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Full Paper

Repellent Effect of Homemade Vaporizer on *Aedes aegypti* (Diptera: Culicidae); using Essential Oil of *Tagetes erecta* (Asteraceae) Flowers

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Abstract

Aedes aegypti (Diptera: Culicidae) mosquito, a major vector for dengue fever, poses a significant public health issue in Sri Lanka. Synthetic repellents are commonly used; concerns over their safety and environmental impact highlight the need for effective natural alternatives. This study evaluates the effectiveness of essential oil derived from African marigold (*Tagetes erecta*) flowers as a natural mosquito repellent, presenting an eco-friendly alternative to synthetic repellents. The essential oil was extracted using steam distillation. Approximately 100 g of air-dried marigold flowers were used per extraction, yielding about 0.25 mL of oil per 100 g of flowers. The repellent efficacy was assessed following World Health Organization guidelines, using one control cage and three treatment cages [size (30×30×30) cm^3, temperature 28 ± 3 °C, and a humidity of 80 ± 5%] each containing 50 *Aedes aegypti* mosquitoes. Six different concentrations of the essential oil diluted with ethanol (5, 6, 7, 8, 9, and 10%) v\v were applied to a 600 cm^2 area on the forearms of a single volunteer, restricting the mosquitoes' landing area to the hands. Each concentration was tested in triplicate, with a 30-minute interval between applications to maintain consistency. Mosquito landings were counted, and percent repellence was calculated as a proportion of the number of mosquito landings on the treated arm to the number of landings on the control arm of the same individual. Probit values of repellence were plotted against log doses. Statistical validation of the equation was tested through linear regression analysis. The essential oil of *Tagetes erecta* demonstrated high repellent efficacy in both laboratory and field settings, with ED_{50} and $ED_{99.9}$ values of 5.98 ± 0.92% and 9.64 ± 1.63%, respectively, and provided complete protection for up to 150 ± 10 minutes under controlled conditions. Field application using a homemade vaporizer containing the essential oil (25 mL) extended protection up to 180 minutes, confirming the effectiveness of African marigold essential oil as a natural, eco-friendly repellent against dengue vector mosquitoes.

Keywords: *Aedes aegypti*, African marigold, homemade vaporizer, mosquito repellent

Introduction

Mosquito-borne diseases, including dengue fever, Zika virus, and chikungunya, pose significant global health risks, primarily transmitted by *Aedes aegypti* (Diptera: Culicidae) mosquitoes [1]. When mitigating the transmission of these diseases, repellents decrease contact between mosquitoes and their hosts

and may even lower the rate of disease transmission in many instances.
Female mosquitoes, specifically attracted to carbon dioxide (CO_2), detect it using their olfactory sensillum on their antenna. When a mosquito detects and encounters CO_2 molecules, an electrical impulse is sent to the insect's brain. These receptors are probably expressed in similar numbers of classes of odorant receptor neurons (ORNs) housed in sensilla on the antenna, maxillary palps, and proboscis [2].

Among the commonly used mosquito repellents, synthetic compounds such as Ethyl butylacetylaminopropionate (IR3535®), Icaridin (picaridin), and N, N-diethyl-meta-toluamide (DEET) are widely recommended by health authorities. DEET, in particular, is considered the "gold standard" repellent, offering up to 8 hours of protection [3]. However, despite its effectiveness, DEET has drawbacks, including rare but severe adverse reactions, damage to plastic materials, and an unpleasant odor and skin sensation [4]. The use of more synthetic pesticides to control mosquitoes causes ecosystems to become increasingly contaminated and poses a risk to human health [5].

Natural products, such as herbal products, are increasingly preferred for their natural origin and effectiveness against mosquitoes. These natural repellents, made from essential oils, are eco-friendly and biodegradable, making them an effective alternative to synthetic repellents. African marigold (*Tagetes erecta*) is a prime example of a natural repellent [6].

African marigold is also a popular medicinal plant from the Asteraceae family that has been used for millennia [7]. This plant contains flavonoids, carotenoids (lutein, zeaxanthin), essential oils (limonene, ocimene, tagetone), and alkaloids, which contribute to its medicinal properties [8]. These chemical constituents confer multifaceted biological effects such as anti-inflammatory, antimicrobial, antioxidant, antifungal, and wound-healing properties [9]. It is traditionally used to treat skin infections, wounds, digestive issues, respiratory conditions, and joint pain. The flower extracts help boost eye health, improve immunity, and act as a natural insect repellent [10].

Plant-based essential oils have shown promising larvicidal and repellent effects against mosquitoes. Studies have demonstrated that essential oils from various plants [11], including *Tagetes erecta* [12], are effective against *Aedes aegypti* larvae. However, most existing research focuses on larvicidal activity, with limited data available on the repellent effects of *T. erecta* essential oil against adult mosquitoes. In particular, there is an insufficiency in published data assessing its repellent efficacy in the Sri Lankan context, where dengue is a growing public health concern. This study aims to fill this gap by evaluating the repellent effect of *Tagetes erecta* flower essential oil on adult *Aedes aegypti* mosquitoes using a homemade vaporizer.

Materials and Methods

Study Site

Experiments were conducted at the School of Entomology, Colombo 08 and a Field experiment was conducted at Rajagiriya house premises (GPS Locations: 6.917239, 79.877935 and 6.918306, 79.891203*)* from December 2023 to February 2024.

Collection of Marigold Flowers and Extraction of Essential Oil

Fresh, mature African marigold flowers were air-dried for two weeks at room temperature, 29 °C, avoiding direct sunlight and high heat (Figure 1). The dried petals underwent steam distillation, with 100 g of flowers producing approximately 0.25 mL of essential oil. The oil was stored in an airtight container at 4 - 8 °C.

Figure 1. Fresh and air dried Marigold flowers

Steam distillation was performed following the method described by [13]. Distillate was collected in a glass container as two separate layers: oil and water. The essential oil was collected using a separating funnel. The extracted essential oil was stored in an airtight container for further analysis [14].

Design of the Homemade Vaporizer

A homemade vaporizer was built using common household materials like A dome-shaped, large plastic LED Bulb cover, CPU Fan Protector, Battery Case, 3.8 V Rechargeable Batteries X3, A CPU Fan, Pin Switch, PCB 18650 BMS Circuit Board. The cylindrical part of the LED bulb was separated. The top of the cylindrical part was cut and removed to make a ring-like cylinder. Another dish-like part of the bulb was used to house the rechargeable batteries, CPU fan, Pin Switch, and Female port. All of these materials are fixed to it using silicone glue. Two numbers of CPU fan protector covers were fitted to the two sides of a ring-like cylinder part, made with the bulb cover, placing a cotton wool layer in between the fan covers as shown in Figure 2 (a) and 2 (b).

The essential oil was placed onto the cotton wool layer situated between the two CPU fan protector covers. When the device is turned on, the air blown by the fan passes through the cotton wool soaked with essential oil, helping to diffuse the aroma effectively.

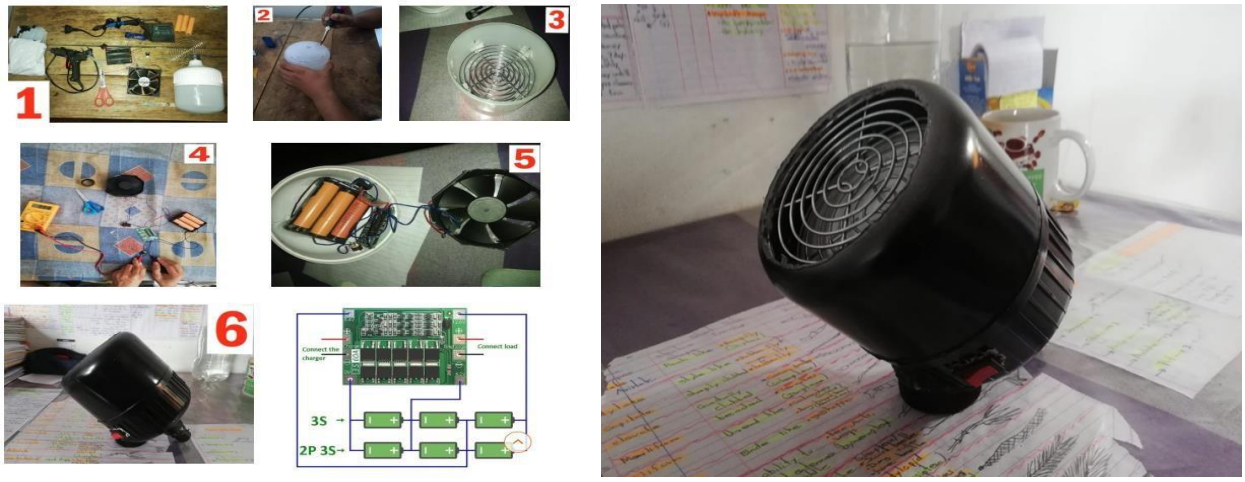

Figure 2. (a) Product Making Process and (b) the homemade vaporizer

Rearing of Testing Mosquitoes (Aedes aegypti)

Mosquito larvae and pupae were collected by placing 30 ovitraps in the field. The collected specimens were placed in trays and allowed to develop into adult mosquitoes in a controlled laboratory setting 28 ± 3 °C temperature and 80 ± 5% humidity level with a 12:12 hour light-dark cycle. The reared colony consisted of approximately 600 adult mosquitoes, of which 350 females were selected for testing. The adult mosquitoes were transferred in 4 mesh cages measuring (30×30×30) cm^3 constructed using metal frames and nylon mesh. The cages were kept under the same laboratory conditions mentioned above. The mosquito species (Aedes aegypti) was identified using standard pictorial keys as described in [15] and transferred to another of the above-mentioned 4 similar cages. They were kept at a temperature of 28 ± 3 °C and a humidity of 80 ± 5%. The mosquitoes were fed with a 10% sugar solution following the standard mosquito rearing procedure described by [16] and kept for up to 5 days before being fed blood by a volunteer. After feeding, the female mosquitoes were allowed to rest for two days to develop their eggs. Oviposition was induced using dark plastic containers filled with dechlorinated tap water and lined with filter paper as an oviposition substrate. These containers were placed inside the rearing cages under above-mentioned laboratory conditions. These eggs were collected and placed in an enamel tray, where they were allowed to develop. When the larvae emerged, the larvae were fed a suitable diet consisting of finely ground fish food powder and liver powder in a 3:1 ratio, which supported their development into adult mosquitoes. The F1 generation of these adult mosquitoes was used for the test. The F1 adults were transferred into the above-mentioned cages, measuring and maintaining following the above-mentioned condition. They were fed with a 10% sugar solution.

Evaluation of Marigold Essential Oil's Repellent

Effect estimation of effective dosage

Six different concentrations of oil, 5, 6, 7, 8, 9, and 10% were prepared using ethanol. Treatments (1 mL of each concentration of oil) were administered to a 600 cm^2 area of skin of the forearm between the wrist and

elbow. A group of 200 female mosquitos (5 days old) that had never received a blood meal were placed 50 mosquitoes per cage inside the 3 test cages and one control cage measuring (30x30x30) cm^3. These mosquitos were denied their sugar diet for 12 hours before the start of the test. During testing, the exposed part of the hand was covered by gloves constructed of a substance that mosquitoes cannot bite through (Figure 3).

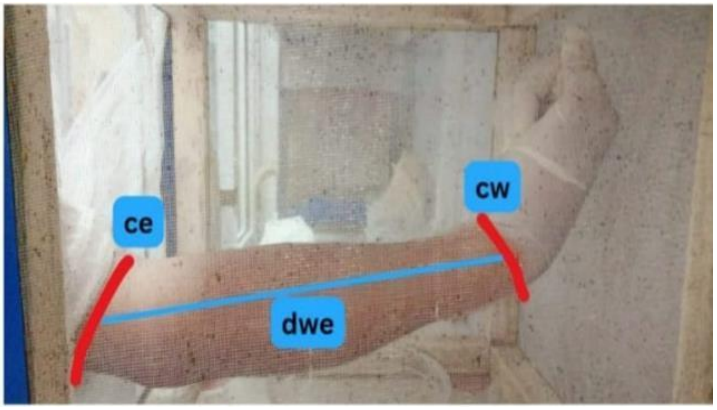

Figure 3. Arm Insertion Method

The readiness of mosquitoes to land was assessed by placing an untreated arm inside the cage for 30 seconds. The prepared mixtures will be applied evenly on the surface of the skin, between the wrist and elbow of the left arm and allowed to dry for one minute. First, the left forearm that had been treated with the lowest dilution was inserted into the cage. Then the number of mosquitoes that landed on the skin within 30 seconds was recorded. This procedure was repeated for each additional incremental dose of repellent. Successive tests were carried out one after the other without delay. For the control, only ethanol (the same diluent used in repellent preparation) was applied to the right forearm using the same procedure. A single test was comprised of continuous use of the same mosquitoes by the same volunteer and was completed in one day. One concentration was tested within one day, and different concentrations were tested on different days with three replicates. The minimum dosage (concentration) that provides 100% protection was determined by this analysis.

Estimation of Complete Protection Time (CPT)

The minimum dosage (concentration) that provided a 100% repellent dose was used for this test. Two hundred human host-seeking nulliparous (not egg-laid) 5-day-old female mosquitoes were placed in the cage 1 hour before the test. The treatment consisted of an ethanolic solution of the repellent active ingredient applied to the forearm of a volunteer (between the wrist and elbow) at the rate of 1 mL/ 650 cm^2 of skin surface area and dried for approximately 1 minute. The treated forearm was inserted into the cage, and the number of mosquitoes that landed and probed the skin in 3 minutes was observed and recorded. If no mosquitoes landed, the procedure was repeated every 30 minutes until the first mosquito landed on the hand. Protection time was calculated as either the time elapsed between repellent application and the first confirmed mosquito bite. The experiment was replicated 3 times [17].

Evaluate the effectiveness of the homemade vaporizer

The evaluation was conducted in two selected rooms (with the same area) in a house, each with an area of approximately 12 m^2, (3 m × 4 m) and a similar layout, containing minimal furniture and no objects that could obstruct mosquito movement. Where one was selected as the treatment room and the other as the control room. Both rooms had similar environmental conditions: average temperature was maintained at 27 ± 1 °C, and relative humidity 75 ± 5%, measured using a digital thermo-hygrometer. Natural lighting was used in both rooms. The test was conducted during the mosquito- biting time (typically between 6:00 a.m. and 10:00 a.m.) in the house that was pre-evaluated. One window was kept open during the observational period.

Two volunteers who were known to be highly susceptible to mosquito bites were randomly selected from a group of pre-screened individuals. Each volunteer was assigned to one room, either the treatment room or the control room. The entire body of each volunteer was covered with a suitable protective outfit, leaving only the upper side of the left thigh exposed for mosquito landing. In the treatment room, the mosquito repellent vaporizer was switched on 30 minutes before the experiment to allow the vapor to disperse evenly. After this 30-minute pre-treatment period, the vaporizer was switched off, and both volunteers simultaneously entered their respective rooms. Each volunteer was seated in a relaxed position in a chair, exposing the upper thigh area for mosquito landing. Whenever a mosquito landed, it was collected using a mouth aspirator by the volunteer. The experiment continued until the repellent's protective effect wore off, as indicated by the first mosquito landing on the exposed area. This time point was recorded as the end of the complete protection period. Additionally, the total number of mosquitoes collected in both rooms was recorded and compared to assess any significant differences. This experiment was repeated on three different days to facilitate replication. During the replication, volunteers were interchanged [18].

Data analysis

The percentage of repellency (P) was calculated using the formula:

$$P = 1 - \left({}^{T}/_{C}\right)$$ [17],

consistent with the WHO Guidelines for Efficacy Testing of Mosquito Repellents for Human Skin. Where T is the landings on the treated arm, and C is the number of landings on the untreated (control) arm of the same individual. The mean repellency percentage was calculated by averaging the protection values from three repeated trials for each treatment.

The Complete Protection Time (CPT) was recorded as the time (in minutes) from repellent application until the first mosquito landed on the treated arm. The mean repellency percentage data converted into the probit values using the standard probit table (Probit repellency). Concentration series were converted into log dosage values. The Y axis is probit repellency, and the X axis is Log dosage.

Data were analyzed using probit-plane regression analysis from which the ED_{50} and $ED_{99.9}$ were estimated. All data were analyzed using SPSS Software and Microsoft Excel.

Results and Discussion

The repellent efficacy of extracted oil was evaluated against female *A. aegypti* mosquitoes in both laboratory and field settings. The study determined the mean repellence percentages at various concentrations of marigold essential oil, as well as the complete protection time (CPT) at the most effective concentration.

The Effective Dosage of Extracted Marigold Essential Oil

Table 1 shows the mean repellence percentage at different concentrations. The repellent effect increased with concentration, reaching 100% repellence at 9% and 10% concentrations.

Table 1. Mean Repellence Percentage of *A. aegypti* at different concentrations of marigold essential oil

Concentration [%]*	Mean Repellence Percentage %	Mean Control Data (No: of *A. aegypti* Mosquitoes landed on the control arm)
5	22	44
6	39	46
7	61	40
8	99	37
9	100	45
10	100	43

*Essential oil diluted with ethanol and concentration given as v/v percentage;

The log-probit analysis revealed that a concentration of 9.64 ± 1.63% (v/v) provided 99.99% repellence (Figure 4), which was identified as the effective dose (ED 99.9). ED 50 value was identified as 5.98 ± 0.92%.

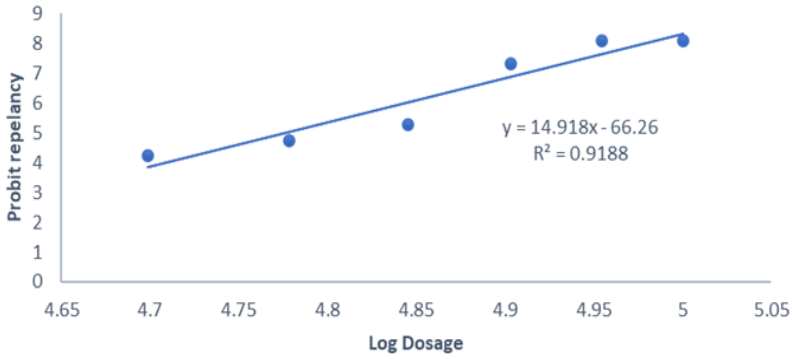

Figure 4. Log-Probit curve for repellence percentage

Some studies showed that 99.9% repellence of Marigold (*T. erecta*) essential oil gives for a 17.5% (v/v) concentration level [19]. Furthermore, marigold Flower (*T. erecta*) methanol extracts lotion against *Culex quinquefasciatus* repellent extraction of 50% and 90% give 11.078% and 32.131%, respectively [20].

Another researcher shows Phlai (*Zingiber cassumuna*), Sweet basil (*Ocimum basilicum*) both substances 0.8% deterrent to *Aedes aegypti* mosquitoes at concertation 10%-30% and citronella grass *(Cymbopogon nardus*) essential oil 0.8% deterrent to *Aedes aegypti* mosquitoes at concentration between 10% - 30% level [21].

According to Table 1 and Figure 4 Marigold oil concentrations of 5% to 10% showed increasing repellence, with a 100% repellence rate between (9-10)%, ED 99.9% value was (9.64 ± 1.63)% (v/v). The past studies revealed that 9.9% repellence of Marigold (*T. erecta*) essential oil gives a 17.5% (v/v) concentration level [19].

The Complete Protection time of extracted marigold Essential oil

As shown in Table 2, the marigold essential oil (9.64 ± 1.63% v/v) provides a mean complete protection Time (CPT) of 150 ± 10 minutes against mosquito bites.

Table 2. Complete Protection Time (CPT) of marigold essential oil (9.64 ± 1.63%) v/v)

Essential oil concentration	Mean Complete Protection Time (CPT) (± 10 minutes)
9.64 ± 1.63% (v/v)	150

These findings are consistent with earlier studies, which reported CPTs ranging from 116 to 122.3 minutes for *Aedes albopictus* using marigold oil, although the specific concentration used in those studies was not specified [19].

The Efficiency of Vaporizer

According to Table 3 and Figure 5, the vaporizer reduced mosquito landings in the test rooms, offering up to 180 minutes of continuous protection.

Table 3. Mean number of adult *A. aegypti* mosquitoes caught in the control and test rooms after marigold essential oil was dispersed using a homemade vaporizer (T-Test, C-control)

Time period (6:00 a.m. to 10:00 a.m.)	Room 1		Room 2	
	Test	Control	Test	Control
1st Hour (6-7)	0	3	0	2
2nd Hour (7-8)	0	2	0	2
3rd Hour (8-9)	0	2	0	3
4th Hour (9-10)	1	2	1	2

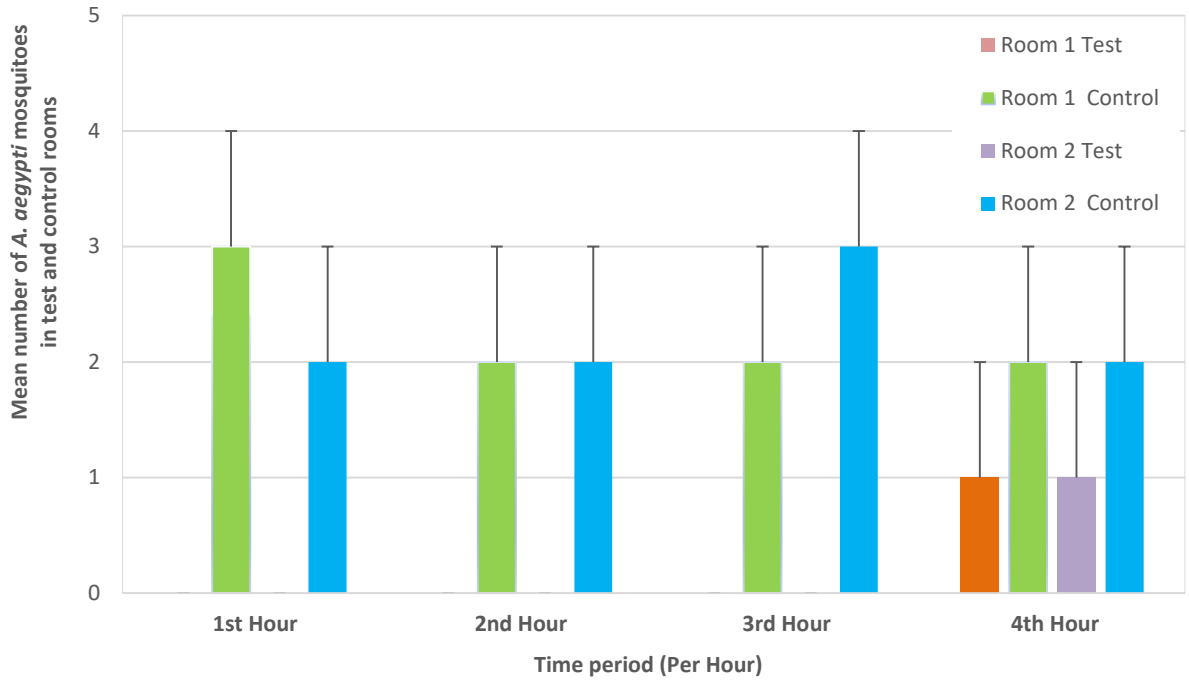

Figure 5. *A. aegypti* mosquito landing comparison between test and control rooms

The ongoing tests in Rooms 1 and 2, as mentioned in Table 3 and Figure 5, showed that marigold essential oil effectively reduced the landing preference of *Aedes aegypti* mosquitoes. In both rooms, no mosquitoes were caught in the test rooms for up to three hours on the first day, with only a few caught in the subsequent hours and days. In contrast, more mosquitoes were caught in the control rooms across all days, indicating the oil's efficacy in repelling mosquitoes. However, there are no published studies discussing the vaporiser-based repellence of marigold essential oil.

Conclusion

This study demonstrates that African marigold essential oil exhibits strong repellent activity against *Aedes aegypti* mosquitoes. At a concentration of 9.64 ± 1.63% (v/v), the oil achieved 99.99% repellence under laboratory conditions, with a protection time of 150 ± 10 minutes. Field tests confirmed that the homemade vaporizer provided 180 minutes of continuous protection. These results highlight the potential of marigold essential oil as a sustainable and eco-friendly alternative to synthetic repellents for *Aedes aegypti* control. While the study is limited by the absence of chemical analysis to identify the active compounds, the findings offer promising evidence for further development of plant-based mosquito repellents.

Conflicts of Interest

We declare that we have no conflict of interest.

Institutional Review Board Statement

The study was conducted under the direct supervision of the Principal of the School of Entomology, with strict adherence to safety, ethical standards, and standard entomological practices, and was subsequently evaluated by the scientific board of the Education, Training, and Research (ET&R) Unit of the Ministry of Health.

Acknowledgement
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