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Smoke repellency effect of *Wrightia tinctoria* (Roxb.) R.Br. (Apocynaceae) on mosquitoes

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Abstract
Plants and its derivatives have been used as a fumigant traditionally to repel and kill mosquitoes and for protection from mosquito bites. Several field evaluations, where plants were burned to produce smoke to repel mosquitoes have shown good reduction in mosquito landings. In the present study, the crude fruit and leaf extracts of *Wrightia tinctoria* were tested for its repellent efficacy against mosquitoes under field conditions. Repellent coil with one per cent concentration of fruit/leaf extract prepared was evaluated for 24, 48 and 72 hours during the peak hours which was standardized. The repellent coil of each plant part and its extract was burned and the protection time for the same were recorded. The overall assessment indicated that the aqueous fruit extract of *Wrightia tinctoria* provided a protection time of 192 and 176 minutes in petroleum ether followed by its leaf extract with 143 and 135 minutes respectively against mosquitoes. The control of the fruit and leaf extract provided a protection time of 37 and 26 minutes respectively. Further studies on the isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth field bioassays are needed as the present study shows that there is scope to use *Wrightia tinctoria* extracts to repel mosquitoes.

Keywords: *Wrightia tinctoria*, fruit and leaf extracts, mosquitoes, smoke repellent

1. Introduction

The major obstacle in mosquito control programme is the development of resistance in mosquitoes to conventional insecticides. WHO [1] felt the resistance in vectors was probably the "biggest single obstacle in the struggle against vector-borne diseases". Synthetic insecticides used to control mosquitoes have created a criticism of ecological impact, non-target organisms being influenced and most mosquito species getting physiologically impervious to them [2]. As of late, the utilization of naturally agreeable, biodegradable insecticides from plants to control insect vectors is picking up significance [3] and botanicals have been seen as successful. Plant products have been utilized customarily to repel mosquitoes in many parts of the world [4] and a huge number of them have been tried as potential sources of insect repellents [5, 6]. Repellents of plant sources are not toxic to humans and domestic animals and can quickly be biodegraded relative to synthetic compounds [7, 8].

Plants of terrestrial origin have also been reported to be a source of mosquito repellents [9-11] and its derivatives have been used as a fumigant for protection from mosquito bites. The first method man used to repel insects was with smoke wherein fresh and dried plants were frequently added to fires to enhance the repellent properties of the smoke as it is still the most widely used means of repelling mosquitoes utilized throughout the rural tropics [12]. A variety of substances, including smoke, plant extracts, oils, tars and mud have been used over the centuries to repel mosquitoes [13]. Several field evaluations, where plants were burned to repel mosquitoes, have shown good reduction in mosquito landings [14, 15]. Most households in the developing world rely on personal protection measures of limited effectiveness, such as burning coils made from plant parts [16]. Keeping in view of afore mentioned features, the smoke repellent efficacy of *Wrightia tinctoria* extracts against mosquitoes were tested in the present study since only scanty research has been reported for mosquito property by this plant [17-21].

2. Materials and Methods

2.1 Plant collection and preparation of phytoextracts

Mature, healthy fruits and leaves of *Wrightia tinctoria* were collected from Chennai, Tamil Nadu, India. Taxonomical identity of the plant was confirmed at Department of Plant Biology and Plant Biotechnology, Madras Christian College, Chennai, Tamil Nadu, India. The fruits and leaves were then brought to the laboratory, washed in dechlorinated water, shade dried and pulverised with the aid of an electrically powered mixer individually. The powdered plant parts (500g) were extracted with petroleum ether and distilled water (1.5L) each for a period of eight hours in a Soxhlet apparatus [22]. The crude solvent fruit and leaf extracts thus obtained were then stored in air tight sterilized amber coloured bottles at 4 °C for bioassay.

2.2 Phytochemical screening

Harborne [23] procedure was adopted to qualitatively determine the major phytochemical constituents present in the crude solvent extracts of *Wrightia tinctoria* fruits and leaves.

2.3 Site selection for field study

The field area selected for the present study was Madambakkam, East Tambaram, Chennai, Tamil Nadu, India (12.9208° N, 80.1306° E) based on the density of mosquitoes (irrespective of species) present. Observations were made based on the bionomics of mosquitoes including swarming, landing and biting behaviour prior to the start of the experiment to justify the site selection and also to standardize the peak hours' time during which the study was conducted.

2.4 Repellent bioassay

Kazembe and Nkomo [24] methodology was adopted in the present study with minor modifications since plant parts burnt on glowing charcoal produce smoke which can act as a repellent mosquitocide. Repellent coil with one per cent concentration (wood powder + charcoal powder + potassium nitrate as binding material + desired dose of fruit/leaf extract) prepared was evaluated along with the control during the peak hours which was standardized. A total of three trials were carried with three replicates per trial. The repellent coil of each plant part and its extract was burnt and the protection time for the same were recorded for 24, 48 and 72 hours.

3. Results

The observations of mosquito landing and biting behaviour in field conditions of the selected study site are presented in Figure 1. The phytochemical components of *Wrightia tinctoria* fruit and leaf extracts tested positive for bioactive constituents like alkaloids, glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids. Amongst these phytochemicals, alkaloids, flavonoids and steroids were present in high concentration and dominated the other phytoconstituents present. *Wrightia tinctoria* fruit and leaf extracts were found to show repellence against mosquitoes in field conditions. In fruit extract, the aqueous part showed more mean repellence (192 minutes) than petroleum ether (176 minutes) (Figure 2). Similarly, in leaf extract, the same result was observed with a mean protection time of 143 and 135 minutes respectively (Figure 3). Among the plant parts tested, fruit was found to be effective than leaf. In comparison with solvent extracts, aqueous exhibited more repellence than petroleum ether. The overall assessment of the repellent study indicated the aqueous fruit of *Wrightia tinctoria* to be effective followed by petroleum ether fruit; aqueous leaf and petroleum ether leaf extract. The control of the fruit and leaf extract provided a protection time of 37 and 26 minutes respectively (Figure 4).

4. Discussion

Repelling mosquitoes is one of the measures to control the transmission of infectious diseases that are transmitted through the bite of infected mosquitoes, thereby preventing mosquito-borne diseases by reducing man–mosquito contact. Further, mosquito bites may also cause allergic responses including local skin reactions and systemic *viz.*, utricaria and angioedema [25]. A repellent to be effective should be helpful in reducing man vector contact as they act regionally or from a distance, in deterring an insect from flying to, landing on or biting human or animals [26,27]. Repellent compounds ought to be non-toxic, non-irritating and long lasting as they're thought-about together of the foremost effective tools for shielding human from vector-borne diseases and nuisance caused by mosquitoes [28-31]. Repellents of chemical origin could cause skin irritation and have an adverse effect on the skin [32]. Commercial repellents, *viz*., allethrin, N,N-diethyl-m-toluamide (DEET), dimethyl phthalate (DMP) and N, N-diethyl mendelic acid amide (DEM) manufactured by using chemical substances are not safe for public use [33,34] because of their unpleasant smell, oily feeling [35,36] and potential toxicity [37-39].

Natural products of plants origin are safe and have been used traditionally to repel and kill mosquitoes in many parts of the world [40]. Plants were first recorded of being used against biting insects by the ancient Greeks, and are still used by enormous number of people today. Plants have been used for centuries in the form of crude fumigants where plants were burned to drive away nuisance mosquitoes and later as oil formulations as applications to the skin which was first recorded in writings by Roman and Indian scholars [41]. The first principle mentioned was applied and observed in the present study. Most households in the developing world rely on personal protection measures, *viz*., burning coils prepared from plant parts, despite the wide range of effective control measures available [16] which was practiced and experimented in the present study. Plant-based repellents are still extensively used in this traditional way throughout rural communities in the tropics as "natural" smelling repellents are preferred because plants are perceived as a safe and trusted means of mosquito bite prevention. Plants suitable for mosquito repellent use should be naturally abundant and the source of repellent must be perennial, sustainable, replaceable parts preferable, e.g., leaves or seeds rather than parts that when removed kill or damage the plant, *viz.*, roots or shoots [42].

Non-polar and polar extracts contain effective ingredients which could possibly alter the physiology of mosquitoes. Mukandiwa *et al*. [43] reported that the hexane and acetone leaf extracts of *Clausena anisata* exhibited smoke repellent activity. An analogous result was also observed in the present study, wherein, the comparison was between a non-polar and a polar solvent, whereas Mukandiwa *et al*. [43] compared it between a non-polar and mid polar solvent. Burning exhausts the amount of active phytochemicals quicker than allowing the extract to vapourise on its own. Thus, the method of burning *Wrightia tinctoria* extracts in the form of a repellent

coil was chosen since it reflects the mode in which local community use these repellents as the methodology was based on traditional knowledge. However, it is to be noted that differences, if any, in results which arise may attribute to the amount of phytochemical constituents and the geographical region where the plant grew and the leaves were collected.
The effectiveness and duration depend on the type of repellent (active ingredients), the mode of application, local conditions (temperature, humidity and wind) and the sensitivity of the insect to repellents since each species has its own specific sensitivity [44, 45]. Allelochemicals have been considered as potential natural insecticides and can be used for insect/mosquito management in integrated control [46] because products of secondary plant metabolism may be responsible for the chemical communication between plants and insects. Vivekanandhan *et al*. [47] reported that the seed pod extract of *Acacia nilotica* smoke toxicity test caused 80, 82 and 90% mortality in *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* respectively. Similarly, the results of the present study indicated *Wrightia tinctoria* crude extracts to be effective against mosquitoes and its smoke repellent activity might be due to the presence of phytochemicals that are able to irritate the olfactory senses of the mosquitoes. These chemicals are non-volatile and release insecticidal smoke when the plant materials in the form of repellent coil containing the active ingredients are burnt as tested in the present study. These bioactive chemicals might be the alkaloids and flavonoids which were found to be dominating when *Wrightia tinctoria* was screened for its phytochemical compounds. Ansari and Razdan [48] reported that alkaloids, flavonoids, phenolics, saponins and tannins present in the phytochemical extracts exert some inhibitory effect on lactic acid receptor cells by masking or changing the lactic acids that normally attract mosquitoes thereby confusing or distracting the mosquitoes, thereby preventing blood feeding contact. These phytocompounds have the capacity to repel mosquitoes through direct toxicity by affecting the acetylcholine receptors in the nervous system or membrane sodium channels of nerves [49] and hence, would have been the reason for repellent activity in the present study. Keeping in view of above mentioned factors it is indicated that chemicals released from burning plants play an important role in repelling host-seeking mosquitoes which was witnessed in the present study.
Repellents vaporize quickly than insecticides. Insecticide last longer by killing or knocking down insects after contact but repellents perform by avoiding human insect contact. The period of protection by a repellent may range from 15 minutes to 10 hours and at times the effect lasts much longer. Besides, Amer and Mehlhorn [50] defined that if the protection time of repellent is extended and the biting percentage is little, the repellent had good competence in repelling mosquitoes and prevents biting. In contrast, the protection time is short but the biting percentage is little, then the repellent is more a feeding deterrent than a repellent. If the protection time is extended but the biting percentage is high, then the repellent is more a repellent than a feeding deterrent. This principle was helpful in evaluating the repellent efficiency of *Wrightia tinctoria* extracts in the present study and as the mean protection time ranged between 135 and 200 minutes with low biting percentage, it might be housed under a feeding deterrent category. This section needs in-depth investigation as a separate study.

Smoke production most likely has a long-range effect on mosquito host-seeking
behaviour [14]. The vapour in the air in the form of smoke affects the central nervous system of mosquitoes since they contain toxic principle which plays a vital role in the control of vectors [51]. Smoke disguises human kairomones particularly carbon dioxide and disrupts convention currents essential in mosquito host location and thereafter releases repellent irritant molecules [52]. Additionally, mosquitoes depend on on heat and moisture in linking currents as a short-range cue for approach to hosts [53] and these too may be transformed by combustion. Smoke production also depresses humidity by dropping the moisture carrying capability of the air. This enables mosquitoes vulnerable to desiccation and lessens sensory input since mosquito chemoreceptors are more receptive in the presence of moisture [54] and undeniably, heat alone is repellent to mosquitoes. The mode of action of spatial repellency occurs through knock down activity or binding or disruption of orientation towards the host. The latter may be categorized as a sub lethal effect that results from neutral excitement which appears to occur at the earlier stage of toxication or at the dosage required to knock down as repellents disturb the capacity of receptors in mosquito antennae to respond to the post stimuli [55]. This would have been the reason for the mosquitoes to be repelled in the present study.

5. Conclusion
Natural products as extracts from parts of plants of insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their repellent toxicity. The findings of the present investigation revealed that the crude extracts of *Wrightia tinctoria* possessed repellent activity against mosquitoes and in addition to this, it may also be strongly noted that this plant being well known and documented for its antipsoriasis property for psoriasis treatment and other skin diseases [56], would certainly come in handy for its application to skin allergies caused due to mosquito bites. Therefore, further studies on isolation and purification of bioactive phytochemical constituents / compounds followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use *Wrightia tinctoria* extracts to repel mosquitoes.

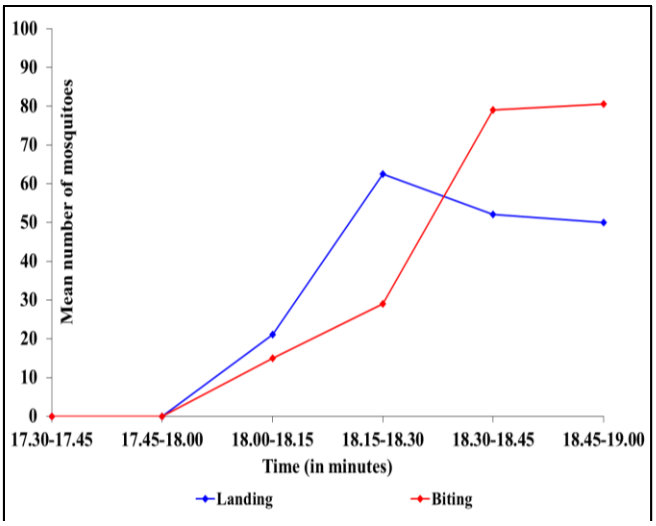

Fig 1: Mosquito landing and biting behaviour in field conditions of the study area

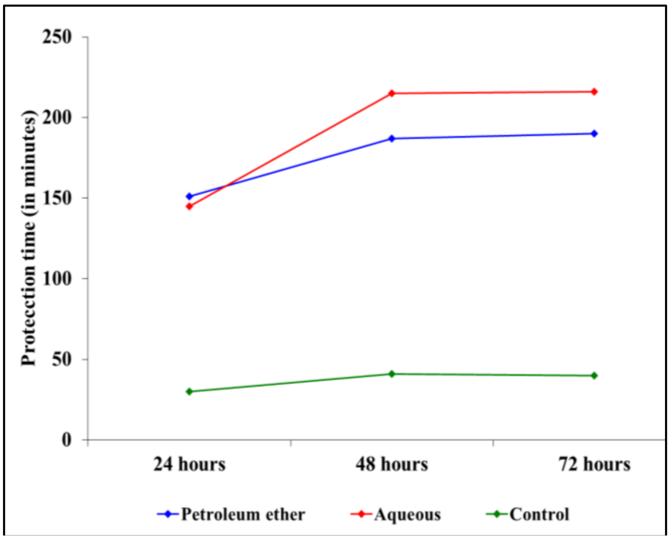

Fig 2: Protection time of *Wrightia tinctoria* fruit extract

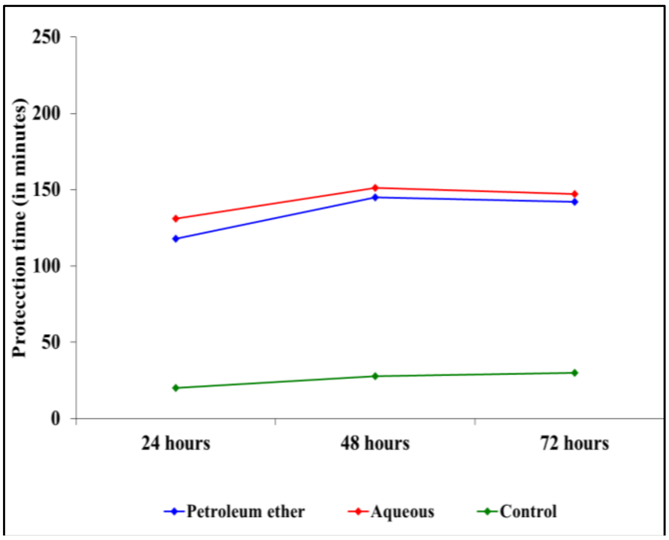

Fig 3: Protection time of *Wrightia tinctoria* leaf extract

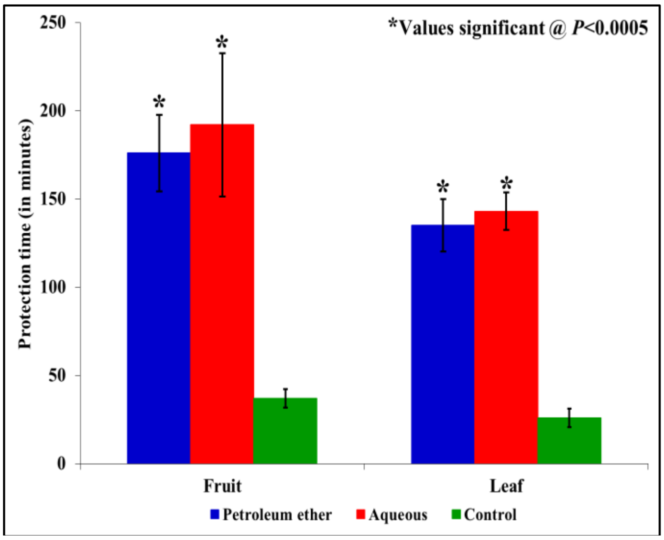

Fig 4: Mean protection time of *Wrightia tinctoria* extracts

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