



FORMULATION AND EVALUATION OF *CARICA PAPAYA* AND *NICOTIANA TABACUM* CONTAINING SHAMPOO FOR ANTI-TICKS ACTIVITY

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Abstract

The aim of this research was to formulate and evaluate shampoo containing *Carica Papaya* and *Nicotiana Tabacum* as a chief herbal drug for the acaricidal activity. *Carica Papaya* and *Nicotiana Tabacum* known for their anti-inflammatory, antioxidant, diuretic, antibacterial, vermifuge, hypoglycemic, antifungal activity, antihelmenthic and immunomodulatory activity, respectively. The shampoo formulations were formulated using extracts of *Carica Papaya* and *Nicotiana Tabacum* leaf. **The formulated shampoo was evaluated for visual appearance, ph, physicochemical characterization, solid content, surface tension, foam volume, foam stability and Anti-Ticks Activity Of Shampoo.** Ethanolic Extract of *Carica Papaya* and *Nicotiana Tabacum* leaf were taken for in acaricidal activity against ticks. Different concentrations (2%, 4%, 6%) of plants extracts were used for the anti-ticks activity and Shampoo concentration (2.5%, 5%, and 10%) were used for activity. shampoo was compared against standard Amitraz. Normal water was used for the control treatment. *Carica Papaya* and *Nicotiana Tabacum* leaf Shampoo showed 100% mortality rate within 04 minutes while standard takes 15 minutes. The results obtained from the study indicate the strong anti-ticks activity, supporting folk use of the plants when compared with the standard. Both the extracts of *Carica Papaya* and *Nicotiana Tabacum* leaf Shampoo were found to show a potent Acaricidal effect better than standard amitraz.

Keywords- Anti-Ticks activity, Amitraz, *Carica Papaya*, *Nicotiana Tabacum*, Shampoo

Introduction

Parasitic diseases is a global problem and considered as a major obstacle in the health and product performance of animals. These may be due to endo-parasites that live inside the body, or ecto-parasites such as ticks, mites, flies, fleas, midges, etc., which attack the body surface.^[1] Ticks transmit economically important haemoprotozoan diseases which is a major threat to livestock population worldwide.^[2] Amongst ectoparasites, about 80% of the cattle population is affected by the ticks in the world. Ticks causes the deleterious effects on the health of animals which includes

tick bites, sucking of blood, tick worry, tick toxicosis, tick paralysis, transmission of diseases hence affects the health of animals and ultimately responsible for economic losses.^[3] also these may result in cattle skin damage, weight loss, abortion, and mortality, leading to substantial economic losses. Ticks are also responsible for indirect effects through the transmission of pathogens responsible for bacterial, viral, and protozoan diseases worldwide.^[4]

The main weapon for the control of ticks is the use of chemical acaricides. Acaricide usage is not sustainable in the long run because of the ticks becomes resistant and presence of residues in the milk and meat.^[5] Currently, various synthetic acaricides are used to control ticks. However, the continuous use of these acaricides has resulted in the selection of acaricide-resistant and multi-drug-resistant tick populations.^[6]

The issues mentioned above have motivated the search for alternative parasite control strategies that are potentially environmentally friendly with fewer negative consequences to the animal being treated. Principal among these alternatives are the plant-based treatment protocols as the healing effect of plants has been explored for thousands of years. Other proposals for the full development of medicinal plants as tick repellents/acaricides has been advocated as plants inherently have a number of protective mechanisms to combat predator and pathogen attacks.^[7]

Carica Papaya- *carica papaya* Linn. from the Caricaceae family, is *indigenous* to Central America and South of Mexico, and commonly grown in India has been used for its medicinal properties around the world . Different parts of papaya plant viz. fruit, bark, roots, seeds, peel, pulp, and leaf have many known therapeutic uses around the world. The papaya plant is a nutritionally abundant source of vitamins A, B and C and also a fair source of calcium and iron.^[8] Various pharmacological actions and medicinal uses of different parts of papaya are well reported. The plant shows antimicrobial, anthelmintic , anti-amoebic , antimalarial, antifungal, diuretic, hepatoprotective and immunomodulatory activities.^[9]

Nicotiana Tabacum- *Nicotiana tabacum* (family Solanaceae), commonly known as tobacco, is a world popular plant used for its narcotic properties. Dried leaves, stalks and the whole herb of tobacco are widely used as an anti-inflammatory, antirheumatic and anthelmintic agent.^[10] Tobacco (*Nicotiana* spp.) leaves, powder, extracts, or fumigants have been used for centuries to control agricultural pests or parasites of medical and veterinary importance. Studies reported that, from tobacco plants around thousands of metabolites have been chemically and biologically characterized and researches are continuing to explore more about its chemical compounds.^[11]

The current study demonstrated that *Carica Papaya* and *Nicotiana Tabacum* leaf shampoo had a strong acaricidal effect on ticks. The anti-ticks activity of ethanolic extract of *Carica Papaya*, *Nicotiana Tabacum* leaf and amitraz liquid were performed in laboratory condition. Amitraz liquid used in this study as a standard agent. Tobacco leaf and papaya leaf contain alkaloids which is possibly responsible for the acaricidal effect of the plant extract.

Using above natural plants extract to prepare herbal shampoo which shows anti parasitic activity. It protects cattle from nuisance of ticks, fleas and lice to ensure a healthy, shiny and luxurious appearance for cattle. It is enriched with ayurvedic ingredient and ectoparasitocidal in nature.

Materials and methods:

1. Collection of plant materials :

The fresh leaf of plants of *Carica Papaya* and *Nicotiana Tabacum* were collected from Modnimb region of Solapur district of Maharashtra state, India. The plant were identified and authenticated by, Department of Botany, KBP College Pandharpur, India.

2. Preparation of extract :

The collected leaf were washed under running tap water, dried under shade and coarse powdered in mechanical grinder. Then powders were passed through sieve no 16, and used for further study. The dried powder 100 gm was macerated successively with 250 ml of Ethanol for seven days with intermitted stirring and then it was filtered and concentrated to get thick slurry. This slurry was then vacuum evaporated to yield solid extract. The percentage yield of ethanolic extract was found to be *Carica Papaya* extract was 29.21%w/w and *Nicotiana Tabacum* extract was 33.94%w/w. The dried extracts were stored in a well-closed, air tight and light resistant borosil glass container.

3. Phytochemical screening of ethanolic extract^[12]

In order to determine the presence or absence of primary and secondary metabolites, phytochemical screening of plant extracts has been carried out using a standard procedure to verify the purity of herbal medicinal products. Phytochemical tests for Tannins, Flavonoids, Anthocyanins, Anthraquinones, Glycosides, Reducing sugar, Steroids, Saponins, Alkaloids, Proteins, Amino Acids, Terpenoids, Carbohydrates and Phenol were carried out adopting standard procedures.

4. Collection of ticks-

The ticks were collected from different Indian buffalo and cow located in different places of Modnimb region of Solapur district of Maharashtra state, India. The collected ticks were put in glass vials and labeled with date of collection and place. The mouth of glass vials with ticks were wrapped in cotton muslin cloth for oxygen supply and authenticated by, Department of zoology, KBP College Pandharpur, India.

5. Standard Drug-
Amitraz

6. Formulation Of Shampoo –

Formulation Of Normal Shampoo Using Following Procedure:

Sodium Lauryl sulphate is a surface-active agent used as a cleansing and foaming agent. Sodium citrate was added as viscosity modifier. Glycerine was added to prevent the-dryness of the formulation and a conditioning agent. HPMC was added as a thickening agent.

Weigh all the solid ingredients. Dissolve SLS in 75% of purified water. Then add Methyl Paraben, Propyl Paraben Sodium citrate and glycerine to the above-prepared solution. Disperse HPMC in the above solution with continuous stirring for 20 minutes at 60°C. Cool the preparation and last add the water up to required volume.

Formulation Table –

Table no.1 - Formulation Table Of Shampoo

Sr. No	Ingredients	Quantity Taken(10ml)	Use Of Ingredients
1	Sodium Lauryl Sulphate	1.0 g	Surfactant
2	Sodium Citrate	0.1 g	Viscosity Enhancer
3	Hydroxy Propyl Methyl Cellulose	0.3 g	Thickening Agent
4	Glycerin	0.5 g	Viscosity Enhancer
5	Methyl Paraben	0.18 g	Preservative
6	Propyl Paraben	0.02 g	Preservative
7	Perfume	q.s	Fragrance
8	Colour	q.s	colour
9	Purified Water	q.s	Vehicle

Formulation Of Anti-ticks Shampoo-

Once a prepare normal shampoo then preparation of 2.5%, 5%, and 10% Anti-ticks shampoo using *Carica Papaya* and *Nicotiana Tabacum* leaf extract added in normal shampoo.

Evaluation-

1. Evaluation Of Shampoo

The prepared formulation was evaluated for product performance which includes organoleptic characters, pH, physicochemical characterization, and for solid content. To guarantee the nature of the items, particular tests were performed for surface tension, foam volume, and foam stability using standard protocol.

Visual assessment-

The prepared formulation was assessed for color, clarity, odor, and froth content.

pH determination-

The pH of the prepared herbal shampoo in distilled water (10% v/v) was evaluated by means of pH analyzer at room temperature.

Determination of solid content percentage -

The percentage of solid substance was determined by weighing about 4 g of shampoo in a dry, clean, and evaporating dish. To confirm the result, the procedure was repeated again. The liquid portion of the shampoo was evaporated in a dish by placing on hot plate. The percentage and the weight of the solid contents present in the shampoo were calculated after drying completely.

Surface tension measurement -

The prepared shampoo in distilled water (10% w/v) was evaluated for surface tension using stalagmometer in room temperature.

Foam stability test -

The stability of the foam was determined using cylinder shake method. About 50 ml of formulated shampoo (1%) solution was taken in a graduated cylinder of 250 ml capacity and shaken for 10 times vigorously. Foam stability was measured by recording the foam volume of shake test after 1 min and 4 min, respectively. The total foam volume was measured after 1 min of shaking.

Dirt dispersion test-

To 10 ml of refined water two drops of cleanser were included and taken in a wide-mouthed test tube. To the formulated shampoo, added one drop of Indian ink and shaken for 10 min after closing the test tube with a stopper. The volume of ink in the froth was measured and the result was graded in terms of none, slight, medium, or heavy.

2. Anti-Ticks Activity of *Carica Papaya* and *Nicotiana Tabacum* Leaf Extract-

The acaricidal activity was performed on the Indian ticks *Rhipicephalus microplus*. Ethanolic extracts of leaf of plant were diluted with water to obtain 2%, 4%, 6% concentrations. All these dilutions were poured into the Petri dishes accordingly. Five groups of ticks (n = 5) were taken for the study. Ticks were placed in each petri dish. Time for mortality was noted down when no movement of any sort could be observed. Then time of death for ticks was recorded after ascertaining that the ticks neither moved when shaken vigorously nor when dipped in warm water (50°C). The mortality were recorded in terms of minutes.

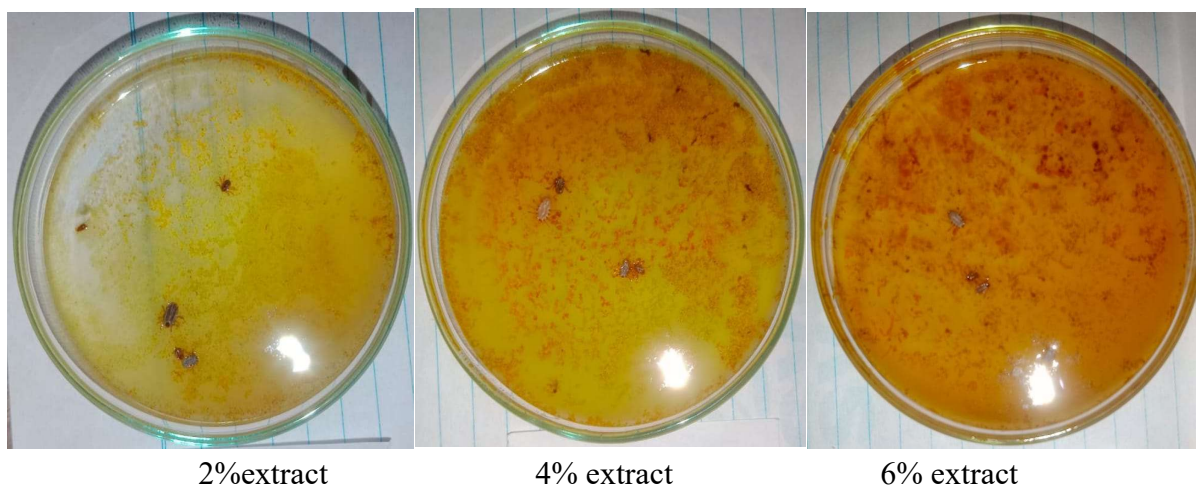


Fig. no 1 : Anti-ticks Activity of Ethanolic Extract Of *Carica Papaya* and *Nicotiana Tabacum* leaf

3. Anti-Ticks Activity Of Shampoo-



fig. no 2 : Negative Control

Fig. no 3 : standard drug

Sufficient Quantity Of 2.5%, 5%, and 10% anti-ticks shampoo was poured into Petri dish. Number of five ticks were placed in petri dish. Time for mortality was noted down when no movement of any sort could be observed. shampoo compared to standart amitraz. Amitraz, the standard , was diluted with water and was poured into Petri dish. Number of five ticks were placed in petri dish. same Time for mortality was noted. Water served as the negative control.

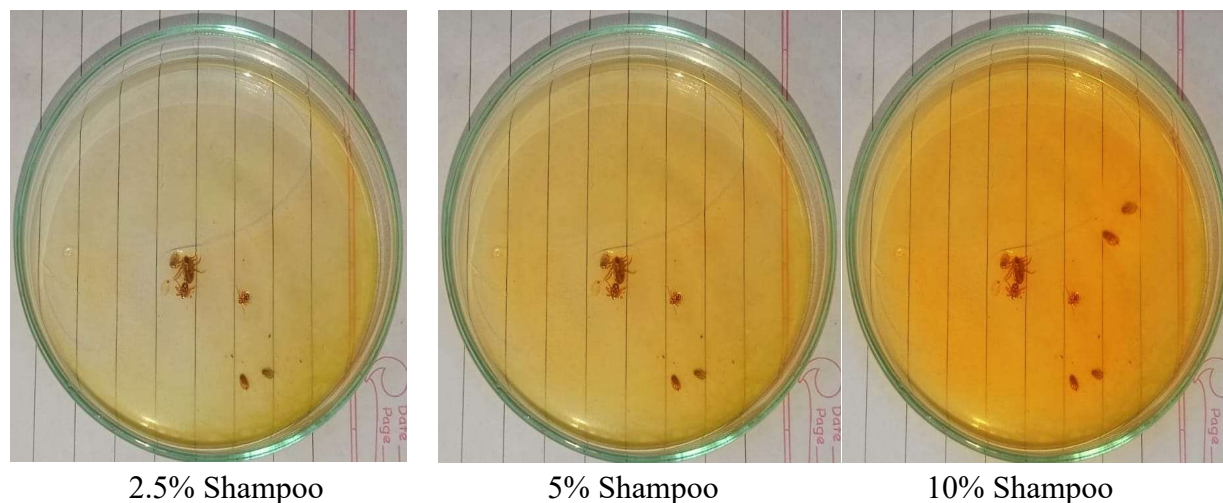


Fig. no 4 : Anti-ticks Activity Of Shampoo

Result and Discussion: -

Evaluation of herbal drugs for purity-

The selected herbal drug has shows the presence of all chief chemical constituent as per earlier studies. Thus it has been certified the purity of authenticity of herbal drug. the results of a phytochemical test for all herbal medicinal drugs show that certain essential phytochemical constituents are present (Table 2)

Table no.2 - Phytochemical screening

Sr.no	Chemical Constituents	Carica Papaya	Nicotiana Tabacum
1.	Alkaloids	+	+
2.	Carbohydrates	+	+
3.	Proteins	+	+
4.	Tannins	+	+
5.	Flavonoids	+	+
6.	Glycosides	+	+
7.	Terpenoids	-	+
8.	Anthroquinones	+	+
9.	Amino Acids	+	+
10.	Steroids	-	+
11.	Saponins	+	+
12.	Phenol	+	+

(+presence of constituents; -

chemical constituents)

chemical absence of

General evaluation of shampoo-

In order to evaluate pharmaceutical ointment, the evaluation parameters are important tests. It has been established that the shampoo is brown colour, pH of shampoo is 5, Percentage of solid content is 23 %, Foaming volume, Foam type of shampoo is 200 ml and Dence.

Table no.3 - General evaluation for shampoo

1	Colour	Brown
2	Clarity	Clear
3	Odor	Good
4	Ph Determination	5.3
5	Percentage Of Solid Content Determination	23%
6	Foaming Volume	200 ML
7	Foam Type	Dence
8	Dirt Dispersion Test	Medium

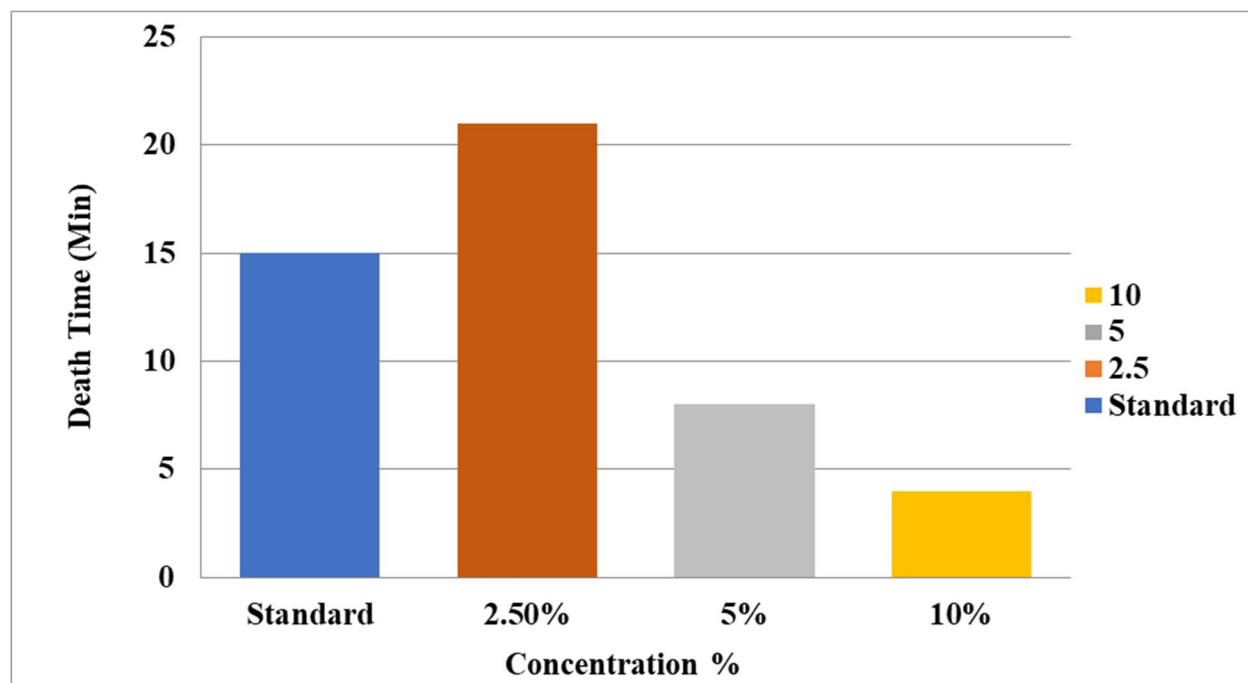
Table no.4 - Anti-ticks Activity of ethanolic extract of *Carica papaya* and *Nicotiana Tabacum* leaves at different concentrations.

Sr.No.	Concentration (%)	Initial No of Ticks	Ticks Mortality Time (min)
1	2	5	15
2	4	5	12
3	6	5	11

Table no.5 – Anti-ticks Activity of Shampoo at different concentration

Sr. No	Concentration (%)	Initial No of Ticks	Ticks Mortality Time (min)
1	Control	5	-
2	Standard (Amitraz)	5	15
3	2.5	5	21

4	5	5	8
5	10	5	4



Anti-ticks Activity (Death Time) of ethanolic extract of *Carica Papaya* and *Nicotiana Tabacum* leaf containing shampoo and standard.

The percentage yield of ethanolic extract was found to be *Carica Papaya* extract was 29.21%w/w and *Nicotiana Tabacum* extract was 33.94%w/w respectively. Preliminary phytochemical screening of both the extracts showed presence of Tannins, Flavonoids, Anthocyanins, Anthroquinones, Glycosides, Reducing sugar, Steroids, Saponins, Alkaloids, Proteins, Amino Acids, Terpenoids, Carbohydrates and Phenol.

So as seen in above table (table No.4&5), as to Evaluation of extract of *Carica Papaya* and *Nicotiana Tabacum* leaf ticks' mortality rate at in 2%, 4%, 6% concentration it shows within 15, 12, 11 minutes. Shampoo concentration 2.5%, 5%, and 10% it shows mortality rate at 21, 8, 4 minutes. Mortality of ticks treated with Amitraz was 15 min whereas no mortality was observed in control treated with water.

Both the extracts of *Carica Papaya* and *Nicotiana Tabacum* leaf shampoo were found to show a potent Acaricidal effect when compared to the standard. It causes death of the ticks at all tested concentrations. As the concentration of the both extracts was increased there was increase in **Acaricidal effect**.

CONCLUSION-

It can be concluded that the present study showed that some indigenous plant based products are very promising against ectoparasites ticks, and can be used as, antimicrobials, insecticides and acaricides. They offer a safer alternative to synthetic chemicals and can be obtained easily at a very low cost. *Carica Papaya* and *Nicotiana Tabacum* leaf extract can be used alone or in combination with other products for effective protection against ticks borne diseases. Even though the plant extract have shown, larvicidal and acaricidal activities, their potential under field conditions needs to be evaluated. The present study was to formulate and evaluate shampoo using plant extract.

Extact of *Carica Papaya* and *Nicotiana Tabacum* leaf concentration (2%, 4%, 6%) shows ticks mortality rate at 15, 12, 11 minutes, respectively. Shampoo concentration (2.5%, 5%, and 10%) it shows mortality rate at 21, 8, 4 minutes, respectively. *Carica Papaya* and *Nicotiana Tabacum* leaf Shampoo showed 100% mortality rate within 04 minutes while standard takes 15 minutes. whereas no mortality was observed in control treated with water. The results obtained from the study indicate the strong anti-ticks activity, supporting folk use of the plants when compared with the standard. Both the extracts of *Carica Papaya* and *Nicotiana Tabacum* leaf containing shampoo were found to show a potent Acaricidal effect when compared to the amitraz. It causes death of the ticks at all tested concentrations. Thus,the phytoacaricides would be possible to produce solution in combating the effective tick control and avoid the cases of resistance associated with the use of acaricides.

In future, studies should be designed to isolate the active principles to explore the exact mechanism of action.

Compliance with ethical standards -

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Conflict of interest-The authors declare that they have no conflict of interest.

Informed consent- Not Applicable

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