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Evaluation of Mosquito Repellencies of *Capsicum Frutescens, Carica Papaya* and *Cyanodon Dactylon* Extracts and Extract Mixtures

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ABSTRACT

The high mortalities due to malaria, the developing mosquito resistance to insecticides, the parasites' resistance to antimalarial drugs and the rising costs of mosquito repellents, insecticides and indoor spraying with insecticides have reawakened the desire for affordable malaria control measures. The objective of this study was to document the efficacies of mosquito repellents from extracts of plants which are traditionally known to repel mosquitoes. The plant parts used in the experiments were dried in the shade and ground to powder. The volatile constituents of the powders were isolated by dry distillation, and the distillates used in repellency tests on A. aegypti mosquitoes at the Research Institute, Ministry of Health, Harare, using the hand-in-cage technic.

The distillates of the fruits of C. frutescens and C. papaya were effective for 2.5 hours, whilst that of C. dactylon was effective for 1.5 hour. The mixture of C. frutescens and C. papaya was effective for 4 hours, whilst that of C. frutescens and C. dactylon was effective for 3 hours. The mixture of C. papaya and C. dactylon was effective for 2.5 hours compared to that of C. papaya (2.5 hours) and C. dactylon (1.5 hours), and the mixture of all three extracts was effective for 4 hours, i.e. giving the same duration of protection as the mixture of C. frutescens and C. papaya. Mixtures of highly repellent extracts are likely to give high repelling products although the repellency of the mixture is not likely to be a simple additive product of the repellencies of the constituent extracts. Thus the interactions of constituents of a mixture to give the repellency of a mixture are likely to be dependent on complex factors, and are not likely to be simply additive. Suitably chosen high repellency plants could provide economically attractive repellents suitable for use in formulations.

Key Words: *Antimalarial drugs, Capsicum frutescens, Carica papaya, Cyanidon dactylon, plant extracts, protection time, mosquito repellency*

INTRODUCTION

Malaria is one of the world's most common and serious tropical diseases. It causes more than one million deaths annually, more than 80 % of which occur in sub-Saharan Africa, mostly among children under five [1]. It kills more people than any other disease including HIV/AIDS [2], even though it is a preventable and curable disease [3]. Malaria, one of the leading causes of death in the world, killing 1.5 to 2.7 million people worldwide [4] is one of the diseases transmitted by mosquitoes. Mosquitoes transmit diseases to more than 700 million people worldwide annually, including arboviruses responsible for yellow fever, dangue haemorrhagic fever, epidemic polyarthritis and several forms of encephalitis and bacroftian filiriasis. The most effective methods for malaria control in third world countries are the use of mosquito nets treated with insecticides and the use of DDT spraying [5]. These methods have, however, been found to be effective in preventing mosquito bites indoors leaving people vulnerable outside their homes. Personal protection methods, such as the use of mosquito repellents applied to the skin and to clothes, may protect people from mosquito bites outside their homes. DEET, the most effective and most widely used synthetic mosquito repellent, is believed to have undesirable side effects, causing rashes, swelling, eye irritation, brain damage and in some cases death [6], and its activity on plastics and synthetic fabrics [7]. Ethnomedicine has been practiced all over the world for a long time. Phytochemists, pharmacists, and ethnobotanists utilize the knowledge from traditional healers to be able to select and test new medicinal plants [8]. The Portuguese discovered that slaves in South America were using the bark of the Cinchona plant to cure malaria and fever [9]. Quinine was eventually commercially isolated from the bark of these large trees indigenous to South America [10] and many other drugs are now commercially produced from other plants [8, 10, 11]. Thousands of plants which have been tested as potential sources of insect repellents have not yielded chemicals with the broad effectiveness of DEET [12], but a mixture of neem oil and coconut oil is a more effective insect repellent than DEET, affording 100 % repellency of Anopheles mosquitoes for up to 12 hours when DEET gives 100 % repellency for 8 hours. Catnip is also claimed to be a better repellent than DEET because the nepetalactone it contains is about ten times more effective at repelling mosquitoes than DEET [6]. Pepermint oil is another effective repellent and mosquiticide which could be a new cheap weapon in the fight against mosquito-borne diseases such as filariasis, dengue fever, and west Nile viruses [13]. A commercial repellent, Bite-Brocker, combines soybean oil and coconut oil. It has been observed to give more than 97 % protection against Aedes mosquitoes under field conditions when 6.65 % DEET afforded 86 % and Avon Skin-so-Soft, a citronella based repellent, gave only 40 % protection [14]. Permethrin, from the dried flowers of daisy is also an effective insect repellent and insecticide. In general, however, plant based repellents require frequent re-application as a result of their high volatility [15]. Thus, the possibility to control malaria through use of plant-based products exists. UNISEF aims to ensure that by 2010, 80% of the people at risk from malaria are protected from mosquito bites. It hopes to achieve this through use of locally available and appropriate vector control methods. This study investigates the possibility of using some plant extracts in the development of affordable, effective mosquito repellents for use in combating malaria.

Plants that repel mosquitoes have been used traditionally in communities, offering advantages such as being cheap, readily available, and renewable. Plant derived chemicals are generally considered as more environmentally safe than their synthetic counterparts since they are part of the ecosystem [2, 3]. Ethnobotanical and chemotaxonomic studies have been carried out in Zimbabwe to document local plants with mosquito repellent properties. The increasing incidence of malaria coupled with increasing worldwide drug failure rates necessitates the search for alternative drugs for combating malaria. One important strategy for reducing outdoor mosquito bites is to control the malaria vector by developing plant-based mosquito repellents [2-4].

Plants Under Investigation In This Study

(i) ***Capsicum frutescens***

Some *Capsicum* species are hoy, but most have soothing effects on the digestive system, relieve symptoms of colds, sore throats and fevers, and hangover. They can also act as heart stimulants and regulators of blood flow and improve circulation especially to cold feet and hands. They also strengthen arteries, possibly reducing heart attacks [16]. Capsacin, the heat generating constituent in chilli peppers, causes prostate cancer cells to kill themselves. The hotter varieties of chilli peppers can also be used externally as a remedy for painful joints, for frost bite, and may be applied directly to stop bleeding. They stimulate blood flow to the affected area, thus reducing inflammation and discomfort [17]. They also heal ulcers, relieve arthritis & muscle pain. When applied around the base of plants, chilli powders repel ants, earthworms, slugs, snails and a wide range of soil pests. Some rural people in Zimbabwe use capsicum frutescens powders to repel mosquitoes [18].

(ii) *Carica papaya*

Water extracts of seeds repel various kinds of insects. Juice obtained by pressing the roots destroy nematodes in soil and the juice extracted from the immature fruit controls termites [19 and the leaves of the plant tenderize meat if placed in water with the meat or if used to wrap the meat [18].

(iii) *Cynodon dactylon*

Cynodon dactylon stays green even in hot, dry wether, hence used for lawn in most parts of the world. It is also reported to be alterative, anabolic, demulcent, depurative, diuretic, emollient, sudorific, and vulverary [18, 20].

Research Design

The study is a scientific investigation into the mosquito repellencies of crude plant extracts and their mixtures. It is an experimental study where hands treated with different extracts are exposed to mosquitoes and the repellency and inhibition of the extracts determined [18].

METHODS

Collection and treatment of plant material samples

The leaves and fruits of *Capsicum frutescens*, leaves and seeds of *Carica papaya,* and the leaves of *Cynodon dactylon* were collected from Chiredzi, Masvingo Province, Zimbabwe, in December, 2006, and identified at the Botanical Gardens, Ministry of Agriculture, Harare, Zimbabwe. The seeds of *Carica papaya*, the fruits of *Capsicum frutescens,* and the leaves of *Cynodon dactylon* were sun dried. The leaves could easily dry, but the seeds of *Carica papaya* and the fruits of *Capsicum frutescens* required vacuum oven drying to ensure nearly complete moisture removal. The dried samples were ground to powder using mortar and pestle [18; 21, 22].

Isolation of volatile constituents of plant materials

The volatile constituents of the powders were isolated by dry distillation of 20g powder of each the plant parts under investigation, collecting the distillates into sample bottles, yielding *Capsicum frutescens* distillate (2.7350g), *Carica papaya* distillate (2.5112g.), and *Cynodon dactylon* distillate (2.6121g) after drawing off the aqueous layer using a separation funnel.

The solutions of extracts were mixed in equal proportions by volume and used to prepare the different repellent combinations [18].

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 [23](Lukwa, 1999).

Determination of landing time / exposure time

The minimum concentration giving 100% protection was taken as a realistic amount and was determined according to Tunon [24]. 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 thirty five mosquitoes which had been starved for I hour. The time taken by the first mosquito to land was recorded and this was repeated until ten readings had been recorded. The average time was calculated as shown below and was 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. Ideally, a repellent should give 100 % protection over the desired duration, normally 8 hours. In the absence of such a repellent, a cut off protection time has to be decided. In our laboratory, a cut off of 70 % repellency has been decided as the repellency of an effective repellent. Any repellent that gives at least 70 % protection is described as effective. A repellent that gives less than 70 % protection is taken as being ineffective [25[. All mosquitoes landing, whether just landing or probing to bite, are recorded. Repellence will be calculated according to Mehra [26] 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

A special glove with an opening measuring 5cm by 5cm was used for all experiments [27]. 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.

Dose finding experiments were conducted for each of: (i) *C. frutescens, C. papaya,* and *C. dactylon* extracts, (ii) *C. frutescens/ C. papaya, C. frutescens/C. dactylon, and C. papaya/C. dactylon* mixtures and *for C. frutescens/ C. papaya /C. dactylon* mixture. The appropriate dose in each case was found to be $2cm^3$.

Experimental Design

The design of the experiment will be a blocked design experiment whereby experimental subjects act as blocks to remove all the random variation between subjects since the attractiveness of different persons to the same or different species of mosquitoes varies substantially [12]. This will also minimize unexplained variation, or variation that is not due to the treatment effects.

Repellence Tests

A special glove with an opening measuring 5cm by 5cm was used for all experiments [27]. The area was cut out and the edges lined with masking tape. 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 liter cage containing 35 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 and used in the determination of repellence.

RESULTS AND DISCUSSION

Repellence of the extracts of Capsicum frutescens, Carica papaya, and Cynodon dactylon extracts and their mixtures against A. aegypti mosquitoes (Table 1) and (Figures 1, 2 & 3).

The hands were treated with the prepared repellents and exposed to hungry mosquitoes for forty seconds as described in the exposure time determination. The numbers of mosquitoes landing were counted during the exposure period and this was repeated after every 30 minutes for six hours. Percentage protection is the same as repellence and was calculated using the formula: $100(B_c-B_t)/B_c$, where B_t = number of mosquitoes landing on the test hand, and B_c = number of mosquitoes landing on control hand. To offset any personal differences in attractiveness to mosquitoes, the nine treatments were rotated among the nine volunteers on a daily basis for the duration of the experiments so that each subject experienced each treatment. The mean percentage protection was calculated from the nine sets of replicated data (Table 1) and (Figures 1, 2 & 3).

Table 1: **Average repellence of extracts with time**

Time/hours	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
C. frutescens	94	94	90	91	81	73	62	58	43	32	29	33	6
C. papaya	93	89	95	89	78	70	65	51	48	23	21	11	6
C. dactylon	95	94	84	69	59	42	38	24	20	6	19	3	7
Cf / Cp	94	94	93	82	96	76	71	86	75	57	29	32	11
Cf /Cd	96	91	82	92	81	83	77	67	52	32	27	14	4
Cp /Cd	94	91	85	84	76	73	60	58	47	37	23	18	10
all	98	94	90	91	91	96	86	82	82	63	51	36	9
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

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 [18].

Repellence of C. frutescens, C. papaya, and their mixtures

C. frutescens and *C. papaya* were effective for 2.5 hours, whilst *C. dactylon* was effective for 1,5 hour. The mixture of *C. frutescens* and *C. papaya* was effective for 4 hours. Analysis of variance (ANOVA) showed that there was a significant difference amongst the three repellents at the 5 % level of confidence ($p = 0.003$ and $F = 7.57$). There was no significant difference between *C. frutescens* (mean = 60.46) and *C. papaya* (mean = 56.85). However, the mean protection time of their composite mixture (mean = 68.85) differed significantly from the individual repellents. Thus the constituents of the two extracts blended and gave a composite mixture whose repellency was longer than the repellencies of the individual extracts.

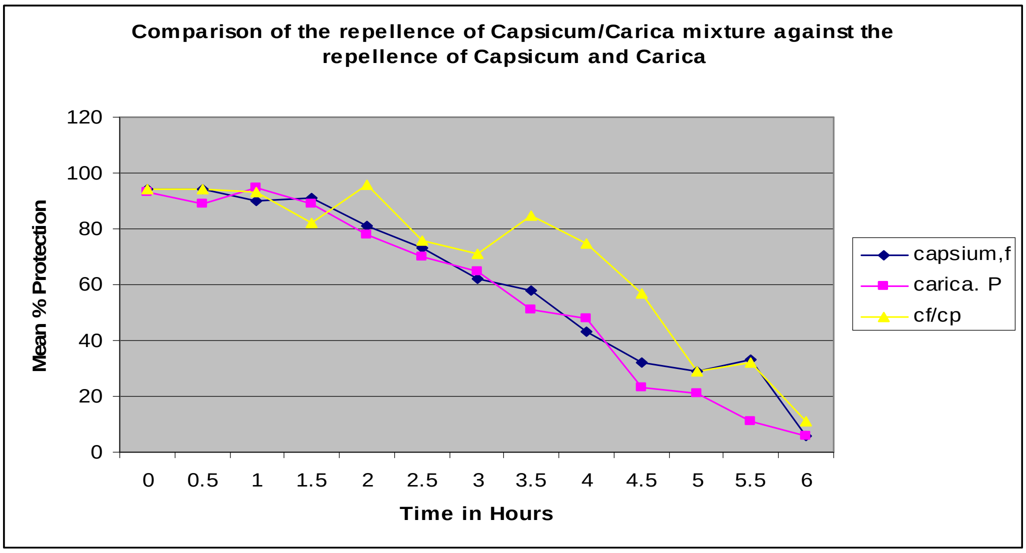

Figure 1

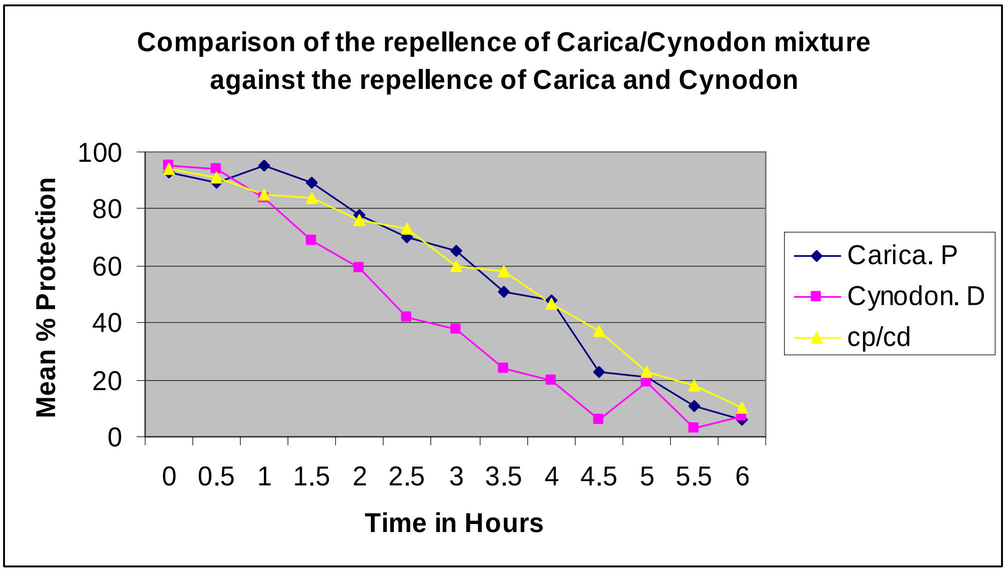

Figure 2

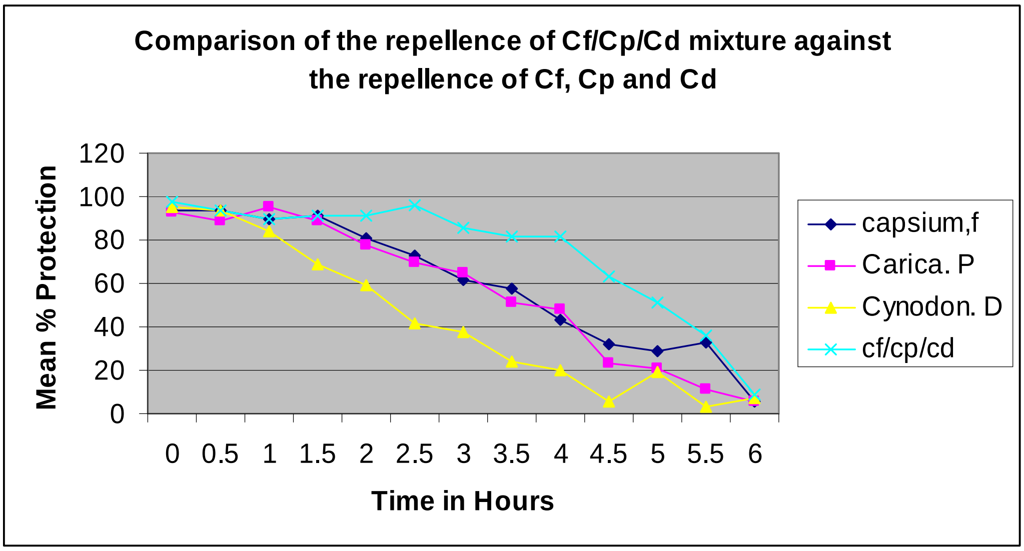

Figure 3

The mixture of *C. frutescens* and *C. dactylon* was effective for 3 hours. Thus the constituents of the two extracts blended to give a mixture with a longer protection time than those of either extract. ANOVA indicated that there was *n*o significant difference between *C. frutescens* (mean = 60.46) and the mixture (mean = 61.38).

The mixture of *C. papaya* and *C. dactylon* was effective for 2.5 hours compared to that of *C. papaya* (2.5 hours) and *C. dactylon* (1 hour). ANOVA indicated that there was no significant difference between the repellency of *C. papaya* (mean = 56.85) and the composite mixture (mean = 58.15). However, *C. dactylon* (mean = 43.08), differed significantly from the other two repellents. ANOVA indicated that there is significant difference among the three ($p = 0.00$ and $F = 14.84$).

The three repellents perform almost equally during the first 1 hour but differences begin to show after 1.5 hours post application, *C. dactylon* being the worst performer.

The mixture of all three extracts was effective for 4 hours, i.e. giving the same duration of protection as the mixture of *C. frutescens* and *C. papaya.* However, the mixture of the three extracts, giving 82 % protection at 4 hours post application, was a better repellent than that of the two extracts which was 75 % effective at 4 hours post application. ANOVA indicated that there was a significant difference amongst the four repellents: *C. cynodon* (mean = 43.08), *C. papaya* (mean = 56.85), *C. frutescens* (mean = 60.46) and *C. frutescens / C. papaya / C. cynodon* mixture (mean = 74.54).

Conclusion

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. Mixtures of highly repellent extracts are likely to give high repelling products although the repellency of the mixture is not likely to be a simple additive product of the repellencies of the constituent extracts. Consider the case for *C. frutescens* (repellency = 2.5 hours) and *C. papaya* (repellency = 2.5 hours) giving a mixture whose repellency is 4 hours; and *C. papaya* (repellency = 1 hour) mixed with *C. frutescens* (repellency = 2.5 hours) to give a mixture whose repellency is 3 hours, compared to *C. frutescens* (repellency = 2.5 hours), *C. papaya* (repellency = 2.5 hours) and *C. cynodon* (repellency = 1 hour) giving a mixture whose repellency is 4 hours. Thus the interactions of constituents of a mixture to give the repellency of a mixture are likely to be dependent on complex factors, and are not likely to be simply additive [26]. Identified high repellent extract yielding plants should be subjected to experimentation in order to come up with recommendations for candidates for development of repellents. Thus, with suitable choices of high repellency plants which could be suggested by traditional practitioners, mixtures of extracts with economically attractive repellencies could be formulated.

CONTRIBUTIONS OF AUTHORS

TK conceived the study, participated in its design, coordination, and implementation. He drafted and produced the manuscript.

CM took part in the conception and design of the study, collected samples and materials, performed laboratory studies and statistical analysis.

NL supervised laboratory studies and participated in the draft of the manuscript.

All authors read and approved the final manuscript.

Conflict of interest

There is no conflict of interest in this article.

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