Original Research Article

IN VIVO CHEMOSUPPRESSIVE EFFECTS OF COMBINATIONS OF THREE NIGERIAN ETHNOMEDICINAL ANTIMALARIAL PLANTS

Abstract:

Aims: Appropriate ratios for combining some African ethnomedicinal plants with proven anti-plasmodial activity were determined with the aim of obtaining herbal remedies with higher efficacies.

Place and duration of the study: Study was conducted in the Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife Nigeria.

Study Design: Infusions of dried powders of three Nigerian ethnomedicinal plants, *Eugenia uniflora* leaf, *Gongronema latifolium* root and stem and *Artocarpus altilis* stem bark, were separately evaluated and in varied combination ratios for their anti-plasmodial activities

Methodology: The infusions were separately prepared, concentrated *in vacuo*, freeze-dried and evaluated at 400mg/kg against *Plasmodium berghei berghei* parasites, using the Peter's four-day chemosuppressive mice model. Normal saline and chloroquine (10mg/kg) were negative and positive controls, respectively. 2- and 3-combinations of the infusions were similarly tested.

Results: Of the 2-plant combination ratios, 1:1, 1:3, 2:3 of EG and GA elicited percentage chemo suppressions which were comparable (P=.28; P=.07) to those of the single drugs. Only the EG ratios gave activities that were comparable (P=.28) to the positive control in

addition to double survival times and high survivor values by EG 2:3 and GA 1:3. However, those of the EA group, gave relatively low values, barely above 20% with only the ratios 2:3 and 3:2 giving values which were significantly (P=.00) higher than negative control with double survival times. The 3-plant combination ratios, EGA 2:1:2 and 3:3:2 gave suppressions that were significantly (P=.00) higher than the negative control with others but comparable (P=.33) activities to those of the individual drugs. The other ratios with low suppression values were relatively inactive. But three ratios, EGA 3:1:2, 2:1:1, 1:1:1 elicited survival times doubled (204, 242 and 202 %) that of the negative control without commensurate high antiplasmodial activities.

Conclusion: Ethnomedicinal antimalarial plants should not be combined without a data of previous scientific evaluations.

Introduction

The antiplasmodial and antimalarial activities, as well as the safety, of *E. uniflora* leaf, *A. altilis* stem bark, *G. latifolium* root and stem have been previously established in various studies [1-8**]**. However, such studies have not taken into consideration the possibility of preparing a combination of these plants as an antimalarial recipe, which may be used in African ethnomedicine [9]. Since, most traditional herbal remedies are prepared as decoctions of more than one plant for improved effects [10-11], there should be increased investigations into the anti-plasmodial activities of Polyherbal remedies used ethnomedicinally as antimalarial and combinations of plants with demonstrated anti-malarial activities [6, 7].This would multiply the number of bioactive plants available to the traditional medicine practitioners or Nigerian citizens for use in treating malaria. Such studies would also increase the number of combinations of such

plants with improved antimalarial properties, as well as list of those combinations that would lead to increased plasmodium resistance due to their reduced efficacies [6, 12]. Plants have multiple chemical components and when combined can exhibit many and different pharmacological profiles (polypharmacological effects) [13]. Therefore, in this study, *E. uniflora* leaf, *A. altilis* stem bark, and *G. latifolium* root and stem were variously combined in twos and threes and tested for chemosuppressive antimalarial activities in a *Plasmodium berghei berghei*–infected mouse model. This was with a view of identifying the best combination ratio of these medicinal plants for traditional treatment of malaria.

Materials and Methods

Equipment: Grinding machine (Christy Norris), Digital weighing balance (Mettler Toledo AB204S), Hot plate, Rotary evaporator (Heidolph Laborota 4010, Germany), Vacuum pump (v-700, Buchi), recirculating chiller (F-108, Buchi), Binocular Light Microscope (Olympus, UK), Dissecting set, Thermometer (Kwest digitherm). **Materials:** Round bottom flask (1L), Aluminum cages, Feeding and water troughs, Mice, Animal feed (Grower pellets, Vital Feeds, Brand Cereals and Oil mills Ltd, Bukuru, Jos), Metal oral cannula, Normal saline, Chloroquine tablets (SIGMA Chemical Company, USA), Giemsa stain, Microscope slides and Immersion oil.

Plant collection and authentication

The leaf of *E. uniflora* Linn (Myrtaceae), stem bark of *A. altilis*, and the stem & root of *G. latifolium* were collected from the Medicinal plant garden and Teaching and Research Farm Area, Obafemi Awolowo University (O.A.U.), Ile-Ife, Osun state, Nigeria on 8th June, 2018. The plant parts were identified and authenticated by Mr. I. I. Ogunlowo, Department of Pharmacognosy, Faculty of Pharmacy, O.A.U. Ile-Ife, and their respective voucher specimens with numbers FPI 2208, 2207 and 2209 were deposited in the Faculty of Pharmacy herbarium,

Department of Pharmacognosy, O.A.U., Ile-Ife. The plants were separately air-dried, powdered and kept in a cool and dry place until needed

Extraction

Powdered samples (40.0g) each of *E uniflora* leaf, *G latifolium* root & stem and *A. altilis* stem bark were separately weighed into different jars. Thereafter, a 500 mL of boiling water was added to each powder, stirred and left to stand for 30 minutes, before it was filtered. The filtrates (infusions) were concentrated to small volumes *in vacuo* at 50°C, using the rotary evaporator, freeze dried, weighed and kept in desiccators until needed. The yields of the infusions were calculated. Also, powdered samples of each plant were weighed into different glass jars to make 40.0g mixtures of *E uniflora* :*G. latifolium* **(EG),** *E. uniflora*: *A. altilis* **(EA)** and *G. latifolium:A. altilis* **(GA)** in 1:1, 1:2, 1:3, 2:1, 2:3, 3:1, and 3:2 ratios, as well as 40 g mixtures combinations of *E. uniflora*: *G. latifolium :A. altilis* **(EGA)** in the ratios of 1:1:1, 1:1:2:, 1:2:2, 2:1:1, 2:1:2, 2:2:1, 3:1:2, 3:2:2, 3:3:2, respectively. They were similarly extracted, treated as given above and weighed to obtain their respective dried infusions [14].

Animals

Swiss albino mice, weighing between 18.0 and 22.0g (male and female), were purchased from the Animal house of the Faculty of Basic Medical Sciences, College of Health Sciences, O.A.U., Ile-Ife. Based on their sexes, mice were differently housed in aluminum cages under a 12-hour day/night cycle and fed with Grower pellets and clean water. They were made to acclimatize for at least 10 days before dividing them into groups of five mice each, for the singly and combined test groups, positive and negative control [15, 16].

Parasite inoculation

The rodent parasite, chloroquine-sensitive *Plasmodium berghei berghei* NK 65 was obtained from the Institute of Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan. Sufficient volume of blood was obtained through cardiac puncture from a euthanized donor mouse with about 30 % parasitaemia. The blood was diluted with normal saline so that 0.2 mL will contain $1x10^7$ infected erythrocytes. A 0.2 mL of the diluted blood was inoculated intraperitoneally into each of the experimental mice [16].

Chemosuppressive antiplasmodial test

Fifty (50) acclimatized and inoculated mice were grouped into 10 groups (**I – X**) of five mice each. They were each administered with 400.0 mg/kg/day of **EG** in ratio 1:1, 1:2, 1:3, 2:1, 2:3, 3:1, 3:2, *E. uniflora* leaf extract and 10 mg/kg chloroquine and distilled water as negative control. In the same way, **GA** was also administered to another set of 50 mice in the ratio 1:1, 1:2, 1:3, 2:1, 2:3, 3:1, 3:2, *G. latifolium* stem and root extract, 10 mg/kg chloroquine and distilled water as negative control. **EA** was also combined in the same ratio of 1:1, 1:2, 1:3, 2:1, 2:3, 3:1, 3:2, and similarly tested. Also, **EGA** was administered to another set of 60 mice in 12 groups of five mice each in the ratio 1:1:1, 1:1:2: 1:2:2, , 2:1:1, 2:1:2, 2:2:1, 3:1:2, 3:2:2, 3:3:2, *A. altilis* stem bark extract, 10 mg/kg chloroquine and distilled water as negative control. These were administered orally two (2) hours after inoculation and daily for three consecutive days (D_0 - D_3). On the fifth day (D_4), blood smear was taken. The percentage parasitaemia was determined by recording the number of parasitized cells out of total red blood cells counted in 10 random fields of blood smear views under light microscope using the oil immersion objective. Average Percentage parasitaemia was calculated using the formula: % Parasitaemia= 100* Average number of parasitized red blood cells)/ Total number of parasitised and unparasitised red blood cells. Also, the average percentage chemosuppression was calculated from the Average

Percentage parasitaemia using the formula: 100{(A - B) / A}, where A is the average percentage parasitaemia in negative control group and B is the percentage parasitaemia in the test group [16, 17, 18].

2.4 Survival time, percentage survival time and percentage survivor

The survival time for each mouse was estimated by recording the number of days each mouse survived, post-treatment after observing for 28 days. The mean survival time ± SEM for each group was calculated and recorded. Percentage survival time relative to that of negative control (% of NC) was obtained by expressing the survival time for each group as a percentage of the value for the negative control group of mice. The percentage survivor, which is the percentage of the number of mice that elicited survival time that fall within the mean survival time of the group was also determined for each combination ratio in mice.

2.5 Data analysis

One-way analysis of variance with Student Newman Keuls as *post hoc* was used for comparison to determine the source of significant differences for all values. Values of $p<0.05$ were considered to be of statistical significance. The data analysis was performed using Vinstat Instant Demo Graphpad Software, Inc.11452, El Camino Real No 215, San Diego, 92130 USA.

RESULTS AND DISCUSSION

Since creation, medicinal plants have occurred in nature, to serve medicinal and other purposes [19-20]. Most, if not all the drugs employed in the treatment of diseases have their origin directly or indirectly in natural products including plants [21, 22]. Malaria is a deadly disease for which breakthrough in control or treatments have not been found [7]. The drugs, quinine and artemisinin that have been used the treatment of malaria were obtained from plants [23]. One

major challenge of orthodox drugs used in the treatment of malaria is resistance of the parasite to the drugs, whereas multicomponent medicinal plants used in ethnomedicine rarely give resistance to the disease but rather give complimentary therapeutic actions [24]. Therefore, the adoption of combination therapy in malaria and other disease conditions was to avoid parasite resistance to treatment [23, 24]. More so, multicomponent nature of medicinal plants should offer a distinct advantage in the management of malaria. Therefore, further combination of different plants may further increase the options of a multicomponent recipe for the treatment of malaria. Also, most of the herbal recipes for malarial in ethnomedicine are a cocktail of different medicinal plants, the basis of which must be investigated. Earlier investigation of combination of medicinal plants [5,12] have focused on the three modes of malarial but the endemic nature of malarial in Africa may suggest concentration on recipe that that have chemosuppressive effects. Also, the recent outbursts in the investigations on the combination of medicinal plants in the treatment of malaria should guide the selection of medicinal plants and the various doses to be used in the management of malaria. This could also be extended to other diseases managed by the Traditional medical practitioners contrary to the common unspecified combination in quantity and quality being practiced for self-medication by the populace and treatment by supposed professional herbalists [12]. Herbal drug development in Africa could be a way to prepare for the eventual co recognition or integration of herbal medicine [25] into the health care system in terms of the availability of recipes to be prescribed for the various ailments. The employment of a combination of plants in this work may therefore lead to a recipe or recipes that will avert the problem of resistance. The current work is to explore the possibility of three investigated antimalarial plants, when combined together in twos or threes to elicit better activity than the single plants. It could at the same time give information as to the therapeutic

compatibility of some plants being combined together in ethnomedicine. It was also thought to prepare the extracts of these combinations as infusions so that the chosen combination can be easily used as a dosage form by patrons. Therefore, extracts obtained from the infusions of a mixture of *E. uniflora* leaf + *A. altilis* stem bark (EA), *E. uniflora* leaf +*G. latifolium* root and stem (EG) and *G. latifolium* root and stem +*A. altilis* stem bark (EA) were each prepared in different ratios of 1:1, 1:2, 1:3, 2:1, 2:3, 3:1 and 3:2 while the three combinations, *E. uniflora* leaf+ *G. latifolium* root &stem +*A. altilis* stem bark (EGA) were in the ratios 3:1:2 , 2:1:1, 2:1:2, 2:2:1, 3:2:2, 3:3:2, 1:1:1, 1:2:2, 1:2:1 with a view to optimizing the combination. The ratios were tested alongside the individual drugs, chloroquine and normal saline as positive and negative controls respectively using the chemosuppressive model of antiplasmodial testing in *Plasmodium berghei berghei*–infected mice [26]. The percentage parasitaemia with percentage chemosuppression, survival times and percentage survivors were used as parameters for assessment of the activities of the different combination ratios [27].

***In vivo* antimalarial activity of individual plant extracts**

The dose, 400 mg/kg, at which the extracts of the individual plants and the combinations were tested was obtained by a comparison of the percentage chemosuppression and the effective doses elicited by the plants in the previous experiments [5, 6]. It should be possible to utilize previous results of experiments to guide the use of medicinal plants in ethnomedicine instead of the unspecified dosages still being used by traditional medical practitioners. The percentages chemosuppression values obtained from the infusions of individual plants as follows: *Eugenia* 49.28±13.80, *Gongronema* 51.63±7.72, and *Artocarpus* 37.87±5.48 portrays their relative antiplasmodial activities and so justify the use of the parameter of percentage chemosuppression for evaluation of antiplasmodial activities of the combination in this work. Infusion is a ready to

prepare and use dosage forms in ethnomedicine and so was used for the extraction of the individual plants and the combinations in this experiment. The parameters of % chemosuppression or reduction of parasite population have been used in the evaluation of *in vivo* and *in vitro* antimalarial activities of medicinal plants and their combinations in previous experiments [1, 5]. The extracts of the three plants gave comparable (*P*=.57) antiplasmodial activities to each other (Figs. 1&2). This serves as a good basis for comparison of the activities of their various combinations.

In vivo* antimalarial activity of the binary combination ratios of Eugenia and *Gongronema*, *Gongronema* and *Artocarpus* and *Eugenia* and *Artocarpus

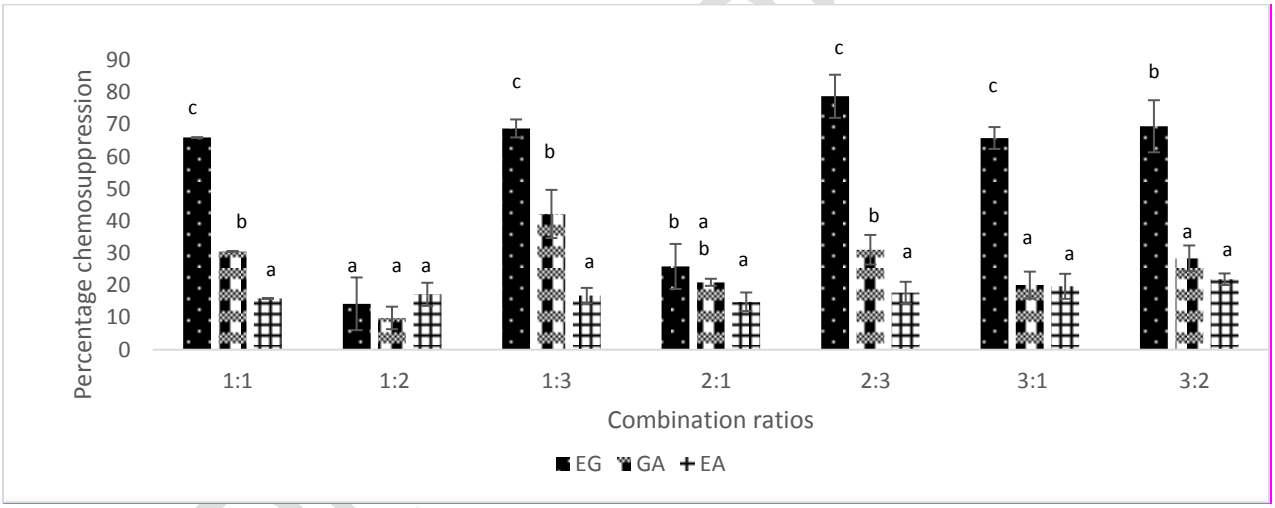

Fig. 1: Comparative Percentage chemosuppression elicited by the different 2-plant ratio combinations of *Eugenia uniflora* leaf, *Gongronema latifolium* stem root and *Artocarpus altilis* extracts. **Keys:** Data show the mean ± SEM, *n* = 5: **G**= *Gongronema latifolium*, **E**= *Eugenia uniflora;* **A** = *Artocarpus altilis;* Only values with different superscripts of alphabets within each combination ratio are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student Newman Keul's post hoc test).

In Fig. 1, the drug combinations, **EG**, **GA** and **EA** were compared within each ratio combinations in order to highlight their relative effectiveness in suppressing the parasites at those tested ratios.

Among all the ratios, it was obvious that **GA** and **EA** elicited lower percentage chemosuppression in comparison to **EG** (Fig 1). This imply that such combinations are less effective and also most likely to give rise to parasite resistance, if so used. Also, the very low response given by the ratios 1:2 and 1:2 for all the drug combinations indicate that doubling the proportion of the first component drug over the other and vice versa could not give better effect but rather reduced the activities considerably. Furthermore, if the proportion was trebled, only **GA** elicited a marginal increase in activity. This indicate that arbitrary increase in the proportion of drug combination components without resort to empirical tests may be counterproductive in combination of medicinal plants for antimalarial therapy. It is also noticeable that ratios 3:2 and 2:3 for the **GA** and **EA** combinations were not active. Among the **EG** ratios tested, only 1:2 (14%) gave comparable ($P=.55$) % chemosuppression to the negative control (Fig. 1). This implies that *Eugenia* and *Gongronema* should not be combined in this tested ratio in ethnomedicine. This was closely followed by the ratio combination 2:1 (26%). However, the ratio combinations **EG** 1:1, 1:3, 2:3, 3:1 and 3:2 with relatively high percentage chemo suppressions: 66, 68.8, 78.8, 65.8, 69.5%) respectively were significantly ($P=.001$) different in activity from the negative control and so possessing significant activities against the malaria parasite (Figs1& 2). Various ratio combinations of medicinal plants have elicited various levels of activities when tested against parasites in mice for example, the ratio combinations of *Mangifera indica, Alstonia boonei, Morinda lucida* and *Azadirachta indica* in **MAMA** (1:1:1:1), **MAMA-1** (1:2:2:2), **MAMA-2** (2:1:2:2), **MAMA-3** (2:2:2:1) and **MAMA-4** (1:1:2:2) as

antimalarial combinations, elicited varying activities as indicated by their respective median effective doses and percentage chemosuppressions [28]. These active ratio combinations of **EG** were also comparable (*P =.55)* in activities to each other and the positive control with the % chemosuppression of 74 (Fig.1). These combination ratios were also found to elicit relatively higher % chemo suppressions than each of the individual plant extracts in their uncombined state. Lower doses of combinations of medicinal plant extracts are needed to elicit similar responses in those of individual plants in similar antiplasmodial tests. For example, 170.0 mg /kg of **MAMA-1** (1:2:2:2) was required to elicit a similar response of 68.2% chemosuppression given by 200.0mg/kg of *Alstonia boonei* while 400.0 mg of *M. lucida* elicited a similar suppression of 63 % also given by 85.0 mg of **MAMA** (1:1:1:1) [29].

In the **EA** group, all the tested combination ratios gave relatively low percentage chemosuppression barely above 20 %. The individual drugs gave 49 and 38 % respectively. The ratios 2:3 and 3:2 which gave relatively better % chemo suppressions of 17.7 and 22.2 respectively were comparable (*P=.66*). Both possess comparable (*P=.35*) activities to the individual drugs. Other ratios also elicited comparable (*P=.35*) activities to the negative control and so were inactive. Either plants must have been inhibiting each other at the ratios employed in the combination. This was not the case when *Artocarpus* was replaced with *Gongronema* (**EG 2:3 or 3:2**). An arbitrary combination of these two plants (**E** and **A**) for the management of malaria which though may be suggestive in ethnomedicine could not have given a good effect. Many such combinations sold to the public in the name of effective medicines' pose unnecessary dangers to health and are prone to encouraging parasite resistance among the population [30].

In the **GA** group, most of the combinations here do not possess better activities than the individual drugs (Fig. 2). This implies that either of the components could have been used to

treat malaria in mice instead of attempting to combine them. Other tested combination ratios, **GA** 1:2, 3:2, 2:1, 3:2 gave comparatively lower % chemosuppression, hence, the high parasitaemia level was easily comparable to that of the negative control. This confirms the inappropriateness of combining these two plants in the treatment of malaria. Such combination could do more harm than good. The order of activity is 1:3= 1:1 = 2:3=E=G=PC>3:2= A >3:1>2:1=1:2> NC. Only **GA** 1:3 gave survival time that was significantly different ($P<.01$) from that of the negative control (Fig. 4) and was also comparable ($P=.58$) to that of the positive control, with the percentage survivor of 80 %. A departure from the assertion that the % parasitaemia reduction are better correlated with % survivor rather than with survival time [31]. Here percentage chemosuppression, percentage survivor and survival time were relatively pronounced together in an extract.

In Fig. 2. The combinations **EG** and **GA** were compared in order to identify which of the two combinations was more effective in suppressing the malarial parasites

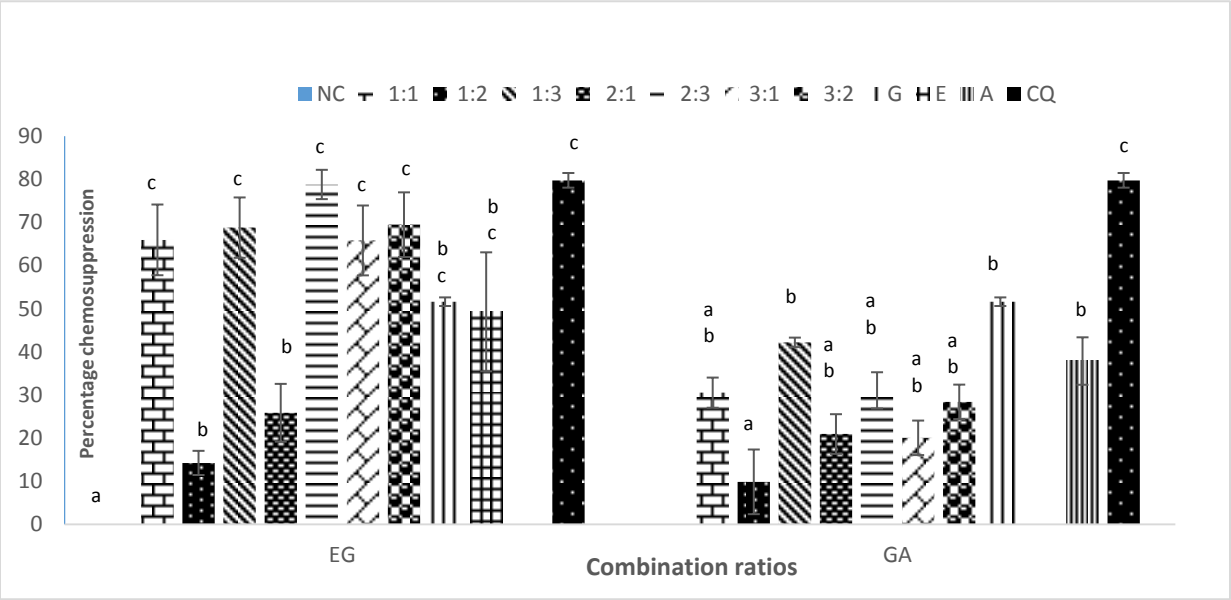

Fig. 2: Comparative antiplasmodial activities of EG and GA combinations of *Eugenia uniflora* leaf, *Gongronema latifolium* stem root and *Artocarpus altilis* extracts. **Keys:** Data show the mean ± SEM, *n* = 5: **NC** (negative control Tween 80 in normal saline): **G**= *Gongronema latifolium,* **E**= *Eugenia uniflora;* **A** = *Artocarpus altilis*; **CQ** = Chloroquine (10 mg/kg). Only values with different superscripts of alphabets are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student Newman Keul's post hoc test).

For the ratio **EG,** it was observed that using ratio 1:1 which contains equal ratio of both drugs as reference, equal ratios of **E** or **G** or slightly more of either **E** or **G** (1:1, 2:3, 3:2) or triple of **E** or G (1:3, 3:1) gave the best antiplasmodial activities. But a doubled proportion of **E** or **G** (1:2, 2:1) gave a relatively reduced antiplasmodial activity. From the above pattern it is obvious that the ratio **EG** 2:3 is the optimum for this combination (Fig 1 &2) and could be chosen for further investigation among others that are equally active. This gives credence to the need for careful selection and precise combination of medicinal plants in the amelioration of diseases; for instance, in bio prospecting new plant drugs for antimicrobial activities, it was discovered that direct ethnopharmacological method was better than random, and indirect ethnopharmacological

approach [30]. This work goes further to suggest an assessment of the efficacies of preparation of medicinal plants when combined in different proportions after obtaining their relative activities before giving it out for public use.

Also, using ratio 1:1 of **GA,** which contains equal ratio of both drugs as reference, equal ratios of **G** or **A** or slightly more of either **A** or **G** (2:3, 3:2) or triple of **G** (3:1), doubled amount of **A** or **G** (1:2, 2:1) gave comparable (*P*=.07) antiplasmodial activities. Only a triple of **G** (**GA** 1:3) in the combination gave the best antiplasmodial activities (42%). Ratio **GA** 1:3 also gave comparable (*P*=.07) % chemo suppressions to the single drugs and the positive control. Though **GA** 1:3 gave better activities of all the tested combinations, it was still not better than the individual drugs (Figs 1 and 2).

In Fig 3, the relative ability of each triple combination ratio compared to the individual drugs and positive control was depicted.

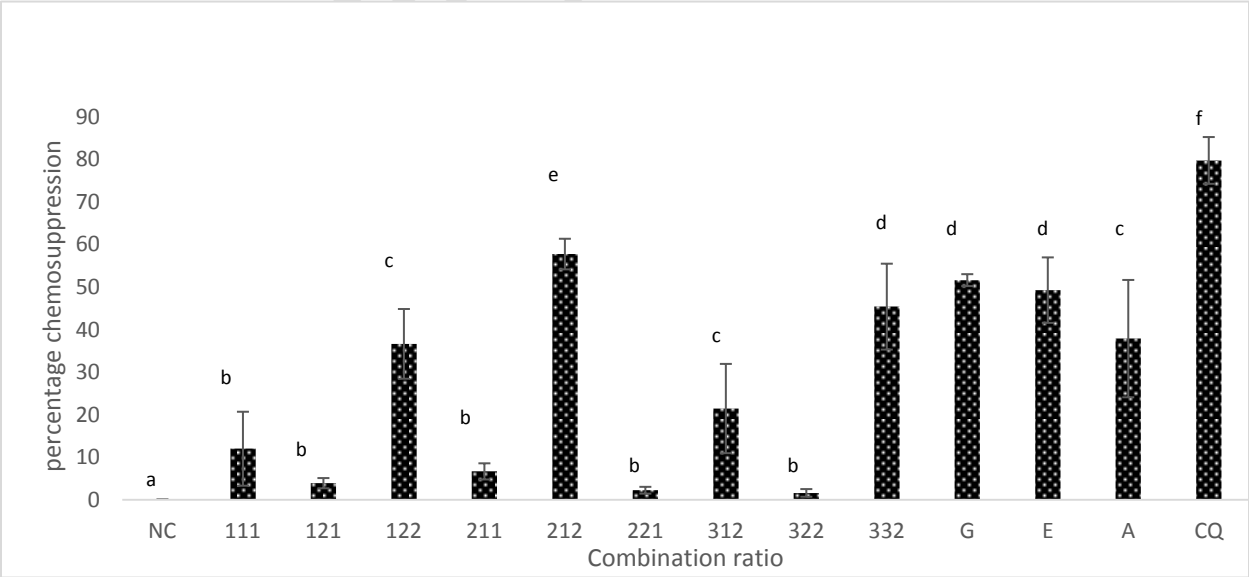

Fig 3: Chemosuppressive antiplasmodial effects elicited by the different trinary ratio combinations of *Eugenia uniflora* leaf, *Gongronema latifolium* stem root and *Artocarpus altilis* extracts **Keys:** Data show the mean ± SEM, *n* =

5: **NC** (negative control Tween 80 in normal saline): **G**= *Gongronema latifolium,* **E**= *Eugenia uniflora;* **A** = *Artocarpus altilis*; **CQ** = Chloroquine (10 mg/kg). Only values with different superscripts of alphabets within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student Newman Keul's post hoc test).

In vivo antimalarial activity of the combination *Eugenia, Gongronema* and Artocarpus

More than two plants may be combined in ethnomedicine for malaria [10, 19], but not all the combinations may be effective. Of all the combination ratios of **EGA** tested, three ratios, 1:2:2, 2:1:2 and 3:3:2 gave % chemo suppressions that were significantly (*P* =.00) higher than the negative control. These were also comparable in value to those of the individual drugs at 400mg/kg tested (*P*=.20) and the positive control (*P*=.20). Other ratios which elicited comparable activities were relatively inactive. Also, three ratios, **EGA** 3:1:2, 2:1:1, 1:1:1 elicited survival times doubled (204, 242 and 202 %) that of the negative control without commensurately high antiplasmodial activities (Figs 3, 5). Doubled survival times is a criteria for curative activity in antimalarial testing [12]. It has also been suggested that a similar activity found in prophylactic and chemosuppressive tests could be used as a criteria for activity [12]. Also, of all the individual drugs tested in this work, only *E. uniflora* elicited doubled survival time but not with the highest chemosuppression (Figs 1, 2, 4). Of the antiplasmodial-active ratios, only 2: 1: 2 with survival time 177 % gave percentage survivor of 60% while of the survival-time active only ratios, 3:1:2 with 202 % survival time also gave a percentage survivor of 60 %. Also, of the individual drugs only *A. altilis* gave a percentage survivor of 80 % without a correspondingly high survival time. Some previous studies have correlated % parasitaemia reduction with % survivors rather than with survival times [31, 32].

The percentage number of mice that survived within the group mean survival time (Percentage survivor) for the two-plant combination was presented in Fig. 4.

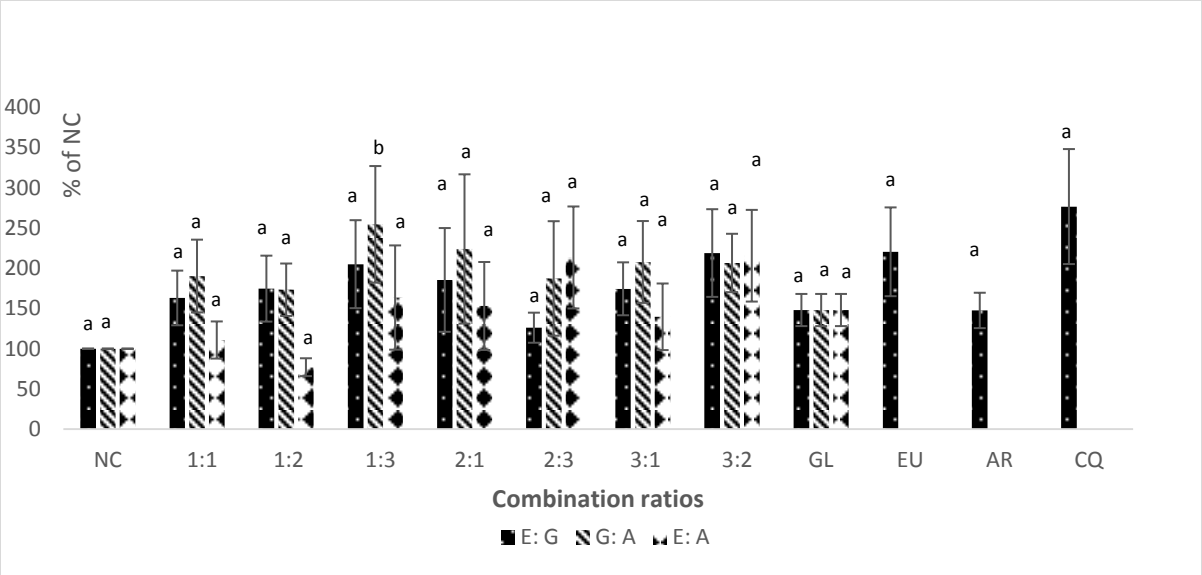

Fig 4: Mice Survival time (as percentages of negative control mice) elicited in the chemosuppressive antiplasmodial studies of *Eugenia uniflora* leaf and *Gongronema latifolium* stem and root and *Artocarpus altilis* extracts and their different binary combinations ratio. **Keys:** Data show the mean ± SEM, *n* = 5: **NC** (negative control Tween 80 in normal saline): **G**= *Gongronema latifolium*, **E**= *Eugenia uniflora;* **A** = *Artocarpus altilis*; **CQ** = Chloroquine (10 mg/kg). Only values with different superscripts of alphabets within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student Newman Keul's post hoc test).

Survival times (in days) given by each 3-plant combination ratio was expressed as percentages of times in days elicited by the negative control in Figure 5

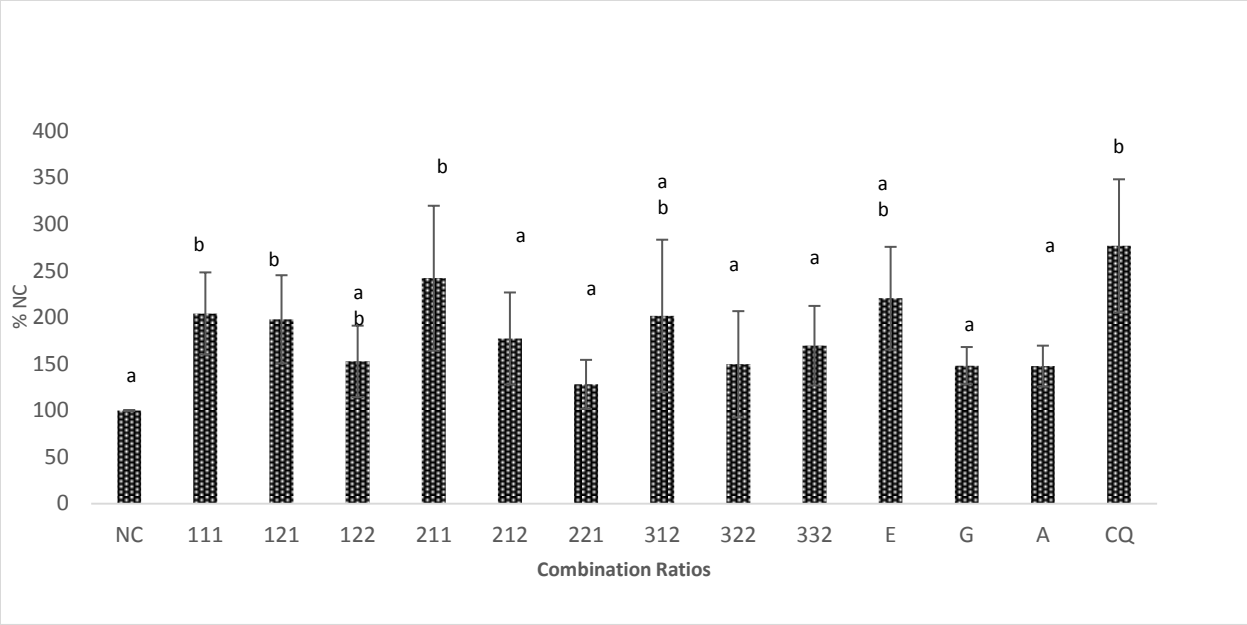

Fig 5: Mice Survival time (as percentages of negative control mice) elicited in the chemosuppressive antiplasmodial studies of *Eugenia uniflora* leaf and *Gongronema latifolium* stem and root and *Artocarpus altilis* extracts and their different three-plant combinations ratio. **Keys:** Data show the mean ± SEM, $n = 5$: **NC** (negative control Tween 80 in normal saline): **G**= *Gongronema latifolium*, **E**= *Eugenia uniflora;* **A**= *Artocarpus altilis*; **CQ** = Chloroquine (10 mg/kg). Only values with different superscripts of alphabets within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student Newman Keul's post hoc test).

The percentage number of mice that survived within the group mean survival time (Percentage survivor) for the 2 and 3- plant combination is presented in Fig. 6.

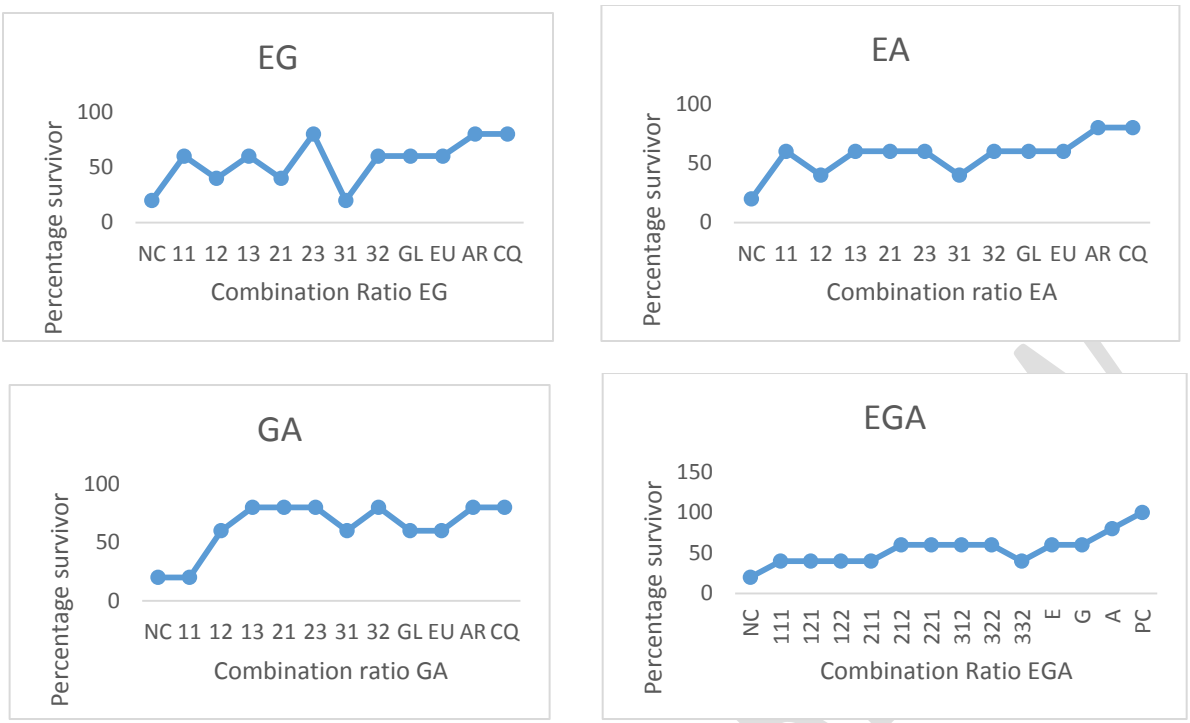

Fig 6: Graphical representation of the percentage survivor pattern of the 2- and 3-plant combinations of *E. uniflora, G. latifolium* and *A. altilis* extracts with individual drugs and positive control.in mice **Keys: NC** (negative control, Tween 80 in normal saline): **G**= *Gongronema latifolium*, **E**= *Eugenia uniflora;* **A**= *Artocarpus altilis*; **CQ** = Chloroquine (10 mg/kg).

Comparative antiplasmodial activities

In identifying, the most active combination(s) of all the tested ratios groups, using the parameters of percentage chemosuppression, percentage survival times and percentage survivors (Figs 1-6), **EG** 1:1, 1:3, 2:1 2:3, **GA** 1:3, 1:1, 2:3, **EA** 2:3, 3:2 and **EGA** 1:2:2, 2:1:2, 3:3:2 were statistically compared. Though all, except **EA** 2:3, 3:2 exhibited chemosuppression levels higher than that of the negative control, **EG 2:3, EG 2:1, EG 1:1** elicited comparable *(P=.55)* activities to chloroquine (10 mg/kg), the positive control drug. Whereas **EG 1:3, GA 1:3, EA 2:3, EA 3:2** and CQ elicited double survival times, only **GA 1:3** and CQ of the group gave the highest PS of 80. Others (**EG 2:3, GA 1:1** and **GA 2:3**) gave PS of 80 % similar to CQ without corresponding doubled survival times, thus confirming the non-correlation of survival time and PS with

antiplasmodial chemosuppressive activities [33,34]. Ratio **EG 2:3** with PS of 80 % and relatively high % chemosuppression similar to chloroquine may be selected as the most active while **EG 1:3** and **EG 3:2** with doubled survival times in addition to survivors of 60% can be next in activity being comparable in chemosuppressive activities. In general, some of the 2- or the 3- combination drugs displayed better than or equal activities to the individual drugs tested. Most of the active ratios were comparable in activity to the positive control. The most active combination was **EG 2:3** while the others were comparable in activities (*P=.55*) to each other.

C**ONCLUSION**

In conclusion, while it seems good to combine plants in the treatment of malaria in ethnomedicine, consideration should be given to their individual relative activities and in combination including their compatibility. This study vividly confirms *Eugenia* and *Gongronema* as the most active individual drugs tested and their ratio 2:3, the most active combination ratio. The inclusion of *Artocarpus* as a third plant in the combination nor its inclusion with either of the other two did not enhance activities.

Ethical Approval

Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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