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Malaria Control with Mosquito Repellent Plants: *Colophospermum mopane, Dicoma anomala* and *Lippia javanica*

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ABSTRACT [ENGLISH/ANGLAIS]

Interviews to establish what rural people use to repel mosquitoes in the absence of modern products were held in Buhera District, Manicaland in 2006, leading to sampling of 3 plants for repellency evaluation. The repellencies of the ethanolic extracts of *C. mopane, D. anomala* and *L. javanica* were determined and compared with the repellencies of *L. javanica/D.anomala, L. javanica/C.mopane, C. mopane/D. anomala* and *L. javanica/C. mopane/D. anomala* extract mixtures using ethanolic DEET as control and ethanol as blank. The ideal repellent would be one that gives 100% protection as is the case with DEET. In this project a cut-off point of 70% was decided on and repellency of 70% and above was deemed effective. In this context *C. mopane* was effective for 2 hours, *D. anomala*, 1.5 hours and *L. javanica*, 3.5 hours. D/C was effective for 2.5 hours, L/D for 3 hours, and L/C for 4 hours. The three-plant mixture system, D/C/L, was effective for 4 hours. All mixtures gave higher repellencies than the respective plant extracts. D/C/L, was superior to either one-plant extracts or two-plant extract mixtures. The higher repellencies observed for extract mixtures were suggestive of component repellency reinforcements either through synergism or potentiation. Extract mixtures could, thus, be formulated to give products with enhanced protection.

Keywords: Acquired immunity, drug resistance, mosquito repellent, repellent plants, repellent plant mixtures

RÉSUMÉ [FRANÇAIS/FRENCH]

Interviews pour établir ce que les populations rurales utilisent pour repousser les moustiques en l'absence de produits modernes ont eu lieu dans le district Buhera, Manicaland en 2006, conduisant à l'échantillonnage de 3 plantes pour l'évaluation répulsion. Les repellencies des extraits éthanoliques de C. mopane, D. anomala et L. javanica ont été déterminées et comparées avec les repellencies de L. javanica / D.anomala, L. javanica / C.mopane, C mopane / D. anomala et L. javanica / C. mopane / D. mélanges d 'extraits anomala utilisant DEET éthanolique que le contrôle et l'éthanol en tant que vide. Le répulsif idéal serait celui qui donne une protection à 100% comme c'est le cas avec le DEET. Dans ce projet, un point de coupure de 70% a été décidée et de répulsion de 70% et au-dessus a été jugée efficace. Dans ce contexte, C. mopane a été efficace pendant 2 heures, D. anomala, 1,5 heures et L. javanica, 3,5 heures. D / C est efficace pendant 2,5 heure, L / D pendant 3 heures, et L / C pendant 4 heures. Le système de mélange à trois plantes, D / C / L, a été efficace pendant 4 heures. Tous les mélanges ont donné plus que repellencies les extraits de plantes respectifs. D / C / L, était supérieur à soit un extraits de plantes ou des mélanges d'extrait de deux végétaux. Les repellencies plus élevés observés pour les mélanges d 'extraits étaient suggestives de renforts de répulsion des composants soit par synergie ou potentialisation. Extrait mélanges pourrait donc être formulée pour donner des produits avec une protection accrue.

Mots-clés: L'immunité acquise, la résistance aux médicaments, moustiques, plantes répulsives, des mélanges de plantes répulsives

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INTRODUCTION

People who lived in malaria endemic areas probably survived malaria through acquired immunity and use of antimalarial drugs, from their environment, especially from plants. These malaria control methods must have been efficacious, otherwise colonialism would have found no inhabitants in these areas. Even David Livingstone, the explorer ahead of colonialist acknowledged the efficacy of the African herbs when he wrote Queen Victoria that some of his crew members had been saved from malaria by the African medicinemen in Mozambique [1].

The indigenous inhabitants did not only survive on immunity and herbal cure against malaria, they also had insect repellents to ward off mosquitoes from areas around them.

The development and spread of antimalarial drug resistance and insecticide resistance, rising cost and operational complexity of indoor spraying with

insecticides, and the need for malaria control intervention tools that can be used in the context of primary health care have reawakened interest in the use of personal protection measures for malaria control [2]. Impregnated bed nets and indoor spraying with insecticides only provide protection against *Anopheles* mosquitoes after people retire to bed which may be long after the transmission of the disease. Protection measures against mosquito bites in outdoor life still need to be developed [3, 4].

 Plants have played a significant role since the early days in the control of malaria with the two most effective antimalarial drugs originating from plants and there is the possibility of the discovery of other plants containing as yet undiscovered anti-malarial substances [5].   By designing suitable experiments, the effectiveness of plant extracts can be determined and the information can then be used to choose the plant(s) that can be used in the development of effective mosquito repellents [6-10].

This research aims to extend the documentation of mosquito repellent plants and create a database of plants which can be used to control malaria transmission, regardless of stereotypes against botanical insectcides in favour of the synthetics [11-15].

The objective of this study was to identify efficacious plants used as mosquito repellents, evaluate and document them.

MATERIALS AND METHODS

Collection of Data on Mosquito Repellent Plants from Rural Areas

Data on mosquito repellent plants were collected from rural areas using interviews. Samples of plants suggested during interviews were collected and identified at the Botanical Gardens in Harare, and the samples were subsequently processed at The University of Zimbabwe, Department of Chemistry.

Preparation of Samples of Plant Parts to be Evaluated as Mosquito Repellents

The leaves of the plants were collected from Buhera District, Manicaland Province, Zimbabwe in March 2006. They were identified at the Botanic Gardens in Harare, dried in the shade for several days in the Department of Chemistry, University of Zimbabwe, Harare, Zimbabwe and ground to a fine powder. Samples of the powder (200g) were soaked in 80% ethanol (1.5 dm3) with agitation (72 hours, mechanical shaker), decanted, filtered and evaporated (rotavap, 40°) giving gums (C.Mopane:

9.60 g, 4.8%; D. anomala: 6.00 g, 3.0%; L. javanica: 11.00 g, 5.5%) from which 0.030 g solutions of each extract were prepared, and their effectiveness as *Aedes aegypti* mosquito repellents determined and evaluated. The *Aedes aegypti* mosquito was used because it is known to be a non-malaria vector in Zimbabwe.

Preparation of Mixtures of Plant Solutions to be evaluated as Mosquito Repellents

The solutions of extracts were mixed in equal proportions by volume and used to prepare the different repellent combinations.

Experimental Design

The experiment was designed as a blocked design experiment whereby experimental subjects act as blocks in mosquito repellency studies which were carried out at The Health Research Institute, Harare, Zimbabwe. The blocked design is similar to a repeated measures design in that each subject performs under all conditions of the experiment so that the effects of subject variables will balance out exactly. The repeated measures design eliminates systematic differences between the conditions of the experiment as far as subject characteristics are concerned. This design also allowed removal of all the random variation between subjects. The attractiveness of different persons to the same or different species of mosquitoes varies substantially [14], each experimental subject must receive each treatment, to allow variation between subjects to be calculated and accounted for. This also minimizes unexplained variation, or variation that is not due to the treatment effects. It is also important to determine the level of attractiveness of each experimental subject before the experiments start.

Bioassays

Aedes *aegyti* female mosquitoes were bred in the Laboratory according to the method of Jensen and Trager [16], ensuring the mosquitoes were malaria parasite-free. The mosquitoes were starved for an hour before any experiments were carried out, to reduce time taken before the mosquitoes start landing in search of a blood meal. Fifty mosquitoes were placed in cages of five-liter capacity to ensure convenient counting of mosquitoes landing and mosquitoes biting. The mosquito cage had a mosquito-netting on top and a sleeve on the side. The sleeve was used to introduce and to retrieve mosquitoes.

Procedure of Repellence Tests

Repellence of the extracts of *L. javanica, C. mopane, D. anomala* and their mixtures against *A. aegypti* mosquitoes DEET is a commercial product available on the market as mosbar which contains 20% DEET. It was applied following the directions on the container of the product. In this study DEET was included to act as the positive control. Studies on DEET have demonstrated that when applied as directed it provides 6 hours protection against biting by mosquitoes. This was also observed in this study as no mosquitoes were observed landing on the hand treated with DEET. The negative control was the hand treated with ethanol only. This provided 0% protection.

RESULTS

Preparation of the mosquitoes

Aedes aegyti female mosquitoes were bred in the Laboratory. The mosquitoes were starved for an hour before any experiments were carried out, to reduce time taken before the mosquitoes start landing in search of a blood meal. Fifty mosquitoes were placed in cages of five-liter capacity to ensure convenient counting of mosquitoes landing and mosquitoes biting. The mosquito cage had a mosquito-netting on top and a sleeve on the side. The sleeve was used to introduce and to retrieve mosquitoes.

Determination of Landing Time and Exposure Time

The minimum concentration giving 100% protection was taken as a realistic amount and was determined according to Tunon [17]. Landing time is the average time required by the first mosquito to land on to the target. The landing time was determined by exposing the hand treated with ethanol only to the fifty unfed mosquitoes. The time taken by the first mosquito to land was recorded and the exposure was repeated 10 times. The average time was calculated and then used as the exposure time in all experiments.

Repellence is defined as the number of mosquitoes that have been prevented from landing compared with the control. A cut off of 70% repellence will be used, any repellence above 70% being considered effective. All mosquitoes landing, whether just landing or probing to bite, are recorded. Repellence will be calculated [18], as follows:

$[(B_c-B_t)/B_c] \times 100$ as a percentage.

B_c = mean number of mosquito bites on the control subject.

B_t = mean number of mosquitoes on the treated subject.

Dose Finding Experiments

Dose finding experiments were carried out [16]. A special glove with an opening measuring 5cm by 5cm was used for all experiments. The total area to be exposed (25cm²) was cut out and the edges lined with masking tape. The plant preparation was applied (0.5 ml at a time) until a dose that gave 100% repellence during the exposure time was achieved. The minimum dose required was that which gave complete protection from mosquito landing. Percentage repellence was then plotted against the concentration of the repellent, Figure 1, from which the volume of the repellent to be applied was determined as 2cm³.

Figure 1: This figure shows dose-finding experiments
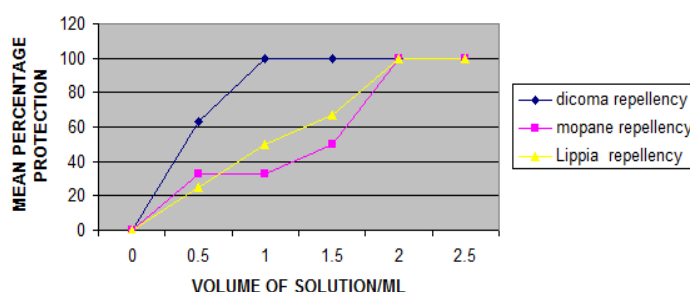
Repellence Tests

The special glove with an opening measuring 5cm by 5cm, as prepared above, was used for all experiments. For each repellent, the minimum dose established in the dose finding experiments, was applied to the exposed skin. The palm was placed in the 5-litre cage containing 50 female mosquitoes that had been starved for 24 hours. The palm was placed in the mosquito cage for the average landing time (exposure time) determined earlier on and then removed. This was repeated at 30-minute intervals until repellence was completely lost. Subjects were allowed to do their day-to-day activities between intervals except that they were not allowed to expose the treated hand to water. The numbers of mosquitoes landing, whether just landing or probing to bite, were recorded in accordance with the definition of repellence given above.

Repelence of aegypt mosquitoes by extracts of *L. javanica, C. mopane,* and *D. anomala*

The hands were treated with the prepared repellents and exposed to hungry mosquitoes for forty seconds. The number of mosquitoes landing was counted during the exposure period and this was repeated after every 30 minutes, for six hours. Repellence was calculated using the formula: $100(c-t)/c$, where t=number of mosquitoes
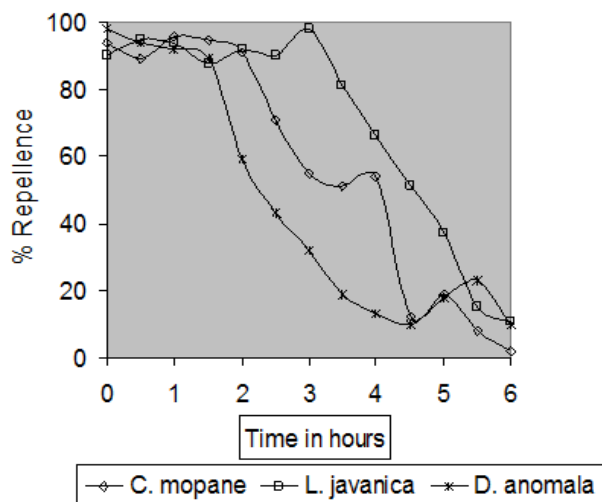
landing on the test hand, and c = number of mosquitoes landing on the control hand. To offset any personal differences in attractiveness to mosquitoes, the nine treatments were rotated daily for the period of the

experiments among the nine volunteers, so that each subject experienced each treatment. The mean percentage protection was calculated from the nine sets of replicated data.

Table 1: This table shows the mean percentage repellence for C. mopane, D. anomala and L. javanica over six hours

Time/hours	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
C. *mopane*	94	89	96	95	91	71	55	51	54	12	19	8	2
L. *javanica*	90	95	94	88	92	90	98	81	66	51	37	15	11
D. *anomala*	98	94	92	89	59	43	32	19	13	10	18	23	10
DEET	100	100	100	100	100	100	100	100	100	100	100	100	100
Ethanol	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 2: This figure shows repellence of plant extracts with time

the control gave 0% repellence throughout. Although the protection time given by *L. javanica* was more than double that given by *D. anomala*, and one hour longer than that given by *C. mopane*, even that of *D. anomala* which was only 1.5 hours may still be considered as being good since the times are comparable with those of most of the repellent plants which are included in commercial products with protection times ranging from as little as ten minutes to two hours [21]. The roots of *D. anomala* have been reported as having no essential oils [19] and this lack of essential oils might explain the observed poor repellency.

Determination of Landing Time / Exposure Time

The minimum concentration giving 100% protection was taken as a realistic amount and was determined [17]. Landing time is the average time required by the first mosquito to land. The landing time was determined by exposing the hand treated with ethanol only to the fifty unfed mosquitoes. The time taken by the first mosquito to land was recorded and this was repeated 10 times. The average time was calculated as shown below and was then used as the exposure time in all experiments.

DISCUSSION

C. mopane, D. anomala and *L. javanica* are readily available from the countryside in Zimbabwe. *L. javanica* had previously been evaluated and found to have repellent properties against mosquitoes [19]. It was, however, included in this study as a quality control strategy to check on the efficiency of the methods being used to study the other two plants, compareing the results with those observed in previous studies. The results obtained for *L. javanica* in the present study were found to be comparable with those reported from previous studies. Confidence in the efficiency of the present study was thus established. However *D. anomala* and *C. mopane* had not been previously evaluated. The mean percentage repellence results for the three plants are presented in Table 1 and plotted in Figure 2. Using 70% as the cut off point for effectiveness [20], *C. mopane* was effective for 2.5 hours, *D. anomala* 1.5 hours and *L. javanica* 3.5 hours. DEET provided 100% protection over the six hour period whilst

Table 2: This table shows determination of exposure time

Replicate	1	2	3	4	5	6	7	8	9	10
Time/sec	40	26	40	90	28	66	29	30	16	37

Exposure time $= \dfrac{40+26+40+90+28+66+29+30+16+37}{10}$

$= 40$ seconds

Repellence is defined as the number of mosquitoes that have been prevented from landing compared with the control .A cut off of 50% repellence will be used. All mosquitoes landing, whether just landing or probing to

bite, are recorded. Repellence will be calculated [18] as follows:

$[(B_c - B_t)/B_c] \times 100$ as a percentage.

B_c = mean number of mosquito bites on the control subject.

B_t = mean number of mosquitoes on the treated subject.

Dose Finding Experiments for Plant Mixtures

A special glove with an opening measuring 5cm by 5cm was used for all experiments. The total area to be exposed ($25cm^2$) was cut out and the edges lined with masking tape. The plant preparation was applied (0.5 ml at a time) until a dose that gave 100% repellence during the exposure time was achieved. The minimum dose required was that which gave complete protection from mosquito landing. Percentage repellence was then plotted against the concentration of the repellent.

**Figure 3:** This figure shows dose finding experiments for plant mixtures
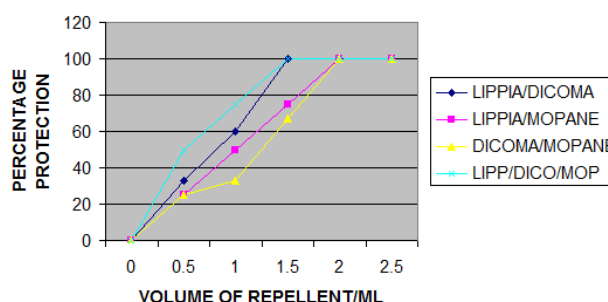
From the results of the dose-finding experiments the volume of the repellent to be applied was determined as 2 cm³.

Table 3: This table shows repellence against *a. aegypti* mosquitoes with time

Time/hours	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
mopane	94	89	96	95	91	71	55	51	54	12	19	8	2
lippia	90	95	94	88	92	90	98	81	66	51	37	15	11
dicoma	98	94	92	89	59	43	32	19	13	10	18	23	10
L/d	94	87	88	96	86	93	86	64	47	35	22	21	12
L/m	100	93	93	88	96	78	88	89	84	49	31	33	23
D/m	92	95	88	92	92	90	58	56	37	26	24	13	18
all	100	89	90	93	88	97	86	90	91	56	31	26	16
deet	100	100	100	100	100	100	100	100	100	100	100	100	100
control	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 5: This figure shows comparison of mean percent protection of lippie and dicoma extracts and lippa/dicoma extracts mixture
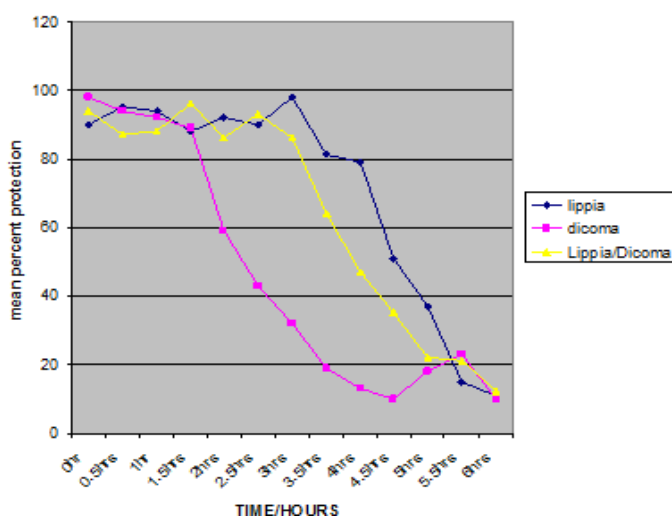
Repellence of *L. javanica and D. anomala* Mixture

The repellence of *L. javanica/D. anomala* mixture was slightly better than that of *D. anomala* but slightly less than that of L. javanica.

The Repellence of Extracts of *L. javanica* and *C. mopane* Compared to that of their Mixture

There is no significant difference between the repellence of L. javanica extract (mean 69.84) and *L. javanica/C.mopane* extract (mean 72.69) at 0.05 level of significance (p = 0.808458). A mixture of *L. javanica* and *C. mopane* was therefore not better than Lippia on its own. There is no significant difference between the repellence of mopane extract (mean 56.69) and *L. javanica/C. mopane* extract (mean 72.69) with a p value of 0.218419 at 0.05 level of significance. Therefore a mixture of the two plant extracts is only slightly better than *L. javanica* on its own and *C. mopane* on its own.

Repellence of *D.* anomala, *C. mopane,* and *D. anomala/C. mopane* Mixture

There is no statistically significant difference between the repellence of a mixture of *D. anomala* and *C. mopane* (mean 60.08) and mopane on its own (mean 46.15) with a p value of 0.309255 at the 0.05 level of significance. There was also no significant difference between *D. anomala/C. mopane* mixture (mean 60.08) and *C. mopane* on its own (mean 56.69). Therefore the mixture of *D. anomala/C. mopane* is only slightly better than *D. anomala* on its own and *C. mopane* on its own.

Repellencies of *L. javanica, C. mopane, D. anomala,* and *L. javanica/C. mopane/ D. anomala* Mixture

There is a statistically significant difference between the repellence of a mixture of *L. javanica/D. anomala/C. mopane* (mean 73.30) and the repellence of *D. anomala* on its own (mean 46.15) at the 0.05 level of significance (p = 0.045474). There is no significant difference between the repellence of *L. javanica/D. anomala/C. mopane* mixture (mean 73.30) and the repellence of *L. javanica* on its own (mean 69.85) with a p value of 0.775414 at the 0.05 level of significance. There is no significant difference between the repellence of *L. javanica/D. anomala/C. mopane* mixture (mean 73.30) and the repellence of *C. mopane* (mean 56.69) with a p value of 0.213902 at the 0.05 level of significance. This means that a mixture of *L. javanica, D. anomala,* and *C. mopane* is slightly better than *L. javanica* and *C. mopane* but significantly better than *D. anomala* in repelling mosquitoes.

Repellence of the Mixtures of Plant Extracts

L. javanica/C. mopane mixture (mean 72.690) is slightly more effective than *L. javanica/Dicoma* mixture (mean 63.92). However at the 0.05 level of significance there is no significant difference in the repellence of these two mixtures with a p value of 0.463707. A mixture of *L' javanica* and *C. mopane* (mean 72.69) is sightly better than a mixture of *D. anomala* and *C. mopane* (mean 60.08) but there is no significant difference with a p value of 0.301907 at 0.05 level of significance.

A mixture of *L. javanica* (mean 73.30) is slightly better than a mixture of *L. javanica* and *D. mopane* (mean 72.69) but there is no significant difference in the means with a p value of 0.95723 at the 0.05 level of significance. A mixture of *L. javanica /C. mopane /D. anomala* (mean 73.30) is slightly better than a mixture of *L. javanica/D. anomala* (mean 63.92) but there is no significant difference with a p value of 0.44764 at

Figure 6: This figure shows mean percentage protection of lippa and mopane extracts compared to the mixture of the two plant extracts
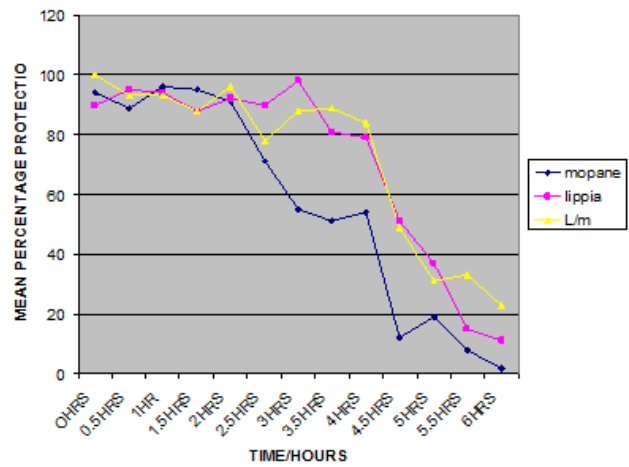

Figure 7: This figure shows repellency of dicoma extract and mopane extract compared to the mixture of dicoma and mopane extracts
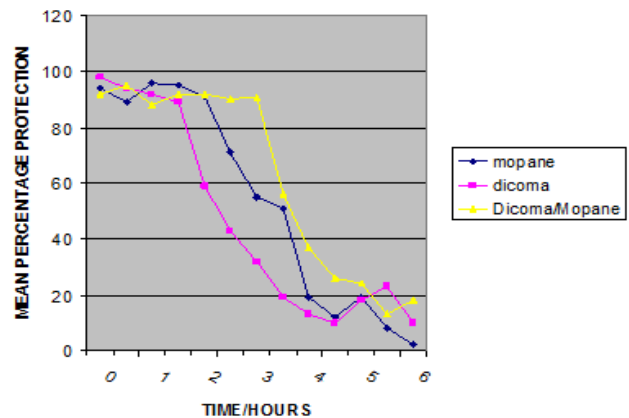

Figure 8: This figure shows repellency of lippa/dicoma/mopane compared to the repellency of extracts of lippa, dicoma and mopane
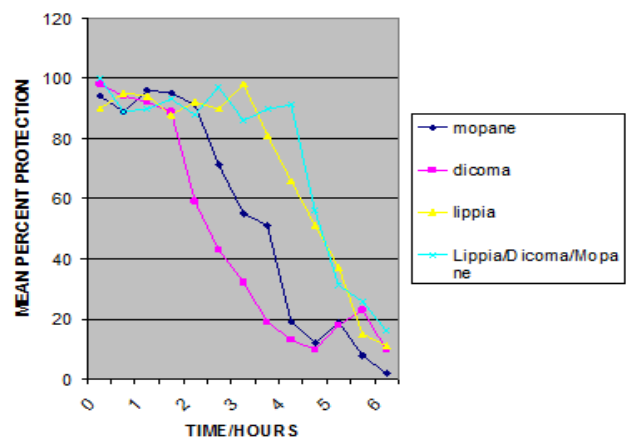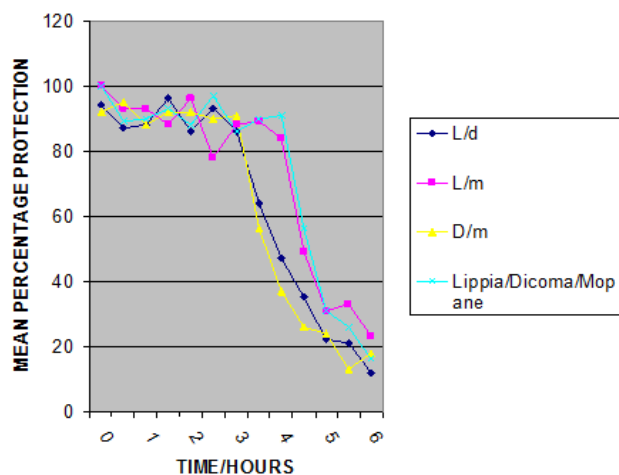
Figure 9: This figure shows comparison of the repellency of mixtures of plant extracts

A mixture of extracts of *L. javanica* and *D. anomala* and a mixture of *D. anomala* and *C. mopane* took 3.5 hours to provide a mean percentage protection of 50% or above. A mixture of *L. javanica* and *C. mopane* took four hours to provide a mean protection of 50% or more. *L. javanica* alone took four hours to provide a mean protection of 50% or more. A mixture of all the three plants took 4.5 hours to provide 50% protection or more. Therefore a mixture of plant extracts is more effective in repelling mosquitoes.

SUMMARY OF THE STUDY

The purpose of the study was to investigate the effectiveness / efficacy of extracts of *D. anomala*, *L. javanica* and *C. mopane* and their mixtures in repelling Aedes aegypti mosquitoes.

Analysis of variance revealed a significant difference between DEET and all the other repellents, DEET being more effective than plant extracts, providing 100% protection for the six-hour duration of the experiments. *L. javanica* provided 66% protection after a four-hour period. *D. anomala* provided 59% protection after 2 hours while *C. mopane* provided 51% protection after 3.5 hours. The repellences of *C. mopane* and *D. anomala* were negligible after 4 hours (19% and 13% respectively).

The question sought to establish the extent to which mixtures of plant extracts were effective when compared to the individual plants.

The repellence of the mixture of *L. javanica* and *C. mopane* was 84% after four hours. This was much higher than that of *L. javanica* (66%) and of *C. mopane* (19%) after four hours.

Thus the combination of the two plant extracts had increased repellence for the same period of time, probably suggesting synergism of the extracts. The same conclusion can be reached for the mixture of *D. anomala* and *C. mopane* (56%), compared to *D. anomala* (19%) and *C. mopane* (51%) after 3.5 hours, supporting the assertion that mixtures of plant extracts offer better protection against mosquito biting compared to individual plant extracts.

CONCLUSION

It is concluded that this study shows that there is a scientific basis for using mixtures of plant-based products in developing mosquito repellents, as they would have higher repellence and longer periods of protection against biting by mosquitoes.

L. javanica had been previously evaluated and found to have repellent properties against mosquitoes. However *D. anomala* and *C. mopane* had not been previously evaluated. This study showed that *C. mopane* had longer protection time and greater repellence than *D. anomala*. This can be attributed to the presence of essential oils in *C. mopane*. The roots of *D. anomala*, which were used in this study, do not have essential oils and this may be the reason why it had poor repellence. The fact that it gave slight repellence against mosquitoes shows that it is not only essential oils that may be responsible for mosquito repellence but that there are other chemicals in plant extracts that repel mosquitoes. *C. mopane* had lower repellence and protection time against biting than *L. javanica*. The results obtained in this study indicate that some people still use plants as mosquito repellents the efficacy of which can be quantified. The people in the rural areas cannot afford the expensive market repellents and have to rely on repellents from their environment to ward off mosquitoes and protect themselves against mosquiyo bites and malaria.

RECOMMENDATIONS

1   The study should be replicated with different plant extracts, in particular, with plant extracts known to have high repellence and protection time against mosquitoes. Extracts of plants known to have high repellence e.g. rapeseed[1] could be mixed with other plant extracts.

2   Field studies are particularly important if the aim is to develop a product that can actually be useful in real life.

3　Further studies are also recommended to find out if the repellents from plants have no ill effects on the health of human beings. While generally plants are regarded as safe, toxicity studies are necessary to ensure that the products would be safe.

4　If resources are available it is also necessary to carry out further studies on individual plants to extract, isolate and characterize the chemicals that have repellent properties against mosquitoes. Such information is important as it can enable complete or partial synthesis of the repellent chemicals, leading to possibilities of factory production and marketing.

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CONFLICT OF INTEREST

No conflict of interests was declared by authors

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