

The combined medicinal plants extracts as alternative for control external parasites of cattle in Vellore district of Tamil Nadu

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Abstract : An aqueous extract of medicinal herbs was prepared from leaves of *Azadirachta indica*, *Acalipa indica*, *Leucas aspera*, *Ocimum sanctum*, *Thespesia populea*, *Datura metel*, *Cynodon dactylon*, *Phyllanthus niruri*, *Morinda pubescens*, *Aegle marmelos*, *Cordia oblique*, *Polygala chinensis*, *Abutilon indicum*, *Sesbania grandiflora*, *Jatropha curcas*, *Pongamia pinnata*, *Kalanchoe pinnata*, *Anona squamosa*, *Vitex negundo*, *Lantana camara*, *Acorus calamus* and *Curcuma domestica* and *Nicotina tabacum* to control the *Hippobosca maculata*, the external parasites of cattle. Trials on the aqueous medicinal plants extract with dilution of 25 per cent, 50 per cent and 100 per cent were conducted for 60 cattle on their external skin surface to assess the insecticidal efficacy on the *Hippobosca maculata*. The chemical insecticide Cypermethrin was tested as a control with a dilution of 1:1000. The *in vivo* and *in vitro* trials using both aqueous medicinal plants extract and Cypermethrin were carried out to compare the insecticidal and repellent effects on external parasites in cattle. The test results of 50 per cent medicinal plants extract shows that the insecticidal effects on *Hippobosca maculata*, the external parasites in cattle are same as that of recommended dose of chemical insecticide. By this study it was concluded that the aqueous extract of medicinal plants can be effectively used as alternative to chemical insecticide for treating the control of *Hippobosca maculata* in cattle for insecticidal and repellent effects and improve the behaviour of cattle towards docile so as to quicker the milking operation in cross bred cattle.

Key words : Extract of medicinal herbs, Medicinal plants extract, Insecticidal, Repellent effects on external parasites in cattle, Milking behaviour, Milking temperament score, *Hippobosca maculata*

How to cite this paper : Ramasamy, M., Pandian, M. and Davidson, S. Joshua (2016). The combined medicinal plants extracts as alternative for control external parasites of cattle in Vellore district of Tamil Nadu. *Vet. Sci. Res. J.*, 7(1) : 16-20.

Paper History : Received : 01.02.2016; Revised : 19.02.2016; Accepted : 10.03.2016

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INTRODUCTION

Ethno-veterinary medicine, especially the traditional herbal remedies are now considered as a potential candidate for developing eco-friendly, safe and cost-effective drugs for animal uses. However, there is a need to

address several challenges to promote the ethno-veterinary medicine as an effective, farmers' friendly and economically viable alternative method of animal treatment in the changing socio-economic and agro-geo climatic conditions. The indiscriminate anthelmintic usage led to chemical residues in milk, meat, eggs, environment, and also the emergence of resistant strains of parasites, causing serious concerns. The search is for alternative methods of control with prime emphasis on safety, sustainability and minimal chemical interventions. There has been dramatic resurgence of interest toward plant based anthelmintics. Phytomedicine is widely used as part of traditional healing and ethno-veterinary practices. Approximately 80 per cent of the world's population depends on traditional medicine for meeting primary health-care needs of humans as well as livestock (Danoe and Bogh, 1999). Phytotherapeutic drugs, especially plant anthelmintics are safe, non-toxic, biodegradable and do not leave any residue in animal products, hence, plants with anthelmintic properties were included in the pharmacopoeias. WHO has also recommended to all member countries to actively promote native medicines and also to initiate steps to conserve and cultivate medicinal plants. The ectoparasitic insects and acarines are widely prevalent throughout the world, particularly in tropical and subtropical countries, which have witnessed a resurgence of epidemic vector-borne infections in the recent past (Atkinson, 2010). These can translate to disease, disability and death in humans and enormous production losses in livestock. To combat this menace, mankind had traditionally relied on various naturally occurring products. The active ingredients of many plants and herbs possess repellent, insecticidal, growth-inhibiting and anti-moulting activities. The criteria for anti-parasitic efficacy *in vitro* had ranged from repellent and cidal activity against adults and/or developmental stages to reduction in reproductive index through reduced egg mass and inhibition of hatchability. *In vivo* studies had relied on the reduced counts on skin surface/scrapings as the measure of efficacy. Adult emergence inhibition (EI) and adulticidal activities of several medicinal plant extracts against *Anopheles stephensi* were studied (Abduz Zahir *et al.* 2010). Ghosh *et al.* (2012) listed many such plants which possess target-specific activities, predominantly larvicidal against different species of vector mosquitoes. *In vitro* effect of 10 per cent aqueous solution of *Annona squamosa* (custard apple) seed preparation on adult houseflies *Musca domestica* (Desai, 1997) was found toxic. Sinthusiri and Soonwera (2013) reported good susceptibility (knock down effect) of housefly *M. domestica* to essential oils of *Cymbopogon citratus* (Lemon grass), *Mentha piperita* (peppermint) and *Lavendula augustifolia* (lavender) at 10 per cent concentration. *In vitro* efficacy of extracts of dried leaf and flower of *Rhinacanthus nasutus* and *Cassia auriculata* in various solvents like acetone, chloroform, ethyl acetate, hexane and methanol (Kamaraj *et al.*, 2010) were tested *in vitro* against the haematophagous fly *Hippobosca maculata* and nymph of goat louse *Damalinia caprae*. Despite numerous plant extracts being reported as promising candidates for acaricidal and insecticidal activity, the feasibility of their usage on infested animals in the field conditions, is yet to be explored adequately (Chhabra *et al.*, 2014). So the present study was carried out to assess utility of locally available medicinal herbal extract and its effect on fly repellency and insecticidal property on *Hippobosca maculata* on body surface the crossbred cattle *in vivo* and in the laboratory by *in vitro* bioassay.

RESEARCH METHODOLOGY

Preparation of medicinal plants aqueous extract :

Green leaves of *Azadirachta indica*, *Acalipa indica*, *Leucas aspera*, *Ocimum sanctum*, *Thespesia populea*, *Datura metel*, *Cynodon dactylon*, *Phyllanthus niruri*, *Morinda pubescens*, *Aegle marmelos*, *Cordia oblique*, *Polygala chinensis*, *Abutilon indicum*, *Sesbania grandiflora*, *Jatropha curcas*, *Pongamia pinnata*, *Kalanchoe pinnata*, *Annona squamosa*, *Vitex negundo*, *Curcuma domestica*, *Lantana camara*, dried leaf of *Nicotina tabacum* and rhizome of *Acorus calamus* were taken each 50 g weight and were grinded with water to prepare aqueous portion in to 6 lit. This was boiled for 3 hours so as to get the volume reduced to 1/3 rd. Then this is allowed for 10 hours for reaction in water and then filtered. The filtrated portion of herbal aqueous extract was used for assess its killing effect on external parasites of cattle by using both *in vivo* and *in vitro* assays. The extract was diluted with water to prepare 25 per cent, 50 per cent concentrations and the 100 per cent concentration were used for both *in vivo* and *in vitro* studies.

***In vitro* bioassay on fly:**

The fly *Hippobosca maculate* with equal size were collected from cattle in the another field before starting the *in vivo* study on cattle. The flies are carried to laboratory and grouped into five, each group consisted of ten number of fly *Hippobosca maculate*. Group I (Distilled water), Group II flies were studied with plant extract with 25 per cent concentrate, Group III with 50 per cent concentrate, Group IV with 100 per cent concentrate and Group V with Cypermethrin as per standard concentration (1:1000).

For group II, one ml of 25 per cent extract was distributed evenly inside of a Petri dish padded with watman filter paper no.1 as done by sharma *et al.*, 2012 for the ticks, *viz.*, Ten number of *Hippobosca maculate* fly were kept inside the covered Petridis. Then the Petridis was slightly and slowly moved up and down so as the plant extract will moisture the fly so as to all the flies get wet. Similarly each one ml of distilled water, 50 per cent plant extract, 100 per cent plant extract and Cypermethrin as per standard concentration (1:1000) were taken into different petridihses and ten number of flies were put inside of each petridihes of respective groups. The number of dead *Hippobosca maculate* fly counted at 10 minutes intervals upto dead of all of the flies in each group. The experiments in each group was replicated 3 times and 10 number of adults *Hippobosca maculate* flies were used per replication (n = 3x10). The concentration showed best killing effect on *Hippobosca maculate* in the petridishes and on the cattle were analysed and compared.

***In vivo* bio assay on cattle :**

Sixty number of cross bred cattle were selected from farmer's field at Vellore district of Tamil Nadu during raining season. These were grouped to Group I (12 number of cows) to as control group, Group II (12 number of cows) for 25 per cent concentrate Group III (12 number of cows) for 50 per cent concentrate and Group IV (12 number of cows) as standard for Cypermethrin.

The animals in the Group I were sprayed on all over the body surface of with distilled water, the plant extract with 25 per cent concentration was sprayed throughout body of cattle of Group II and the plant extract with 50 per cent concentration was sprayed on Group III. Cypermethrin was mixed with water as dosage prescribed by the manufacturer (1:1000) and sprayed on animals of Group IV. The milking temperament score and total numbers of the of fly *Hippobosca maculate* were observed one day before spray and on day 7, 14, 21 post spray treatment. The data were analysed as per the standard procedure.

Milking behaviour of cattle :

The cows were observed for milky temperament score at the time of milking at morning hours from score 1 to 5 according to Tullah (1961) as modified by Mishra *et al.* (1975). One day before start of experiment and days 1, 7, 14 and 21 post spray treatment. The number of fly *Hippobosca maculate* were counted on the visible anatomical sites of body, ears, head, dewlap abdomen, anus-vulva and tail of all of the milch cows one day before spray of plant extract or on the day of measuring milking behaviours. The number of house fly, other flies and mosquitoes per square feet area were also obsrved.

RESULTS AND DISCUSSION

The mean mortality of *Hippobosca maculate* 10 minutes was 0, 7.3, 7.3 and 8.3 for 25 per cent, 50 per cent and 100 per cent concentration and for Cybemethrin, respectively. The mean mortality at 20 minutes interval as 2.3, 10, 8.3 for 25, 50, 100 per cent concentrate while for STD it was 9.6. So the 50 per cent concentration and STD drugs were took time to kill all the 10 fly (Table 1). The 25 per cent concentration took five hours, 100 per cent concentration took 4 hours while 50 per cent concentration took 20 minutes only for killing all the flies. This tier variation on mortality of *Hippobosca maculate* flies could be due to variation on absorption of extract by its different concentration. Since 50 per cent concentration showed 100 per cent lethal effect within 20 minutes, this concentration was used to spray on cattle.

The pattern of motility and mortality of *Hippobosca maculate* was recorded by observing the loss of both

motility and pedal reflex at an interval of 10 minutes. The 50 per cent diluted extract showed maximum cidal effect by inducing complete and irreversible inhibitory effect on both body motility and pedal reflexes at 20 minute post exposure. The 25 per cent diluted herbal extract showed incomplete and reversible inhibitory effect on body motility and pedal reflex upto 5 hours of post treatment. So the corrected number of mortality occurred in this.

Result of *in vivo* study of cattle :

In this study the herbal extract with 25 per cent concentration was sprayed on cattle so as to completely wet the external skin. Immediately after spray the *Hippobosca maculate* flies were gone away from external surface of cattle. This could be due to short time repellent effect of 25 per cent concentration of extract. The *Hippobosca maculate* flies were reemerged at day 14 post spray treatment. Spray of 50 per cent concentration on external surface of cattle has killed the *Hippobosca maculate* flies within 20 -30 minutes post spray. Animals were free from flies upto 21 days. Cypermethim as per manufactures concentration has killed all the *Hippobosca maculate* flies within 10 -20 minutes post spray. They did not remerge upto 21 days post spray. Spay of distilled water did not have killing effect on *Hippobosca maculate* flies and the flies were present all the days of the experiment. From this study it is concluded that both *invivo* and *in vitro* the 50 per cent concentration of the extract showed irreversible motility blocking and pedel reflex blocking effect and long time fly repellency effect on the *Hippobosca maculate* of cattle. The effect of 50 per cent concentration of the extract also showed similar to the effect of STD Cypermethim (Table 2).

One day before spray the milking temperament score of cattle was 0.5 in all the groups. The cattle in Group I showed the milking temperament score range from 0 to 1 on all the days of experiment. The cattle in Group II showed the docile milking temperament (score 0) range from day 1 to 7 post spray treatment thereafter they showed slightly restless milking temperaments from the score ranging from 0 to 1. The cattle in the Group III, IV and IV were showed milking temperament score 0 by behaving docile in nature due to absence of the biting of *Hippobosca maculata* flies on animal body at the time of milking.

Formulations containing *azadirachtin* fraction of neem, viz., Neemrich I 80 EC and Neemrich II 20 EC (CSIR, Pune) at 0.5–1.0 per cent concentrations were 79.0 to 85.7 per cent effective in control of natural infestations of lice and fleas in sheep and goats (Muraleedharan *et al.*, 2003). A similar neem-based commercial formulation ‘Nimbitor’ EC containing tetranotis terpinoid as active component, at 2 - 8 per cent concentration (Maske *et al.*, 2000) resulted

Table 1 : Number of mortality of fly *Hippobosca maculate* at different interval of time during the *in vitro* bioassay

Experimental group		Mean values of numbers of mortality of <i>Hippobosca maculate</i> fly at different interval of time during the <i>in vitro</i> bioassay									
Group	Concentration used	10 Minutes	20 Minutes	30 Minutes	40 Minutes	50 Minutes	1 Hour	2 Hour	3 Hour	4 Hour	5 Hour
1	Distilled water	0	0	0	0	0	0	0	0	0	0
2	25% extract	0	2.3	2.3	3	3.6	4	6	7	9	10
3	50% extract	7.3	10								
4	100% extract	7.3	8.3	8.3	8.3	8.3	8.3	9.3	9.6	10	
5	Cybermethrin(1;1000)	8.3	9.6	10							

Table 2 : Mean milking temperament score of cattle of different experimental groups on different days post spray treatment with aqueous extract of medicinal plants

Group	Concentration used	Day before spray	Mean values of milking temperament score on different days post spray			
		Day 0	1	7	14	21
1	Distilled water	.5	.5	.5	.5	.5
2	25% extract	.5	0	0	.5	.5
3	50% extract	.5	0	0	0	0
4	100% extract	.5	0	0	0	0
5	Cybermethrin(1;1000)	.5	0	0	0	0

in 100 per cent killing of lice *Linognathus vituli* and fly *Hippobosca maculata* of cattle *in vitro* at 4 per cent concentration in 10–20 min. Ponnudurai *et al.* (2007) recorded 100 per cent reduction of lice in birds that were treated with herbal formulation containing ingredients of *A. indica*, *Cedrus deodara*, *Brassica campestris* and *Ocimum sanctum*. All the lice species died within 30 min of spraying the drug and p.t. lice count fell to zero. Additionally, fly repellent activity against house flies with 90–96 per cent fly reduction lasted for 4 hours.

Ethanol extracts of leaf, bark and seed of neem, of leaf and seed of peach (*Prunus persica*), bark of mango (*Mangifera indica*) and leaf of guava (*Tsidium guajava*) were evaluated *in vitro* against *B. microplus* (Srivastava *et al.*, 2008). Of these, 8 per cent extract from *A. indica* seed showed high level of efficacy (80%) after 5th of treatment. The direct effect of reference standard cypermethrin was 92.44 per cent. Besides the immediate effect on adult ticks, the egg laying property of surviving ticks was significantly reduced. Also significant reduction in the reproductive index of ticks fed on animals treated with *A. indica* seed extract was noted.

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