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Antimalarial Property of Tetra Combination (TC) Of Biomaterial with Special Reference to *Spilanthes Acmella*

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Abstract--Malaria is the most threatened disease in almost all the Asian countries and every year about 250 million people suffering from Malaria (Plasmodium falciparum) and kills from this disease; the basic cause of the disease is female anaphelese mosquito arguably the most dangerous insect on our planet. Various mosquito repellents available in market have so many side effects on human health i.e. skin irritation, eye irritation, breathing problem (Asthma, Wheezing etc.).The active ingredient of these repellents is DEET (N, N-diethyl-meta-toluamide and it is a derivative of toluene), it is harmful pesticide and can damage to the brain cells, hence the study and development of alternate antimalarial drug is immense important. During the study we have screened some selected biomaterials derived from MAP's for the study. During the study Spilanthes sp. appeared as a potential antimalarial alternate biomaterial as well as most effective drug. Spilanthes sp. is also used as traditional herbal medicine in Africa and India to treat malaria. The flower heads contain up to 1.25% of spilanthol (N-isobutyl-4, 6-decadienamide), an antiseptic alkaloid. Spilanthol, a major constituent of ethanol extract of flower heads of Spilanthes acmella Murr. It shows potent ovicidal, larvicidal and pupicidal activity. Other test plants like extract of Azadirecta indica, Oil of Cymbopogon citratus (Lemon grass), and extract of Andrographis paniculeta (Kalmegh) also show remarkable insecticidal properties. Different doses have been prepared from these plant extracts in different combination and tested on different samples collected from different areas, where mosquitoes are in high population and infectious in nature. As a final test result we found potential mosquito repellent activity in spilanthol, citronella oil and kalmegh. We found that 7.5 μl concentrations causes 100% motility of eggs, larvae and pupae of Anopheles, Culex and Aedes mosquito.Finally our TC drugs show 100% inhibition of development of larva and also effectively able to killing the mosquito on the concentration of spilanthol (5.3 μl), oil of lemon grass (0.7 μl), Azadirachtin (1.5 μl) and kalmegh. And (1.5 μl) as a tetra combination of the drug .On the other hand, we also found that TC drug effectively reduces the population and larva of insects (Aedes).TC Drug have no harmful toxic effect even when we use it in combination and in the recommended concentration. It is natural and non carcinogenic and degradable. Other advantage of the drug is its more effective, natural and very cheap than other available drugs for controlling the population and larva of insects (Aedes). Hence, we recommended use of TC Drug in rural (low income) areas for control of malaria. This is first report for using as a TC drug for controlling of insects (Aedes).

Keywords-Antimalarial Property , Malaria, Plasmodium falciparum, DEET is N, N-diethyl-meta-toluamide and it is a derivative of toluene, TC =Tetra Combination, Biomaterial Spilanthes acmella, Azadirecta indica, Andrographis paniculeta, Cymbopogon citrates, Ovicidal, Larvicidal and Pupicidal activity, Oil and Extracts

I. INTRODUCTION

According to Spelman (2009), many Countries, WHO, States & Central Govt. as well as NGOs spend about $323 million every year to control the malaria parasite and suppression of mosquito population. In practice, different pesticides and chemicals are used for control of mosquito and their larval development, but day by day mosquitoes develop resistant against these chemicals and pesticides which are used for population control. On the other hand, these chemicals are the major problem to the human health and cause various side effects to the human, animals and insects also. It is not only harmful but it also causes mutation and cancer, lung infection in respiratory tract etc. Due to such economics, lack of healthcare facilities, less awareness and literature analysis shows excellent potential and possibilities to developed new alternative drugs from natural biomaterials to control the malaria in the rural areas. Although in recent year, use of eco-friendly and easily biodegradable plant products having natural insecticidal activity is increased. Spilanthes acmella Murr. is one such plant from the traditional time that is reported to be useful in the treatment of

malaria. The extensively used compound spilanthol against mosquito was obtained from the flower extract of *Spilanthes acmella*. Although the extract is effective, photo degenerative and therefore, low quantity is required to control the insect population. Other medicinal plants i.e. Azadirecta indica, Cymbopogon citratus (Lemon grass), and *Andrographis paniculeta (*Kalmegh*)* also have mosquito repellent property and can control malarial population. In the Azadirecta indica (Neem), Azadirachtin is the main component responsible for the toxic effects in insects. It affects insect reproduction directly and "secondary" anti-federacy. Lemon grass oil citronella is considered as bio-pesticide and insect repellent with non toxic mode of action, if treated with proper concentration. More than 0.7mg/day of citronella oil is harmful for animal and human beings. Kalmegh extract is also used in mosquito repellents. This TC drug appears as a cost effective, non carcinogenic and biodegradable. Hence, these natural compounds are very useful to control the mosquito's growth in modern era. Therefore, the study and drug development from medicinal and aromatic plants have immense importance and futuristic potential. Therefore, we have undertaken ours study. The extracts of spilanthol, citronella oil, Azadirachtin oil and kalmegh are used in different concentration and combination against the tested pathogen (mosquito population). The present investigation was undertaken to find out "Antimalarial Property of TC Drugs with special reference to Spilanthes acmella".

II. MATERIAL AND METHODS

A. *Collection Of Plant Material And Extraction*

The different parts of test materials i.e. S. acmella, Neem, Lemon grass and Kalmegh were collected from MAP's Garden CSIR-AMPRI Bhopal and identified by taxonomist. The different plant's parts such as leaf, roots and flowers are collected and dried in shade. The Powder of these dried plants was prepared by electric blender. This powder is used for extraction by using ethanol as a solvent through Soxhlet apparatus. Same methodology is used for extraction of each plant. Then the extracts were filter by whattman no. 1 filter paper. The residues are re-extracted in Rotary evaporator to remove insoluble impurities from the residues and obtain pure extracts. The ethanol is evaporated or removed from the extracts and collected in another flask. The crude extract is filtered. From the extracts of each plant, different doses are prepared in different concentration for the formation of TC drug in different concentration as mentioned below in Table 1.

TABLE-1:
Preparation of Different doses of TC drug according to different ratio/concentration of each plant extracts.

Preparation of Tetra Combination (TC) Drugs					
Doses Per 10 μl	Concentration Of *Spilanthes acmella* (Akarkara)	Concentration of *Azadirecta indica* (Neem)	Concentration Of *Cymbopogon citrates* (Lemon grass)	Concentration of *Andrographis paniculeta (*Kalmegh*)*	Final TC Drugs in different ratio
1st	7 μl	1.4 μl	0.6 μl	1 μl	7:1.4:0.6:1
2nd	6 μl	1.5 μl	0.5 μl	2 μl	6:1.5:0.5:2
3rd	5.5 μl	0.6 μl	0.4 μl	3.5 μl	5.5:0.6:0.4:3.5
4th	5 μl	3.5 μl	0.3 μl	1.2 μl	5:3.5:0.3:1.2
5th	4.5 μl	1.3 μl	0.2 μl	4 μl	4.5:1.3:0.2:4

The table shows combinations of different plant extracts in different concentrations.

The samples of mosquito's larvae collected from different areas (Narayan Nagar, Gautam Nagar, Bhopal Bus Stand, Anna Nagar, Bharat Nagar area Bhopal Madhya Pradesh, India) for testing the prepared TC drug in different ratios shown in Table 3.

III. EXPERIMENT

Different doses of TC drugs in different concentration are tested on sample of mosquito larvae collected from different areas and observe the results under bull lens or microscope and compared the results showing that, which concentration, which dose of the TC

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drug is more effective and gave early & better results.

Table no.2 Effects of TC drug on different larva sample collected from different areas.

Doses	Concentrations of doses	Area 1 (Narayan Nagar)	Area 2 (Gautam Nagar)	Area 3 (Bhopal Bus Stand)	Area 4 (Bharat Nagar)	Area 5 (Anna Nagar)	Result
Dose[1st]	1 μl	-	-	-	-	-	no effectiveness
	2 μl	-	-	-	-	-	no effectiveness
	3 μl	EH	PFI	LD	PFI	LD and PFI	effective
	10 μl	-	PFI	PFI	LD	LD and PFI	effective
	15 μl	EH	PFI	LD	PFI	LD and PFI	effective
Dose[2nd]	1 μl	EH	PFI	EH and PFI	PFI	LD and PFI	effective
	3 μl	EH	EH and PFI	PFI	PFI	PFI	effective
	5 μl	EH	PFI	PFI	PFI	PFI	very effective
	10 μl	EH and PFI	EH,PFI,LD	EH and PFI	EH,PFI,LD	PFI	highly effective
	15 μl	PFI	PFI	PFI	EH,PFI,LD	PFI	effective
Dose[3rd]	1 μl	-	-	-	-	-	less effective
	3 μl	-	-	-	-	-	less effective
	5 μl	-	-	-	EH	EH	less effective
	10 μl	EH	EH	PFI	PFI	EH,PFI, LD	effective
	15 μl	EH and PFI	EH and PFI	PFI	PFI	PFI	effective
Dose[4th]	1 μl	--	-	-	-	-	less effective
	3 μl	-	-	-	-	-	less effective
	5 μl	--	--	-	-	EH	less effective
	10 μl	EH	EH	EH	EH and LD	PFI	less effective
	15 μl	EH	EH	EH	EH and LD	PFI	effective
Dose[5th]	1 μl	-	-		-	--	less effective
	3 μl	-	-	-	-	EH	less effective
	5 μl	-		-	-	EH	less effective
	10 μl	EH and PFI	PFI	EH and PFI	LD	LD	effective
	15 μl	EH and PFI	EH and PFI	PFI	LD	LD	effective

EH= Egg hatched, PFI= Pupa formation inhibited, LD= Larvae died,

This TC drug also tested on sewage where mosquitoes are present in high population (slum area) and analysed the effects of drug.

Table no.: 3 In-vivo effects of TC drug on sewage in slum area/colony

Selected concentration of TC drug for in vivo study	In-vivo effects of TC				
	Effect of drug : Day 1	Effect of drug: Day 2	Effect of drug: Day 3	Effect of drug: Day 4	Effect of drug: Day 5
Control (without TC drug)	all larva, egg and pupa live	all larva, egg and pupa live	all larva, egg and pupa live	all larva, egg and pupa live	all larva, egg and pupa live
2nd dose 10 μ l concentration (10x10 feet area approximate)	egg hatched	pupa died	larva died	all larva, egg and pupa died	all larva, egg and pupa died

IV. RESULT & DISCUSSION

Preparation of different concentrations (in different ratio) of Spilanthes acmella, Neem, Lemon grass and Kalmegh are presented in table 1. We prepared five doses in 10 μ l weight of TF drug with combination and mixing of extracts of Spilanthes acmella, Neem, Lemon grass and Kalmegh in the following given different ratio i.e. 7:1.4:0.6:1, 6:1.5:0.5:2, 5.5:0.6:0.4:3.5, 5:3.5:0.3:1.2 and 4.5:1.3:0.2:4. Finally we prepared five doses of TF drug per 10 μ l concentration for further experimental propose.The result showing the in-vitro Inhibitory effect of TC drug and recommendation for control of mosquito population (larvicidal activity) are presented in table 2. It is evident from these results that the 10 and 15 μ l concentrations of TF drugs showed remarkable inhibitory effect against the development of egg, larvae and pupae formation of mosquito (Ades and anopheles). As result shown in table 2, the inhibition of larvae and pupae formation of mosquito (Ades and anopheles) development was 100 % at 10 μ l concentration of TF drug. According to table -2, 2nd dose's 10 μ l concentration is effective in egg hatching and 3 and 5 μ l concentration was effectively kill the pupa and inhibit larva growth and its 10 μ l is highly effective to kill all the larva, egg and pupa of mosquitoes. In-vivo studies and effectiveness of TC drug on sewage in slum area/colony in respect of five days are presented in table 3. Day 2nd we observed slightly decrease in mosquito larvae population and after 3rd and 4th day mosquito larvae population is successfully died by TC drug. And 4th day no larvae is live in a test sample.Concentration of the drug proved deleterious to the various larval stages of mosquitoes. 100% mortality of larvae of Culex and Anopheles was recorded with in 24 hrs. at 10 μ l concentration respectively. Finally 10 μ l concentrations is appeared as a most effective concentration and we found that no larvae of any test species of mosquito could survive. Result also showing that at 1 and 2 μ l concentration of drug 12 to 48% eggs hatched normally. But in 4-5 μ l concentration of the drug effectively killed the 50% pupae within 3 to 5 hrs. of exposure. It is observed that as the concentration and exposure time increase progressively mortality increases in severity. This is a new observation with marked larvicidal, pupicidal and ovicidal activity of TC Biomaterial/Drug and had never been recorded and reported previous workers.

V. RECOMMENDATION

Finally we recommended our TC Drug for foliar spray/ mixing in sewage water in slum as well as rural areas for large scale trials.

VI. ACKNOWLEDGEMENT

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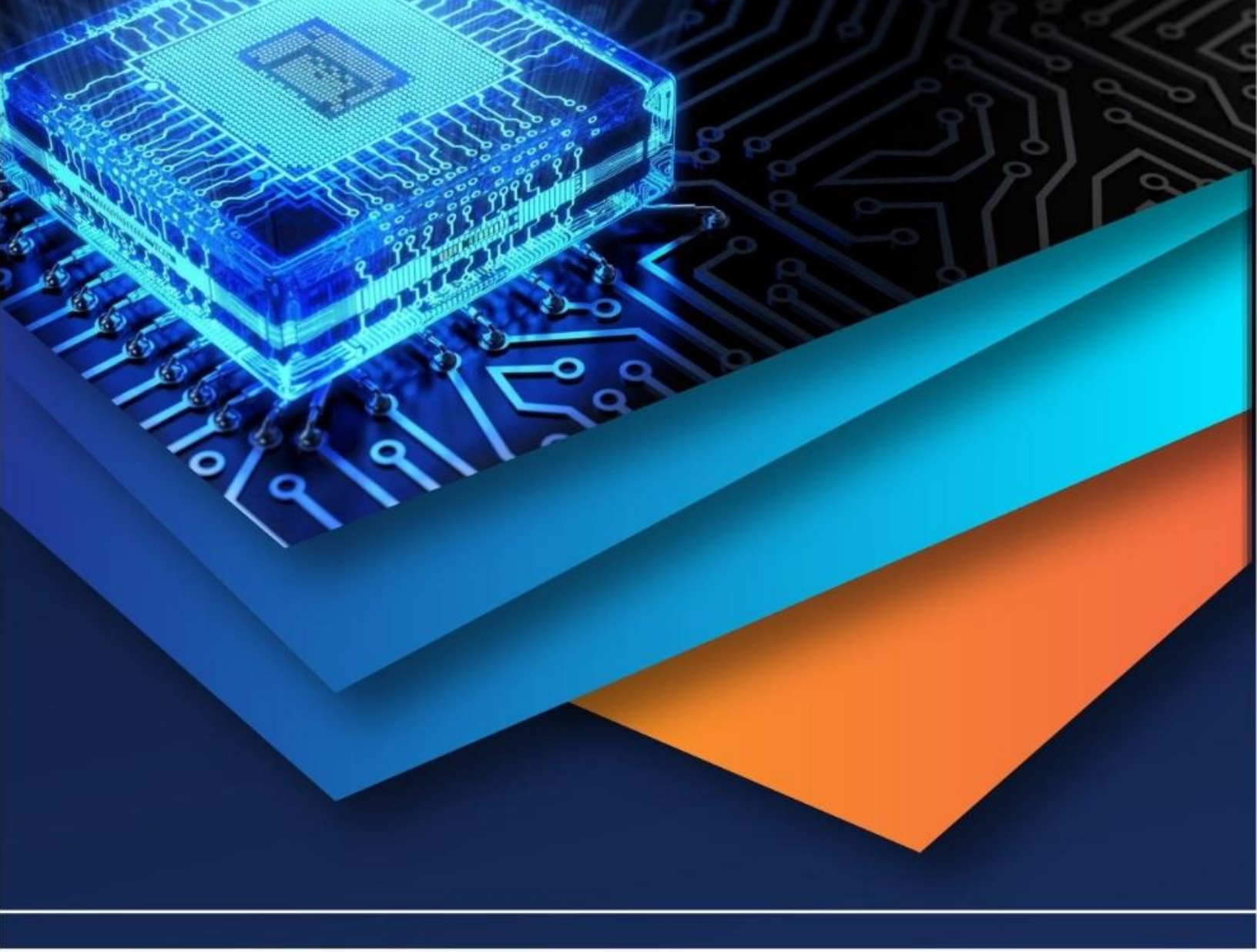

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