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Adult emergence inhibition and adulticidal activities of medicinal plant extracts against *Anopheles stephensi* Liston

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ABSTRACT

Objective: To determine the adult emergence inhibition (EI) and adulticidal activities of hexane, chloroform, ethyl acetate, and acetone leaves extracts of *Anisomeles malabarica (A. malabarica), Euphorbia hirta (E. hirta), Ocimum basilicum (O. basilicum), Ricinus communis (R. communis), Solanum trilobatum (S. trilobatum), Tridax procumbens (T. procumbens)* and seeds of *Gloriosa superba (G. superba)* against *Anopheles stephensi (An. stephensi)*. **Methods:** The EI and adulticidal trials were carried out according to World Health Organization (WHO) procedures with slight modifications. The extracts were diluted in dimethyl sulphoxide in order to prepare a serial dilution of test dosages (15.625, 31.25, 62.5, 125, 250, 500 and 1 000 μg/mL). Five duplicate trials were carried out for every sample concentration, and for each trial a negative control was included and the mortality was determined after 24 h of exposure. **Results:** The highest EI activity was found in ethyl acetate extracts of *A. malabarica*, chloroform extracts of *O. basilicum, S.trilobatum*, acetone of extract of *R. communis, T. procumbens*, and seed extract of *G. superba* with EI_{50} values 143.12, 119.82, 157.87, 139.39, 111.19, and 134.85 μg/mL, and the effective adulticidal activity was observed in chloroform, acetone extracts of *G. superba, T. procumbens, R. communis, S.trilobatum* and ethyl acetate extract of *O. basilicum* with LD_{50} values 120.17, 108.77, 127.22, 163.11, 118.27, and 93.02 μg/mL, respectively. Chi-square value was significant at $P<0.05$ level. **Conclusions:** These results should encourage further efforts to investigate the compounds that might possess good EI and adulticidal properties when isolated in pure form.

1. Introduction

Mosquitoes are the most important single group of insects well-known for their public health importance, as they act as vector for many tropical and subtropical diseases such as dengue fever, yellow fever, malaria, filariasis and encephalitis. To prevent mosquito-borne diseases and improve public health, it is necessary to control them. Mosquito-transmitted diseases remain a major cause of the loss of human life worldwide with more than 700 million people suffering from these diseases annually[1]. *Anopheles stephensi (An. stephensi)* is a major malaria vector in India. With an annual incidence of 300-500 million clinically manifested cases and a death toll of 1.1-2.7 million, malaria is still one of the most important communicable diseases. Currently, about 40% of the world's population lives in areas where malaria is endemic[2]. The failure of chemical insecticides to control the insect and growing public concern for safe food and a healthy environment have catalyzed the search for more environmentally benign control methods for management of these vectors. Bio pesticides provide an alternative to synthetic pesticides because of their low environmental pollution, low toxicity to humans and other advantages[3]. Secondary metabolites of plants, many of them produced for protection against microorganisms and insect predators, are natural candidates for the discovery of new products to combat *An. stephensi*. Several studies have focused on larvicides, adulticides, repellents, and ovipositional attractants activities observed by different researchers[4-6].

The hexane, chloroform, ethyl acetate, acetone, and

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methanol extracts of *A. malabarica* were tested against the fourth instar larvae of *Anopheles subpictus (An. subpictus)* and *Culex tritaeniorhynchus (Cx. tritaeniorhynchus)*[7]. *Euphorbia hirta (E. hirta)* is an anthropogenic herb found in many parts of the world, the larvicidal property of carbon tetrachloride, methanol, and petroleum ether extracts were reported against third instar larvae of *An. stephensi*[8]. The hexane fraction, dichloromethane fractions 1 and 2, and methanol fraction from *Gloriosa superba (G. superba)* were investigated for colchicine-like activity using *Aedes aegypti (Ae. aegypti)* cytogenetic assay[9]. Bagavan *et al*[10] had reported that the leaves hexane, chloroform, ethyl acetate, acetone and methanol extracts of *G. superba* were tested against fourth instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus*.

Murugan *et al*[11] studied the larval toxicity and smoke repellent potential of methanol extract of *O. basilicum* against different instar (I, II, III and IV) larvae and pupae of *Ae. aegypti*. The larvicidal and adult emergence inhibition activities of *Ricinus communis (R. communis)* seed extract against *An. stephensi*, *Culex quinquefasciatus (Cx. quinquefasciatus)* and *Aedes albopictus (Ae. albopictus)*[12]. Senthilkumar *et al*[13] have reported that the larvicidal and adulticidal activities of leaves extract of *R. communis* were tested against *An. stephensi*.

Thoothuvalai [*Solanum trilobatum (S. trilobatum)*] is a thorny shrub widely spread in India, and has been screened for its ovicidal and larvicidal activities against *Culex* mosquitoes[14]. The oviposition deterrent and skin repellent activity of *S. trilobatum* tested against *An. stephensi*[15]. Essential oil extracted by steam distillation from the leaves of *Tridax procumbens (T. procumbens)* (coat buttons) was evaluated for their topical repellency effects against *An. stephensi* in mosquito cages[16].

As far as our literature survey could ascertain, no information was available on the adult emergence inhibition and adulticidal activities of the experimental plants given here. Therefore, the aim of this study was to investigate activity of different solvent extracts of seven plant species from Tamil Nadu, South India. The present study was an attempt to assess the adult emergence inhibition and adulticidal activity of plant extracts against *An. stephensi*.

2. Materials and methods

2.1. Plant collection

The leaves of *Anisomeles malabarica (A. malabarica)* (L.) R. Br. (Lamiaceae), *E. hirta* Linn (Euphorbiaceae), seed of *Gloriosa superba (G. superba)* L. (Liliacea), *Ocimum basilicum (O. basilicum)* L. (Lamiaceae), leaves of *R. communis* L. (Euphorbiaceae), *S. trilobatum* L. (Solanaceae), *T. procumbens* L. (Asteraceae) were collected in and around Melvisharam, Vellore district, Tamil Nadu. The taxonomic identification was made by Dr. B. Annadurai, Department of Plant Biology and Biotechnology, C. Abdul Hakeem College, Melvisharam, Vellore, India. The voucher specimens were numbered and kept in our research laboratory for further reference.

2.2. Preparation of plant extract

The leaves and seed were dried for 7-25 days in the shade at the environmental temperatures (27-37 ℃ day time). The dried leaves (700 g), and seed (450 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with hexane (1 200 mL, Fine), chloroform (1 500 mL, Fine), ethyl acetate (1 800 mL, Qualigens), and acetone (1 200 mL, Qualigens) in a soxhlet apparatus (boiling point range 60-80 ℃) for 8 h. The extract was concentrated under reduced pressure of 22-26 mm Hg at 45 ℃, and the residue obtained was stored at 4 ℃. To prepare the stock solution, one gram of the crude extract was first dissolved in 100 mL of acetone.

2.3. Collection and rearing of mosquito

An. stephensi larvae were collected from rice field and stagnant water area of Melvisharam and identified in Zonal Entomological Research Centre, Vellore, Tamil Nadu. To start the colony, larvae were kept in plastic and enamel trays containing tap water. They were maintained at (27±2) ℃ and 75%-85% relative humidity under 14:10 light and dark cycles. Larvae were fed a diet of brewer's yeast, dog biscuits, and algae collected from ponds in a ratio of 3:1:1, respectively. Pupae were transferred from the trays to a cup containing tap water and maintained in the insectary (45 cm×45 cm×40 cm) where adults emerged. Adults were maintained in glass cages and continuously provided with 10% sucrose solution in a jar with a cotton wick. On day 5, the adults were given a blood meal from a pigeon. They were maintained and reared in the laboratory as per the method of Kamaraj *et al*[4].

2.4. Adult emergence inhibition (EI) bioassay

The inhibition of adult emergence was evaluated by following the WHO standard procedure for testing insect growth regulators[17]. Only 3rd instars larvae were used and followed the method of larvicidal activity. Because of the long duration of the test, the larvae were fed by yeast at two days intervals until mortality counts were made. The yeast powder was prepared as stock suspension in water from which one or two drops added per cup. To make a stock solution, one gram of crude extract was first dissolved in 100 mL of acetone. From the stock solution, 15.62-1 000 μg/mL was prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. All the treated and control cups containing pupae were kept separately in the net cage to prevent successfully emerged adults from escaping into the environment. Mortality of the pupae was recorded at 24 h intervals. Observation was continued in treated and control cups (acetone, polysorbate 80 and de-chlorinated tap water) until the complete emergence of adults. At the end of observation period, the impact was expressed as EI% based on the number of pupae that did not develop successfully into viable adults. In recording EI% for each concentration, moribund and pupae, as well as adult mosquitoes not completely separated from the pupal case, were considered as dead. The experiments were stopped when all the pupae in the controls have died or emerged as adults.The experimental media, in which 100% (EI) pupae occurs alone,

were selected dose response bioassay test[18].

2.5. Adulticidal bioassay

An. stephensi mosquitoes were selected for the testing of adulticidal activities. The bioassay was performed by WHO method[19]. Appropriate concentrations of the plant solvent extracts were dissolved in 2.5 mL of acetone and applied on Whatman No. 1 filter papers (size 12 cm×15 cm) as described by Dua *et al*[20]. The impregnated paper was hung under a shade overnight, by which time all the solvents had evaporated and the extracted medicinal plants solution had been spread evenly. Control papers were treated with acetone under similar conditions. The plant extracts were evaluated at seven concentrations 15.625, 31.25, 62.5, 125, 250, 500 and 1 000 μg/mL to produce a range of mortality from 10% to 100% along with control. Twenty mosquitoes (2-5 days old glucose fed, blood starved) were collected and gently transferred into a plastic holding tube. The mosquitoes were allowed to acclimatize in the holding tube for 1 h and then exposed to test paper for 1 h. At the end of exposure period, the mosquitoes were transferred back to the holding tube and kept 24 h for recovery period. A pad of cotton soaked with 10% glucose solution was placed on the mesh screen. Mortality of mosquitoes was determined at the end of 24 h recovery period. Per cent mortality was corrected by using of Abbott's formula[21].

2.6. Dose response bioassay

From the stock solution, different concentrations ranging from 15.62 to 1 000 μg/mL were prepared. Based on the preliminary screening results, crude different solvent extracts prepared from the leaves of *A. malabarica*, seed of *G. superba*, leaves of *O. basilicum*, leaves of *R. communis*, *S. trilobatum*, *T. procumbens* were subjected to dose response bioassay for percentage EI and adulticidal activity counted after 24 h of exposure, against the *An. stephensi*. The percentage larval, EI% and adult mortality and was reported from the average of five replicates.

2.7. Statistical analysis

Adult mortality counts were made after 24 h exposure. Bioassay test showing more than 10% control mortality were discarded and repeated. However, when control mortality ranged from 5% to 10%, the corrected mortality was calculated using Abbott's formula[21]. LD_{50}, LD_{90} and the 90% confidence intervals (*CI*) of the lethal dosage of 50% and 90% calculated by a computerized log-probit analysis (Harvard Programming; Hg1, 2) were used to measure differences between test samples.

3. Results

Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest managing agents. The botanical extracts from the plant leaves, roots, seeds, and bark in their crude form have been used as conventional insecticides for centuries. The activity of crude plant extracts is often attributed to the complex mixture of active compounds. The preliminary screening is a good means of evaluation of the potential of plants adult emergence inhibition and adulticidal activity of crude plant extracts is often attributed to the different solvent extracts of seven plants are noted and presented in Table 1. Among the crude extracts tested, the present results showed that emergence inhibition of leaves ethyl acetate extracts of *A. malabarica*, chloroform extracts of *O. basilicum*, and *S.trilobatum*, acetone extracts of *R. communis*, *T. procumbens*, and seed of *G. superba* (EI_{50}=143.12, 119.82, 157.87, 139.39, 111.19, and 134.85 μg/mL; EI_{90}=714.92, 705.09, 770.75, 651.34, 691.04, and 690.18 μg/mL); and the maximum adulticidal activity was observed in chloroform, acetone extracts of *G. superba*, *T. procumbens*, *R. communis*, and *S.trilobatum*, ethyl acetate extracts of *O. basilicum* (LD_{50}=120.17, 108.77, 127.22, 163.11, 118.27, and 93.02 μg/mL; LD_{90}=588.38, 472.8, 418.63, 867.64, 450.09, and 378.98 μg/mL), respectively against *An. stephensi*. Chi-square value was significant at $P<0.05$ level (Table 2, 3).

4. Discussion

In view of residue problems in the environment and the development of insect resistance to synthetic insecticides like DDT and other chlorinated hydrocarbons, the recent trend is to explore plants to obtain extracts that are safe for nontarget animals and do not pose any residue problem but are still able to suppress pest populations. Though several compounds of plant origin have been reported as insecticides-larvicides, there is a wide scope for the discovery of more effective plant products. Further research undoubtedly will lead the improved formulations with enhanced activity, which may eventually become environmentally acceptable and replace objectionable conventional insecticides for mosquito control.

Our results agreed with some previous studies, undoubtedly, plant derived toxicants are a valuable source of potential insecticides. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future[22]. Similar study was conducted by Elimam *et al*[23] and reported that aqueous extracts from *R. communis* showed 50% of adult EI_{50} were 374.97 and 1 180.32 μg/mL against 3rd instar larvae of *Anopheles arabiensis* (*An. arabiensis*) and *Cx. quinquefasciatus* and the extract showed oviposition deterrent effect against both species. Rahuman *et al*[24] reported that the larvicidal activity of the petroleum ether extracts of *Jatropha curcas* (*J. curcas*) and *Euphorbia tirucalli* (*E. tirucalli*) were highly effective against the larvae of *Ae. aegypti* (LC_{50} 8.79 and 4.25 ppm) and *Cx. quinquefasciatus* (LC_{50} 11.34 and 5.52 ppm), respectively.

Dysoxylum malabaricum (*D. malabaricum*) leaf extract produced more than 90% mortality of all instars of *An. stephensi* at a concentration of 4% of leaf extract, though larvicidal activity was observed at higher doses, lower doses greatly inhibited the reproductive potential of adults[25]. One or more active components in the *D. malabaricum* extract (Dymalol, Nymania-3, and other triterpenes) acted as an oviposition repellent and/or deterrent to *An. stephensi*[26,27]. The acetone extract of *Nerium indicum* (*N. indicum*) and *Thuja orientalis* (*T. orientalis*) had been studied with LC_{50}

Table 1

Adult emergence inhibition and adulticidal activity of different plant extracts against *An. stephensi* at 1 000 μg/mL (%).

Botanical name/ Family	Solvents	EI	Mortality at 24h
A. malabarica (L.) Sims./Lamiaceae	Hexane	67.00±3.21	71.00±1.30
	Chloroform	78.00±1.87	75.00±1.58
	Ethylacetate	100.00±0.00	78.00±1.34
	Acetone	74.00±1.30	80.00±1.23
E. hirta Linn/ Euphorbiaceae	Hexane	88.00±2.05	86.00±2.17
	Chloroform	74.00±2.39	79.00±2.59
	Ethyl acetate	75.00±2.55	70.00±3.55
	Acetone	86.00±2.17	78.00±1.14
G. superba L./Liliaceae	Hexane	78.00±2.30	73.00±0.89
	Chloroform	60.00±2.92	100.00±0.00
	Ethyl acetate	69.00±1.64	80.00±1.87
	Acetone	100.00±0.00	100.00±0.00
O. basilicum L./Lamiaceae	Hexane	51.00±0.84	56.00±1.48
	Chloroform	100.00±0.00	71.00±1.92
	Ethyl acetate	76.00±2.17	100.00±0.00
	Acetone	73.00±0.90	68.00±2.07
R.s communis L/Euphorbiaceae	Hexane	54.00±1.30	84.00±1.48
	Chloroform	76.00±1.79	100.00±0.00
	Ethyl acetate	80.00±1.87	81.00±1.48
	Acetone	100.00±0.00	86.00±2.17
S. trilobatum L./Solanaceae	Hexane	70.00±3.39	74.00±2.39
	Chloroform	100.00±0.00	100.00±0.00
	Ethyl acetate	66.00±0.84	80.00±2.83
	Acetone	70.00±31.87	87.00±1.82
T. procumbens L./Asteraceae	Hexane	36.00±0.84	62.00±2.70
	Chloroform	54.00±1.30	83.00±1.67
	Ethyl acetate	45.00±1.23	71.00±3.35
	Acetone	100.00±0.00	100.00±0.00

Number of each replicates five, and mortality was recorded at 24 h recovery period.

Table 2

Adult emergence inhibition activity of different plant extracts against *An. stephensi*.

Name of the plants	Solvents	EI_{50}(μg/mL)	UCL– LCL	EI_{90} (μg/mL)	UCL – LCL	Regression coefficient(Slope)	χ^2(df=4)
A. malabarica	Ethyl acetate	143.12±10.33	163.37–122.87	714.92±89.64	890.64–539.21	1.834	13.33
G. superba	Acetone	134.85±9.82	154.10–115.60	690.18±88.19	863.04–517.32	1.807	16.75
O. basilicum	Chloroform	119.82±9.28	138.01–101.62	705.09±101.45	903.94–506.25	1.660	21.59
R. communis	Acetone	139.39±9.86	158.73–120.06	651.34±80.83	809.78–492.89	1.914	16.67
S. trilobatum	Chloroform	157.87±11.32	180.05–135.68	770.75±97.74	962.33–579.16	1.861	13.19
T. procumbens	Acetone	111.19±8.77	128.38–94.00	691.04±101.13	889.27–492.81	1.615	17.62

UCL= upper confidence limit; LCL= lower confidence limit; x^2=Chi–square; df= degree of freedom.

Table 3

Adulticidal activity of different plant extracts against *An. stephensi*.

Name of the plants	Solvents	LD_{50}(μg/mL)	UCL– LCL	LD_{90} (μg/mL)	UCL – LCL	Regression coefficient(Slope)	χ^2(df=4)
G. superba	Chloroform	120.17±8.63	137.10–102.25	588.38±73.83	733.09–443.66	1.857	19.36
G. superba	Acetone	108.77±7.38	123.23–94.30	472.80±53.14	576.35–368.02	2.010	10.25
O. basilicum	Ethyl acetate	93.02±6.31	105.40–80.65	378.98±43.68	464.59–293.36	2.100	11.25
R. communis	Chloroform	163.11±12.12	186.88–139.55	867.64±117.06	1097.09–638.18	1.765	19.29
S. trilobatum	Chloroform	118.27±7.61	131.21–103.34	450.09±47.54	543.27–356.90	2.208	95.53
T. procumbens	Acetone	127.22±7.69	142.31–112.13	418.63±41.68	500.32–336.93	2.477	19.58

UCL= Upper confidence limit; LCL= lower confidence limit; x^2=*Chi*–square; df=degree of freedom. Mosquitoes was exposed for 1 h and mortality was recorded at 24 h recovery period.

values of 200.87 and 127.53 ppm against third-instar larvae of *An. stephensi*[28]. Mosquito larvicidal activity of crude carbon-tetrachloride extract of *Solanum xanthocarpum (S. xanthocarpum)* fruits was the most effective with LC_{50} values of 5.11 ppm after 24 h and 1.27 ppm after 48 h of treatment against *An. stephensi*[29]. The benzene, chloroform, ethyl acetate, and methanol extracts of (*Acalypha indica) A. indica* showed the highest effective attractancy of 90.09%, 94.20%, 85.43%, and 95.75% were observed at 100 ppm against *An. stephensi*, respectively[30]. The compound n-hexadecanoic acid isolated from the leaves acetone extract of *Feronia limonia* (*F. limonia*) was effective with LC_{50} of 129.24, 79.58 and 57.23 ppm on *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* larvae, respectively[31].

The peel chloroform extract of *Citrus sinensis (C. sinensis)*, leaves ethyl acetate extracts of *Ocimum canum (O. canum)* and *Ocimum sanctum (O. sanctum)*, and leaves chloroform extract of *Rhinacanthus nasutus (R. nasutus)* were tested against the larvae of *An. subpictus*, with LC_{50} values of 58.25, 88.15, 21.67, and 40.46 ppm and LC_{90} values of 298.31, 528.70, 98.34, and 267.20 ppm, respectively[32]. The LC_{50} values of the chloroform extract of *Nyctanthes arbor-tristis (N. arbor-tristis)* were 303.2, 518.2, and 420.2 ppm against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus*, respectively[33]. Senthilkumar *et al*[13] reported that the larval mortality between 80% and 100% was observed in the mixture treatment, *C. asiatica* and *Eucalyptus globulus (E. globulus)* at low lethal concentration and lethal time against *An. stephensi*. The isolated flavonoids from *R. communis* showed potential insecticidal, ovicidal and oviposition deterrent activities against *Callosobruchus chinensis (C. chinensis)*[34].

The 50% inhibition of the emergence of adult mosquitoes was observed by the use of the ethyl acetate fractions of *Calophyllum inophyllum (C. inophyllum)* seed and leaves, *Solanum suratense (S. suratense)* and *Samadera indica (S. indica)* extracts and the petrol ether fraction of *R. nasutus* extract on *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* at the 500 ppm[35]. Similarly 88% of the adult mortality was observed by the use of *Pelargonium citrosa (P. citrosa)* extracts at 2% concentration against An. stephensi[36]. This has been observed earlier by Howard *et al*[37] have reported the 50% inhibition of adult emergence (IE_{50}) of all larval instars was obtained with <0.4 g of neem bark chippings of *A. indica* in 1 liter of distilled water against *Anopheles gambiae*. The hexane extract obtained from leaves of *Eucalyptus citriodora (E. citriodora)* tested at lowest concentration viz. 10 ppm, 73% larvae of *An. stephensi* failed to emergence as adult mosquito while in *Cx. quinquefasciatus* and *Ae. aegypti* only 10% and 6% larvae failed to emerge[38]. The root extract of *Valeriana jatamansi (V. jatamansi)* exhibited adulticidal activity and the LC_{50} and the 90% lethal concentration against adult *An. stephensi, Anopheles culicifacies (An. Culicifacies), Ae. aegypti, An. albopictus*, and *Cx. quinquefasciatus* were 0.14, 0.16, 0.09, 0.08, and 0.17 and 0.24, 0.34, 0.25, 0.21, and 0.28 mg/cm^2, respectively[20]. Nathan *et al*[39] considered pure limonoids of neem seed, testing for biological, larvicidal, pupicidal, adulticidal, and antiovipositional activity, *An. stephensi* and the larval mortality was dose-dependent with the highest dose of 1 ppm azadirachtin evoking almost 100% mortality, affecting pupicidal and adulticidal activity and significantly decreased fecundity and longevity of *An. stephensi*. Dua *et al*[40]observed the adulticidal activity of the essential oil of *Lantana camara (L. camara)* was evaluated against different mosquitoes species on 0.208 mg/cm^2 impregnated papers, the KDT_{50} and KDT_{90} values of the essential oil were 20, 18, 15, 12 and 14 min and 35, 28, 25, 18 and 23 min against *Ae. aegypti, Cx. quinquefasciatus, An. culicifacies, Anopheles fluvialitis* and *An. stephensi* with their per cent mortality of 93.3%, 95.2%, 100%, 100% and 100%, respectively.

All toxins used in insect control pose some hazards to the user and also to the aquatic environment[41]. Hence this research is mainly focused on finding newer insecticides which will be more effective, biodegradable and also easily available at low cost. In our observation, chloroform, ethyl acetate and acetone extracts were possessed higher EI and adulticidal activity than the other solvent extracts.

In conclusion, an attempt has been made to evaluate seven plant extracts for adult emergence inhibition and adulticidal bioassay. There are probabilities that the active principal contained in these plant extracts, especially the chloroform, ethyl acetate and acetone extracted fractions are further more potent as mosquito adult emergence inhibition and adulticidal as compared with their crude forms. The identification and isolation of these active components is a part of further search for an efficient, ecofriendly, biodegradable insecticide of plant origin, and is under consideration in the laboratory.

Conflict of interest statement

We declare that we have no conflict of interest.

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