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Research Article

PLANTS SCREENING AND LABORATORY INVESTIGATION OF *AGERATINA ADENOPHORA* METHANOL EXTRACT AGAINST *ANOPHELES STEPHENSI*

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ABSTRACT

Laboratory investigation of *Ageratina adenophora* methanol leaf extract fractions (*A. adenophora*-MLEFr) was controlled and using GC-MS analysis, highlights of the main components on malarial vector, *Anopheles stephensi*(*An. stephensi*). In phytochemical, characterized by GC-MS analysis was carried out to be eligible for the constituents of the MLE. Maximum mortality was exerted by Fr-1 was showed EH/ENH% ovicidal activity of 15.7/37.2% at 10 ppm and 100% mortality at 20, 30, 40 50 and 60 ppm, against *An. stephensi*. The repellent activity of Fr-1 was the highest, showing 100% protection up to 240 min at 0.75 mg/cm^2. In GC-MS analyzes, a total of 21 compounds were identified in the *A. adenophora*-MLE, and the main component was 5-isopropyl-2-methylphenol. The reports revealed the 5-isopropyl-2-methylphenol was the main constituent provides malarial control for the vector mosquito from *A. adenophora*-MLEFr.

Keywords: *Ageratina adenophora*, *Anopheles stephensi*, GC-MS, Repellent activity.

INTRODUCTION

Malaria is a life-threating disease caused by parasites (Genus: *Plasmodium*) that are transmitted to people through the bites of infected *Anopheles*. Presence of the report, according to the latest World malaria report, there were estimated 241 million cases of malaria worldwide, and deaths stood at 627000 in 2020. Continuously, vector control is a vital component of malaria control and elimination strategies as it is highly effective in preventing infection and reducing disease transmission. The two core interventions are insecticide-treated nets (ITNs) and indoor residual spraying IRS (WHO, 2021). At present, imported malaria remains a significant medical and health issue in many European countries, and non-immune travellers from malaria-free areas are very vulnerable to the disease when they become infected (WHO,2021).Medicinal plant products have been making use of conventionally by human being population in variety part rural areas global against vector-borne diseases and parasitology diseases (Baranitharan *et al*., 2019). Biological activity components produced by overall medicinal plants can act as larvicides, and toxin against insects (Irrusappan *et al*., 2022 and Jebanesan *et al*., 2020).The growing awareness of the hazards of excessive use of insecticidal globally has led researchers to search for safer and more environment friendly alternative methods for insect pest control (Baranitharan *et al*., 202; Baranitharan *et al*., 2021; Gokulakrishnan *et al*., 2016; Dhanasekaran *et al*., 2018; Kovendan *et al*., 2013).

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Ageratina adenophora (Asteraceae) is one of the medicinal plants in India, which is now successfully invading large portions of the forest eco-system in North-western Himalaya province in India incorporating the hills of Himachal Pradesh (HPSFD, 2011).The boiled leaves are treatment for malaria and paste of leave were applied on the cut and wounds (Baranitharan *et al*., 2014). The leave are present in the natural antioxidant activity has been associated with the concomitant reduced risks of cancer, cardiovascular disease, diabetes and other diseases related with age as they have the advantage of being almost devoid of side effects (Vanlalhruaii Ralte, 2014). In this investigate; we analyzed twenty one compounds of *A. adenophora*-MLE using GC-MS, compound identification and eggs and adult emerge activity. The compounds were tested as ovicidal and pupicidal activity against *An. stephensi*.

MATERIAL AND METHODS

Plants collection

Fully developed leaves of *A. adenophora* were collected from Nagapattinam District,Tamil Nadu, India, and washed methodically, blotted and shade dried. It was authenticated by plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is the deposited at the herbarium of plant Phytochemistry division, Department of Zoology, Annamalai University, Tamil Nadu, India.

Extraction

The healthy leaves were washed with sterile distilled water, shade dried, and finely ground. the finely ground leaf powder (500 g/ solvent) was extracted with methanol, ethyl acetate, acetone and benzene using Soxhlet extraction apparatus (Borosil 3,840,020- Borosil Glass Works Limited, ISO 9001 Company, Mumbai, India), and the extraction was continued till visibly no further extraction is possible (by observing the colour of the extracted portion). The solvent from the extract are removed using a rotary vacuum evaporator (Cold Trap Condenser Model No. SSI/68, Singhla Scientific Industries, India) to collect the crude extract and stored at 4ºC. Standard stock solutions were prepared at 1% by dissolving the residues in ethanol. From this stock solution, different concentrations were prepared and this solution is used for repellent activity.

Mosquito Rearing

An.stephensi was procured from the Centre for Research in Medical Entomology (ICMR), Madurai, reared in the laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at (28±2)ºC, 70%-85% Relative Humidity (RH), with a photo period of 14 h light, 10 h dark.

Ovicidal activity

The method (Su and Mulla, 1998) was followed to test the ovicidal activity. The leaf extracts was diluted in the respective solvent to achieve different concentrations. The freshly laid egg raft containing 100 eggs of *A. stephensi* were exposed to each dose of leaf extract until they hatched or died. Each concentration was replicated six times. Eggs exposed to respective solvents in water served 48 h post treatment by the following formula.

$$\text{PEH (\%)} = \frac{\text{NHL}}{\text{TNE}} \times 100$$

Repellent activity

Following the method (WHO, 2009: Baranitharan *et al*., 2017). Three day-old blood-starved female *An.stephensi*, mosquito (n=100) were stored in a net cage (45cm × 30cm × 45cm). The volunteer had no contact with lotion, perfumed soaps on the day of the assay. A dorsal facet skin portion on the arms of a volunteer (25 cm^2) was exposed. The extract Fs were applied at 2.5 mg/cm^2 on the forearm exposed area. *An.stephensi* was tested from 8:00 to 16:00. Every check concentration was continual five times. The proportion of repellent was calculated by the subsequent formula:

$$\% \text{ Repellency} = [(T_a\text{-}T_b)/T_a] \times 100$$

Where T_a is the number of mosquitoes in the control group and T_b is the number of mosquitoes in the treated group.

GC-MS analysis

Chromatography analysis was performed using a mass detector Turbo mass gold-Perkin Elmer particular identifier and an Elite-5MS (5% Diphenyl/ 95% Dimethy poly siloxane), 30×0.25mm×0.25µm df) slender segment (Ideal Analutical and Research Institution, Puducherry). The stove temperature was customized from 50 to 280ºC at the rate of 5ºC min-1 and stopped at this temperature for 36 min. The delta and interface temperatures were 200 and 280ºC, respectively. The transporter gas was heat a stream rate of 1.0 ml min-1 (consistent stream). The sample (2µl) was injected at a split of 10:1. Electron sway mass spectrometry was conveyed at 70eV. Particle source and fourfold temperature were kept up at 250 and 200ºC separately (Kumaravel *et al*., 2010).

Statistical analysis

Ovicidal and repellent activity data were analyzed using two-way ANOVA (factors: the tested fraction and the tested dose) followed by Tukey's HSD test. The significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

Fr-1 was showed EH (Eggs Hatchability)/ENH (Eggs No Hatchability)% ovicidal activity of 15.7/37.2% at 5ppm and 100% mortality at 10, 15, 20, 25, 30ppm, against *An.*

stephensi. Moreover, Fr-2 was focused EH/ENH% values of 11.4/54.4%, 23.7/5.2% at 5, 10ppm and 100% mortality at 15, 20, 25, 30ppm. Fr-3 was noticed EH/ENH% values of 9.5/62%, 17.2/31.2%, 24.8/0.8% at 5, 10, 15ppm and 100% mortality at 20, 25, 30ppm. Fr-4 were screened EH/ENH% values of 7.3/70.8%, 13.2/47.2%, 18.4/26.4%, 23.5/6% at 5, 10, 15, 20ppm and 100% mortality at 25, 30ppm. Fr-5 were noticed EH/ENH% values of 5.2/79.2%, 9.4/62.4%, 13.7/45.2%, 17.5/30%, 22.3/10.8% at 5, 10, 15, 20, 25ppm and 100% mortality at 30ppm. Fr-6 was focused EH/ENH% values of 3.8/84.8%, 7.2/71.2%, 10.8/56.8%, 14.2/43.2%, 17.6/29.6%, 21.7/13.2% at 5, 10, 15, 20, 25 and 30ppm, respectively (Table 1).

The repellent activity of Fr-1 was the highest, showing 100% protection up to 240 min at 0.75 mg/cm^2. Followed by, Fr-2, Fr-3, Fr-4, Fr-5 and Fr-6 were noticed 99.2%, 98.4%, 96.8%, 86.3% and 83.7 % protection up to 240 min at 0.75 mg/cm^{2}against dengue vector, *An. stephensi*(Table 2).The MCCs of *A. adenphora*-MLE the retention indices and the percentage of the individual components (Table 3).*A. adenophora*-MLE were in a GC Clarus 500 Perkin Elmer apparatus and were analyzed by GC-MS. A total of 21 compounds were detected representing 100%. The MPCs in MLE are 5-isopropyl-2-methylphenol (32.32%) (Fig. 1a, b), n-Hexadecanoic acid (11.32%) ($C_{16}H_{32}O_2$), Hancolupenone (7.81%) ($C_{30}H_{48}O$), Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+) - (6.33%) ($C_{31}H_{48}O_3$), 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-o1 (5.92%) ($C_{20}H_{40}O$) and á-Amyrin (5.69%) ($C_{30}H_{50}O$). Further, the six compounds were checked for their bio-efficacy against the selected mosquito species.

In results showed that, *A. adenophora*-MLEFr has significant ovicidal and repellent activity against *An. stephensi*. The consequence of present study are comparable with earlier reports to study the 100% mortality (ovicidal activity) was exerted by methanol fraction 4 tested at 40 ppm, and repellent activity at 2.5 mg/cm^2 was tests for at least 320 min against *An. stephensi*. Totally MCCs 9 were identified in the *Coleus aromaticus*-MLE (Baranitharan *et al*., 2017).The concentrations of 1.0, 2.5 and 5.0 mg/cm2 of the *Citrullus vulgaris*-benzene, petroleum ether, ethyl acetate and methanol extract tested gave complete protection ranging from 119.17 to 387.83 min against *An. stephensi* (Mullai *et al*., 2008).

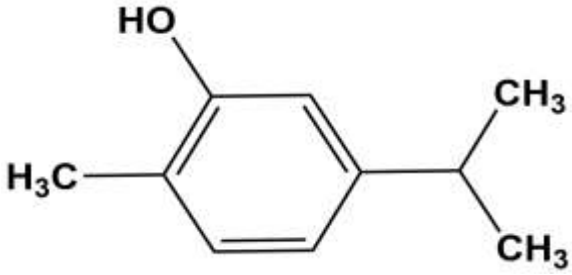

Figure 1a. Shows the 2D structure of 5-isopropyl-2-methylphenolcompound

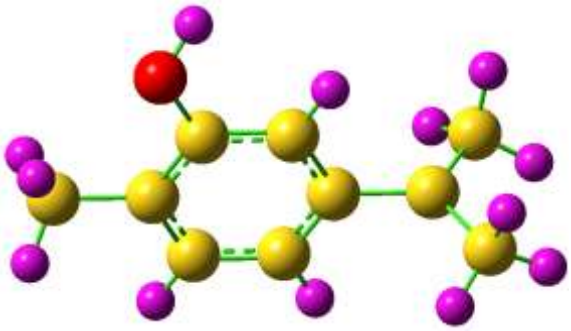

Figure 1b. Shows the 3D structure of 5-isopropyl-2-methylphenol compound.

Table 1.Ovicidal activity of *A. adenophora*-MLEFr tested against eggs (0-6h old) of *An.stephensi*.

Fractions	Percentage of egg hatch ability, concentration (ppm), EH/ENH % (48 hrs)						
	Control	10 ppm	20 ppm	30 ppm	40 ppm	50 ppm	60 ppm
Fr-1	25/0	15.7/37.2%	0/ENH	0/ENH	0/ENH	0/ENH	0/ENH
Fr-2	25/0	11.4/54.4%	23.7/5.2%	0/ENH	0/ENH	0/ENH	0/ENH
Fr-3	25/0	9.5/62%	17.2/31.2%	24.8/0.8%	0/ENH	0/ENH	0/ENH
Fr-4	25/0	7.3/70.8%	13.2/47.2%	18.4/26.4%	23.5/6%	0/ENH	0/ENH
Fr-5	25/0	5.2/79.2%	9.4/62.4%	13.7/45.2%	17.5/30%	22.3/10.8%	0/ENH
Fr-6	25/0	3.8/84.8%	7.2/71.2%	10.8/56.8%	14.2/43.2%	17.6/29.6%	21.7/13.2%

EH- Eggs hatchability, ENH %- Eggs no hatchability, *Fraction with strongest ovicidal effect.

Table 2. Repellent activity of *A. adenophora*-MLE Frtested against *An.stephensi* vector mosquito.

Fractions	Concentration (mg/cm^2)	% of repellency Time post application of repellent (min)					
		40	80	120	160	200	240
Fr-1	0.25	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	97.6±0.0
	0.50	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	99.4±0.0
	0.75	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0
Fr-2	0.25	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	95.8±0.0
	0.50	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	97.4±0.0
	0.75	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	99.2±0.0
Fr-3	0.25	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	93.6±2.4
	0.50	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	96.2±2.2
	0.75	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	98.4±2.8
Fr-4	0.25	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91.6±2.7
	0.50	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	94.4±2.9
	0.75	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	96.8±2.5
Fr-5	0.25	100±0.0	100±0.0	100±0.0	100±0.0	86.6±2.2	83.3±2.6
	0.50	100±0.0	100±0.0	100±0.0	100±0.0	92.9±1.6	85.5±2.2
	0.75	100±0.0	100±0.0	100±0.0	100±0.0	94.2±1.4	86.3±2.9
Fr-6	0.25	100±0.0	100±0.0	100±0.0	100±0.0	82.8±2.6	78.8±2.2
	0.50	100±0.0	100±0.0	100±0.0	100±0.0	88.4±2.8	81.4±2.4
	0.75	100±0.0	100±0.0	100±0.0	100±0.0	91.2±2.7	83.7±2.5

Each value mean ± Standard Deviation represents mean of six values. *Fraction with strongest repellent effect.

Table 3. Components identified from *A. adenophora*-MLE by GC-MS (Code No. 365)**.**

MF	Compounds	RT (min)*	PA (%)	MW	RI
$C_{10}H_{14}O$	5-isopropyl-2-methylphenol	4.14	32.32	150	*RI-MS*
$C_{15}H_{24}$	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene,[1R-(1R*,4Z,9S*)]-	5.92	1.34	204	*RI-MS*
$C_{15}H_{24}$	Trans-á-Bergamotene	6.01	0.43	204	*RI-MS*
$C_{15}H_{24}O$	Caryophyllene oxide	8.01	1.16	220	*RI-MS*
$C_{15}H_{20}O$	Ar-tumerone	9.11	3.81	216	*RI-MS*
$C_{20}H_{40}O$	3,7,11,15-Tetramethyl-2-hexadecen-1-o1	10.48	5.92	296	*RI-MS*
$C_{16}H_{32}O_2$	n-Hexadecanoic acid	12.20	11.32	256	*RI-MS*
$C_{20}H_{40}O$	Phytol	13.51	4.92	296	*RI-MS*
$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-	14.17	2.06	280	*RI-MS*
$C_{15}H_{28}O_2$	Z-8-Methyl-9-tetradecanoic acid	16.66	0.58	240	*RI-MS*
$C_{16}H_{32}O_3$	Methoxyacetic acid, 4-tridecyl ester	18.31	0.53	272	*RI-MS*
$C_{30}H_{50}$	Squalene	22.54	3.86	410	*RI-MS*
$C_{27}H_{46}O_2$	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-	24.55	0.48	402	*RI-MS*
$C_{28}H_{48}O_2$	Ç-Tocopherol	25.83	2.48	416	*RI-MS*
$C_{29}H_{50}O_2$	Vitamin E	26.76	1.67	430	*RI-MS*
$C_{28}H_{48}O_2$	Cholestan-3-ol, 2-methylene-, (3á,5á)-	27.98	1.07	400	*RI-MS*
$C_{29}H_{48}O_2$	Cholesta-22,24-dien-5-ol, 4,4-dimethyl-	28.35	2.64	412	*RI-MS*
$C_{30}H_{48}O$	Hancolupenone	29.26	7.81	424	*RI-MS*
$C_{31}H_{48}O_3$	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	30.07	6.33	468	*RI-MS*
$C_{30}H_{50}O$	á-Amyrin	30.62	5.69	426	*RI-MS*
$C_{27}H_{44}O$	Cholest-4-en-3-one	31.68	4.21	384	*RI-MS*

MF= Molecular formula, *RT= Retention time (min), PA= Peak area, MW = molecular weight.

The MCCs of 24 compounds was identified from *Melissa officinalis* compounds noticing to 98.73%. ovicidal activity of Citronellal compound exerted 45, 60, 75 and 90 mg/L, and repellent activity was contained to be mainly effective and the maximum activity was observed at 0.75 and 1.50 mg/cm^2 concentrations giving 100% protection up to 210 min against *An. stephensi* (Baranitharan *et al*., 2016). Highest ovicidal activity of *Cereus hildmannianus* petroleum ether extract on mosquito eggs with 52.8% EMR at 1000 mg/L at 96 h (Kamakshi *et al*., 2015). Repellent activity of *P. arvensis*-MLE had strong activity against *An. stephensi* as it provided 100% protection at 280 min (Deepa *et al*., 2014). The compound composition in each *Punicagranatum*-MLE was first identified using a GC-MS analysis and important was phenol, 2-methyl-5-(1-methylethyl). Due to the resistance developed by the mosquito against chemical pesticides, scientists need to identify new ovicides and larvicides compounds from the natural products (Benelli, 2016). Repellent activity of *Acalyphaalnifolia*-hexane, benzene, ethyl acetate, acetone and methanol extract was observed at 1.0, 3.0 and 5.0 mg/cm^2 against *Cx. quinquefasciatus* (Kovendan *et al*., 2013).

GC-MS analysis of *Punicagranatum*-ELE was identified seven MCCs, and the important Methyl 4-piperidineacetate. Further, repellent activity to be best and therefore the most activity was ascertained at 3.5 mg/cm^2 concentration provided 100% protection up to 240 min against *An. stephensi* (Baranitharan *et al*., 2019). Synthetic elements 15 compounds were recognized from *Pogoste moncablin* components representing to 98.96%, and repellent activity of patchouli alcohol compound was observed to be better for activity than 2 mg/cm^2 fixation giving 100% security up to 280 min against *An. stephensi*. Liquid chromatography analysis of the chloroform extract provided a hesitant identification of 13 components, and Bis-(3-oxaundecyl) tetrasulfide was known as important compound in A7 fraction (Ke-Xin *et al*., 2015). *Citrus limetta*-MPCs presence of 6 compounds, Corynan-17-01, 18, 19-didehydro-10-methoxy-, acelate (ester) was found as MPC (39.01%) (Baranitharan *et al*., 2020).

CONCLUSION

The finding of the present investigation, 5-isopropyl-2-methylphenol compound of *A. adenophora*-MLE was attempted. Further, we demonstrated the possible application of *A. adenophora*-MLE in medical field as it shows anti-mosquito repellent against *An. stephensi*. The data represented in our study contributes to a novel and unexplored area of *A. adenophora* as an alternative medicine for future.

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