100	0.34±0.05[a]	66	11.33±0.88

a= P< 0.05, b= p< 0.01 n=5DW ---------- distilled water

Table 2: Effect of bi-herbal mixture of *Guiera senegalensis* and *Psidium guajava* extracts on curative treatment of *Plasmodium berghei* induced malarial

Groups	Dose (mg/kg)	Mean±SEM acetic acid	% inhibition
Control (Dw)	0.2 ml	38.33±4.04	–
Indomethacine	20	9.00±1.16[c]	76.5
Bi-herbal	50	38.33±2.33	0
Bi-herbal	100	15.33±2.03[b]	60
Bi-herbal	200	17.00±6.08[b]	55.6

a= P< 0.05, b= p< 0.01 n=5 DW = distilled water

Oral administration of bi-herbal (*Guiera senegalensis* and *Psidium guajava*) leaves extract and reference drug (ibuprofen) significantly reduced the number of writhes induced by acetic acid- in mice, when compared to control (P < 0.05). The extract efficacy is efficient at 50 and 100 mg/kg. At 50 and 100 mg/kg, an absolute prophylactic measured was observed by the absence of abdominal contraction (Table 3). The acetic acid-induced abdominal contraction test is indistinct, since the definite point of action is vague, either peripherally or centrally as understudied by Franzotti *et al*. (2002) and Magaji *et al*. (2008). More ever, the method employed is amenable and efficient to sense the bioactive compounds duly liable for its pharmacological action (Bentley *et al*., 1981). Effective inhibitory effects exhibited by bi-herbal leaves extract recommend analgesic feedback peripherally being stimulated in writhing test, a reported by Okpo *et al*. (2001). Analgesictrial result of bi-herbal extract can possibly be linked with partialanti-inflammatory response. This is due to its mechanisms of action in visceral pain model, triggering processor to release arachidonic acid via cyclooxygenase and prostaglandin biosynthesis to enable its functions in nociceptive mode of action (Franzotti *et al*., 2002).

Table 3: effect of bi-herbal mixture of *Guiera senegalensis* and *Psidium guajava* extract on acetic acid induced peripheral pain in mice

Drugs	Groups	Dose (mg/kg)	Mean±SEM of Parasitemia	% inhibition	Mean±SEM of survival days
DW	Negative control	0.2 ml	1.00±0.00	0	7.67±0.88
Chloroquine	Positive control	10	0.48±0.08[a]	52	17.33±1.45
Bi-herbal	Plant extracts	25	0.54±0.09[a]	46	13.67±0.88
Bi-herbal	Plant extracts	50	0.20±0.07[b]	80	16.00±2.08
Bi-herbal	Plant extracts	100	0.53±0.10[a]	47	14.33±0.88

b= p< 0.01, c= p<0.001 n=5 DW ----- distilled water

Table 4: effect of bi-herbal mixture of *Guiera senegalensis* and *Psidium guajava* extract on formalin induced inflammation on rat sub-plantar paw

Groups	Dose (mg/kg)	Mean±SEM 1hr	Mean±SEM 2hr	Mean±SEM 3hr	Mean±SEM 4hr
Control	Dw	5.53±0.15	5.77±0.15	5.50±0.29	5.27±0.15
Acety-salicyclate	100	4.00±0.23[b]	3.90±0.06[b]	3.90±0.06[b]	3.50±0.17[b]
Bi-herbal	50	4.50±0.29[a]	4.10±0.06[a]	4.00±0.29[a]	3.57±0.15[b]
Bi-herbal	100	4.27±0.15[b]	3.97±0.09[b]	3.97±0.09[b]	3.90±0.06[a]
Bi-herbal	200	4.00±0.29[b]	4.00±0.29[a]	4.00±0.12[a]	3.80±0.12[b]

a= P< 0.05, b= p< 0.01 n=5 DW ---------- distilled water

Frequently, it uses major test to monitor novel anti-inflammatory mediator to evaluate the active compound responsible in reducing local edema triggered by right paw via injection of an irritating agent as shown in Table 4, established the result with diverse treated groups in respect to mean hind paw diameter. The leaf extract of bi-herbal as anti-inflammatory agent showed a decrease in the induced paw edema with significant different of (P<0.05). The extract reduces inflammation, with respective to time interval. These findings concur with anti-inflammatory property of ethanolic leaves extract of *Citrus sinensis* as reported by Omodamiro and Umekwe (2013). Non-steroidal anti-inflammatory agents synthesized their curative actions through inhibition of cyclo-oxygenase (COX), the enzyme that is implicated in prostaglandin-biosynthesis. COX has two isoenzymes (COX – 1 and COX – 2).

Table 5: Effect of bi-herbal mixture of *Guiera senegalensis* and *Psidium guajava* extracts on acute toxicity study

Groups	Dose (mg/kg)	Number of Mice	Mortality (%)
Control	DW	5	0
Guera senegalensis	50	5	0
Guera senegalensis	500	5	0
Guera senegalensis	5000	5	0

Acute toxicity study, the behavioral pattern of the animals observed showed no change in the normal behavior within 24 h of the *Guiera senegalensis* extract exposure, and no mortality although the 14 days the study lasted showed that *G. senegalensis* extracts has no toxic effect across the administered doses 100, 500 and 1000 mg/kg in the study in accordance to Idu *et al*. (2010) and Gasting *et al*. (2010).

Conclusion

In conclusion, the various concentrations of the bi-herbal constituents where found to be very effective against malaria, inflammation, analgesic and biosafety evaluation. Therefore, further studies will be required for compound identification and characterization of the potent principles/substances present in the extract.

Conflict of Interest

Authors declare that there is no conflict of interest.

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