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Research Article

STUDIES ON PESTICIDE REMOVING EFFICACY AND ANTIBACTERIAL ACTIVITY OF DHATHRI EAT PURE NATURAL VEGETABLE CLEANER FOR FRUITS AND VEGETABLES

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ABSTRACT

Fruits and vegetables are highly nutritious and form as key food commodity in the human consumption. They are highly perishable due to their low shelf life. These food commodities are reported to be contaminated with microbes and health hazardous chemicals such as pesticides. Hence, it is very necessary to remove the pesticides and prevent the microbial contamination of fruits and vegetables without changing its nutritional value. For this purpose Dhathri Eat Pure Natural Vegetable Cleaner (DEPNVC) have been used to wash out the pesticides along with prevention of microbial contamination. The present study was performed to study the pesticide removing efficiency and antimicrobial activity against the food borne microbial pathogens. Dhathri Eat Pure Natural Vegetable Cleaner contains natural ingredients such as Coconut vinegar, Neem (*Azadirachta indica),* Turmeric *(Curcuma longa).* The HPTLC studies were also performed to determine the ingredients of DEPNVC. The results shown that DEPNVC has the 75 % (Organo Phosphorous) 90% (organo chlorines) of pesticides and the dose dependent antibacterial activity with mean zone of inhibition was observed as 11.63 ± 3.03 mm.

KEYWORDS: Dhathri Eat Pure Natural Vegetable Cleaner, antimicrobial, pesticide removal, HPTLC, Pesticide, Fungicide.

INTRODUCTION

Most foods are consumed after passing through various culinary and processing treatments, which include peeling, cooking, and washing [1]. Peeling can remove some pesticides mainly adhered on the surface to safe levels [2,3]. However, the dietary value of the peels of some vegetables should be retained [4]. Peeling is also not practical for leafy vegetables. Cooking can reduce some volatile pesticides, but does not work well for less volatile ones and is not applicable to fruits and vegetables cleaning[5,6]. Among all, washing, with tap water, is the most common and practical way to reduce pesticide residues [2]. Chlorothalonil (CHT, 2,4,5,6-tetra chloro-1,3-b enzenedicarbo- nitrile, C8Cl4N2), a nonsystemic fungicide, is highly toxic to fish, birds, and aquatic invertebrates. Its production exceeds 8000 tons year in China. It is the second most Commonly used agricultural fungicide in USA[7]. Once it enters human bodies, it can cause dermatitis, severe eye and skin irritation, and gastrointestinal symptoms [8]. Chlorpyrifos (CHP, O, Odiethyl-O[3,5,6-tuichloro- 2-pyridi-nyl, $C_9H_{11}C_{l3}NO_3PS$), a broad spectrum organophosphate intrinsic insecticide [9], is used to mainly control foliage and soil-borne insects on a variety of food and feed crops (US.EPA, 2000). It is an irreversible inhibitor of cholinesterase, and the inhibition is believed to be the most sensitive for all animal species evaluated and humans [10,11].

Dhathri Eat Pure Natural Vegetable Cleaner is the combination of Coconut vinegar, Neem (*Azadirachta indica),* Turmeric *(Curcuma longa).* In *Siddha* and Ayurveda, Lemon was used as a cleaning agent for pesticide and fungicides. Now a day's all vegetables and fruits are having in some residues of pesticide and fungicides. Dhathri developed eat pure natural vegetables cleaner. The present study was performed to understand the Efficacy and antibacterial activity of eat pure cleaning solution for fruits and vegetables The study reveals that eat pure cleaning solution have the abilities to clean the microbes, pesticide and fungicides.

MATERIALS AND METHODS

Media and Reagents

Nutrient Agar, Nutrient Broth, Nutrient broth, Fluconozole, Tetracycline Himedia laboratories, Malathion, Carbaryl, Permathrin, DDT sigma chemicals. The bacterial and fungal strains were purchased from Microbial Culture Collection Centre, Chandigarh. All the other chemicals and reagents used were of analytical grade and were obtained from SISCO Research Laboratories Pvt Ltd. Mumbai, India.

Antimicrobial efficacy

Extraction:

There were two different extracts of Dhathri Eat Pure Natural Vegetable Cleaner were used. One is Raw Dhathri Eat Pure Natural Vegetable Cleaner and another one is Ethyl acetate extract of the solution were used for the assay. The bacterial and fungal strains were purchased from Microbial Culture Collection Centre, Chandigarh and sub cultured based on their standard protocol[13].

The bacterial pathogens were cultured in Nutrient Agar (Himedia, Mumbai). The fungal pathogens were

cultured in Sabourd's dextrose broth (Himedia, Mumbai), *Cryptococcus laurentii* cultured in Malt Yeast agar (Himedia, Mumbai) and *Fusarium oxysporum* cultured in Potato dextrose broth (Himedia, Mumbai) and the respective agar was used for Agar well diffusion assay[15].

The agar was poured into the assay plate (90 mm in diameter) (100 µl of 500 mg of Tetracycline was pre inoculated on to the plates to prevent the growth of bacterial contamination and Fluconozole to prevent the fungal contamination) and allowed to cool down on a leveled surface. Once the medium had solidified, four wells, each 6 mm in diameter, were cut out of the agar, and 50 µl of Dhathri Eat Pure Natural Vegetable Cleaner was placed into each well at different concentrations. Tetracycline and Fluconozole were used as a positive control Triplicates were maintained. The zone of inhibition was measured from the agar well to the end of the zone (mm)[17]. The average inhibitory concentration of the Dhathri Eat Pure Natural Vegetable Cleaner was also determined.

Treating of fruits and vegetables [12]

100 gm. of Vegetables (Green chili, Tomato and grapes) were purchased from agricultural farm, Chengalpattu, Kanchipuram District. We have segregate into two batches one is for studying the overall washing ability of Dhathri cleaning solution, another batch is used for studying the specific washing ability of individual pesticides commonly used in cultivation of vegetables and fruits.

The four pesticides Malathion, Carbaryl, Permathrin, DDT were purchased from Sigma Aldrich were used as a common pesticides to analyze the overall washing ability of Dhathri cleaning solution. The washing ability was compared with water.

Method I:

The fresh vegetables were segregated into 2 different batches one batch was washed /soak in water for 5 min and another batch was washed/ soak in with Dhathri Eat Pure Natural Vegetable Cleaner (ratio of 1:100 for 10 mins.) Then the solutions were taken for pesticide residue analysis using standard protocols (EPA3501/8141A)

Method II:

A set vegetables were washed/soaked in water and then they were soaked in different pesticides (mentioned above) for 5 mins. Separately. One batch was soaked in water and another batch was soaked in Dhathri cleaning solution. Then the solution was analyzed for pesticide using standard protocol (EPS 3501/8141A)..

Chemical analysis of solution

The ingredients Curcuma, Neem and Coconut vinegar, Dhathri Cleaning solution. The raw solution was spotted on TLC plate. The Hexane: Ethylacetate (6:4) was used as a mobile phase. The following specifications were used for the HPTLC analysis.

Name of the instrument	:	CAMAG HPTLC
TLC plate	:	Aluminium coated Silica gel- MerkF254
Mobile Phase	:	Hexane:Ethyl acteate (6:4)
Wavelength	:	200-400 nm
Lamp	:	Deutrium

RESULT ANS DISCUSSION

Antimicrobial efficacy

The results of antimicrobial efficacy of Dhathri Eat Pure Natural Vegetable Cleaner were shown in Table.1. And Plate 1-3. The dose dependent antimicrobial activity was observed for the cleaning solution with average zone of inhibition 11.63 ± 3.03 mm.

Treating of fruits and vegetables

The results were shown (Table.1 Fig.1 and Fig.2) the efficiency of the Dhathri Eat Pure Natural Vegetable Cleaner for removing the pesticides from vegetables and fruits may vary. The efficiency of Dhathri Eat Pure Natural Vegetable Cleaner was observed more in organochlorine (90%) pesticides than organo phosphorous (75%) pesticide residues [14, 18].

Chemical analysis of solution

The results showed in (Fig. 4 to 9)from the results that we may confirm the presence of Curcuma, Neem and coconut vinegar in the Dhathri cleaning solution. The combination of the formulation (Dhathri cleaning) may be the synergistic effect of all 3 ingredients for removing the pesticides and pathogens. This chemical analysis also confirms that the ingredients are herbal origin and proved to be Neem, curcumin and coconut vinegar.

TABELS

Table. 1. Antimicrobial activity of DEPNVC

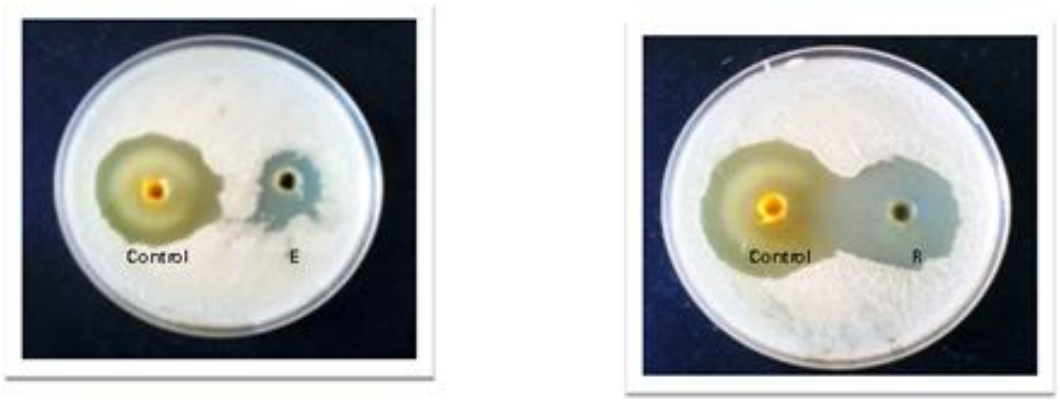
Name of the Strain	Raw solution	Ethyl acetate extract
	Zone of inhibition (mm)	
Escherichia coli	**15**	**12.5**
Streptococcus pneumonia	**10**	**10**
Proteus mirabilis	**16**	**7.5**
Shigella flexneri	**14**	**10**
Staphylococcus epidermis	**10**	**9**
Clostridium perfringens	**11.5**	**8**
Propionibacterium acnes	**8**	**6**
Aspergillus niger (Fungi)	**8.5**	**7.5**

Table.2. Percentage Removal of DEPNVC for different pesticides

S. No	Name of the pesticides	% Removal	
		Dhathri Cleaning Solution	Water
1	Malathian	95	10
2	Carbaryl	85	7
3	Permethrin	85	5
4	DDT	98	10
5	α-BHC	80	5
6	β-BHC	85	5
7	γ-BHC	89	10
8	Cherdane	91	10
9	DDT	98	10
10	Endosulphan	90	5
11	Organophosphorus	95	5
12	Organochlorine	89	7
13	Other Pesticides	85	5

Plate.1. Antimrobial activity of DEPNVC

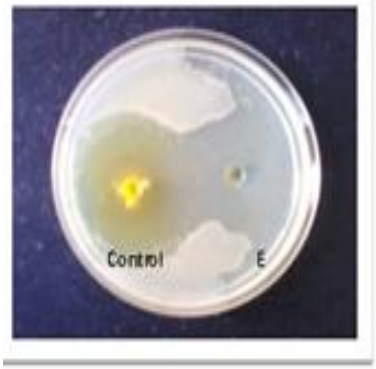
Escherichia coli

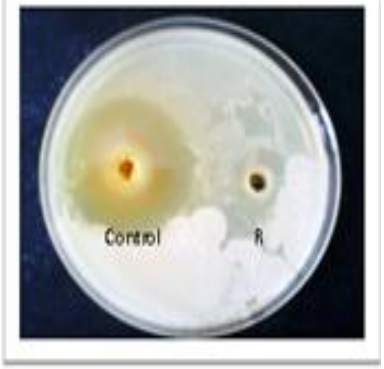


Shigella flexneri

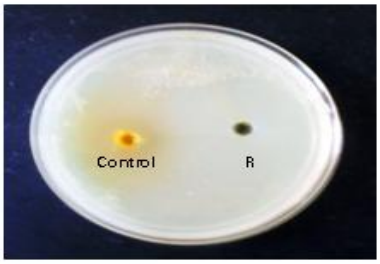
Proteus mirabilis

Streptococcus pneumonia

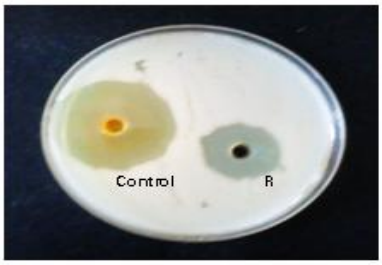




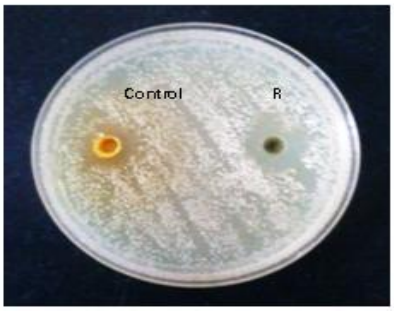
Propionibacterium acnes



Staphylococcus epidermis



Clostridium perfringens



Aspergillus niger

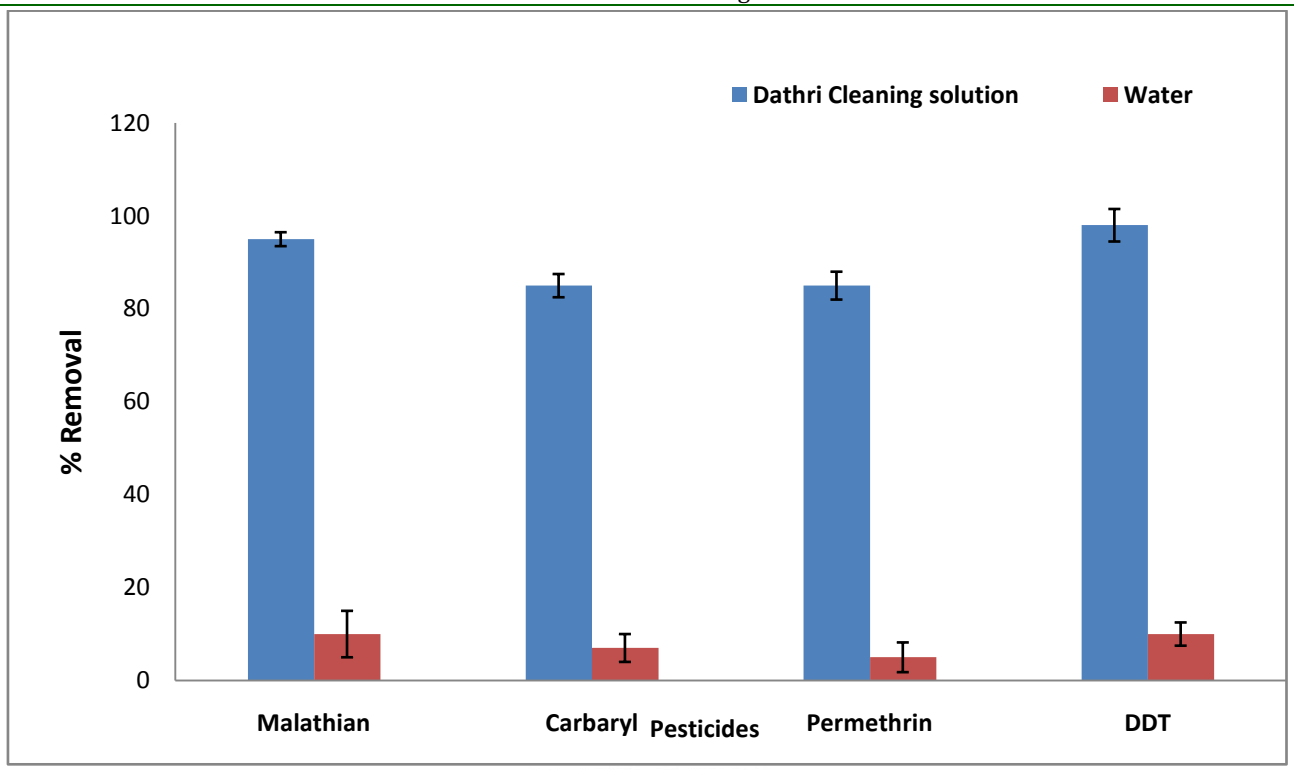


Fig.1.Comparative study of pesticide removing Efficiency of DEPNVCVs water

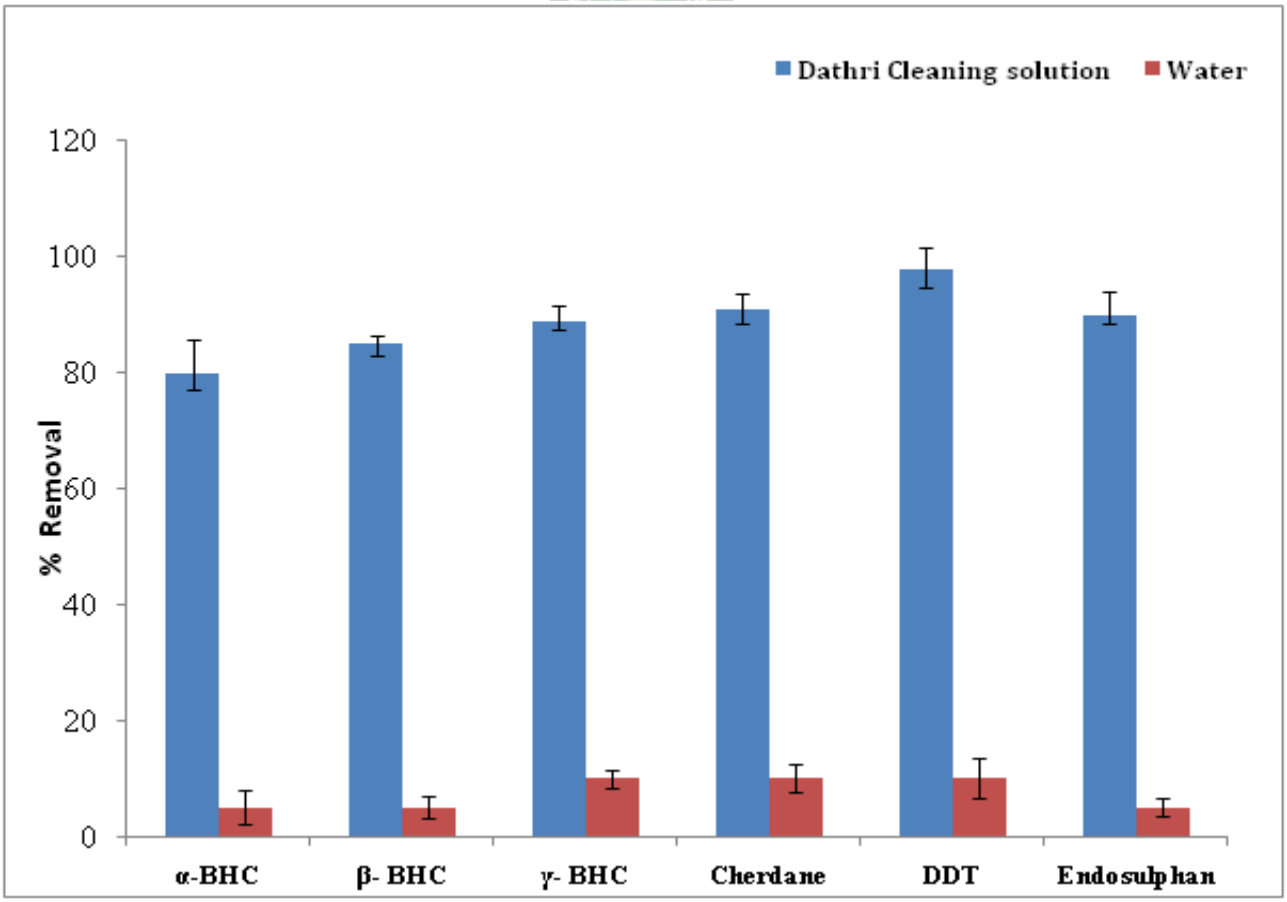


Fig.2. Efficacy of DEPNVC for removing different pesticides

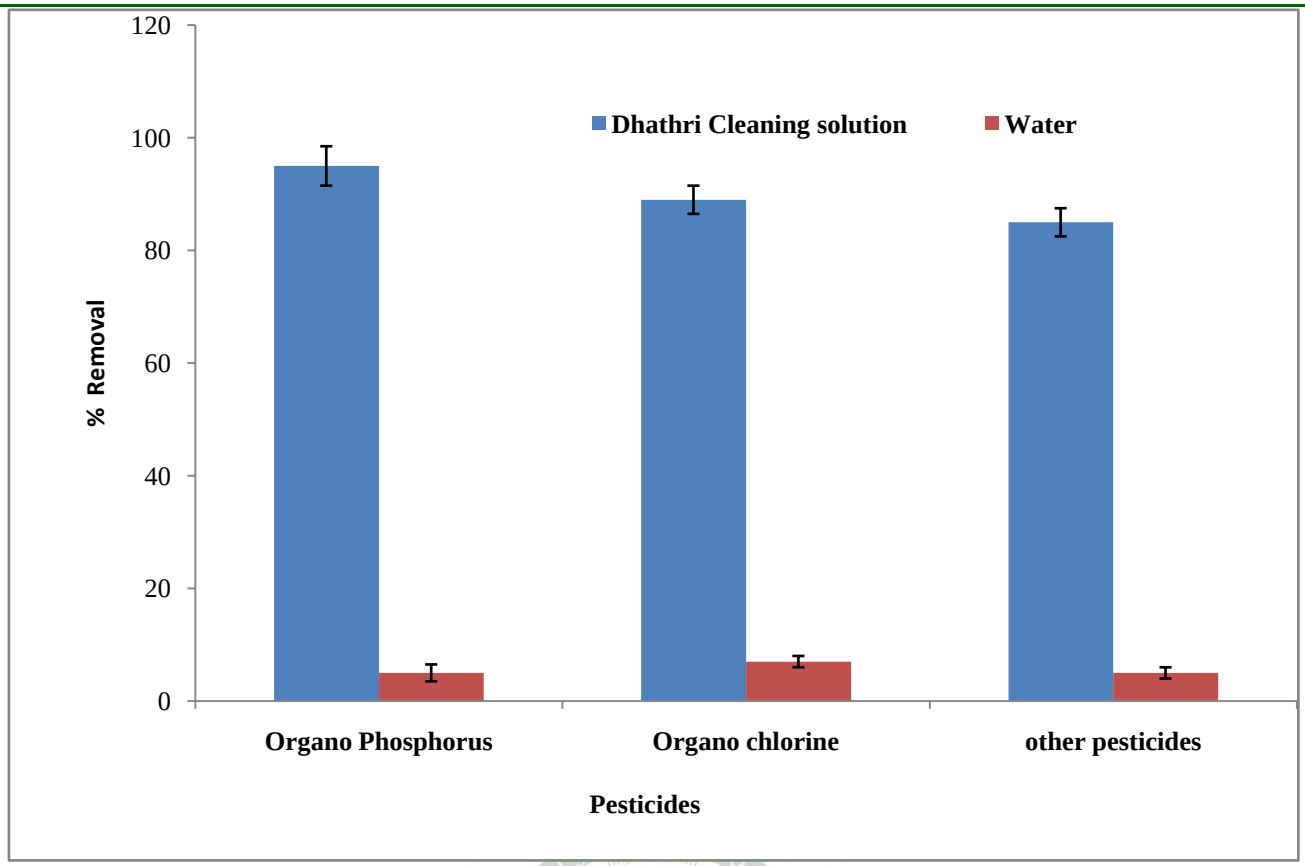


Fig.3. Efficacy of DEPNVC for removing organo phosphorous and organochlorine pesticides

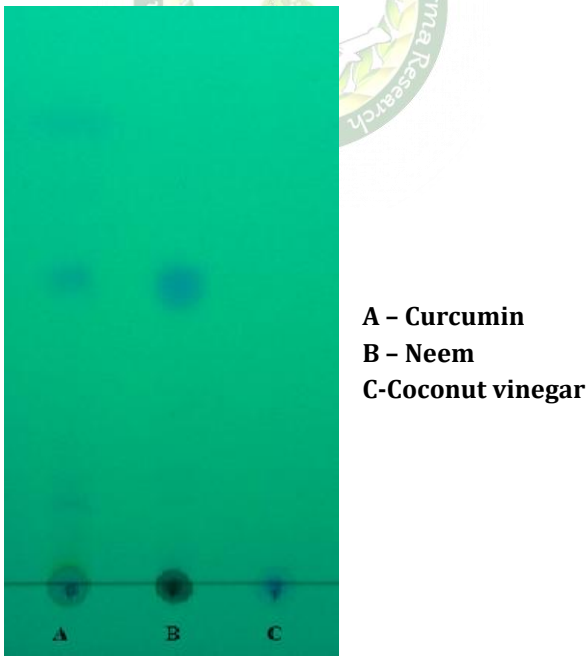


Fig.4. TLC of Dhathri Cleaning solution along with ingredients

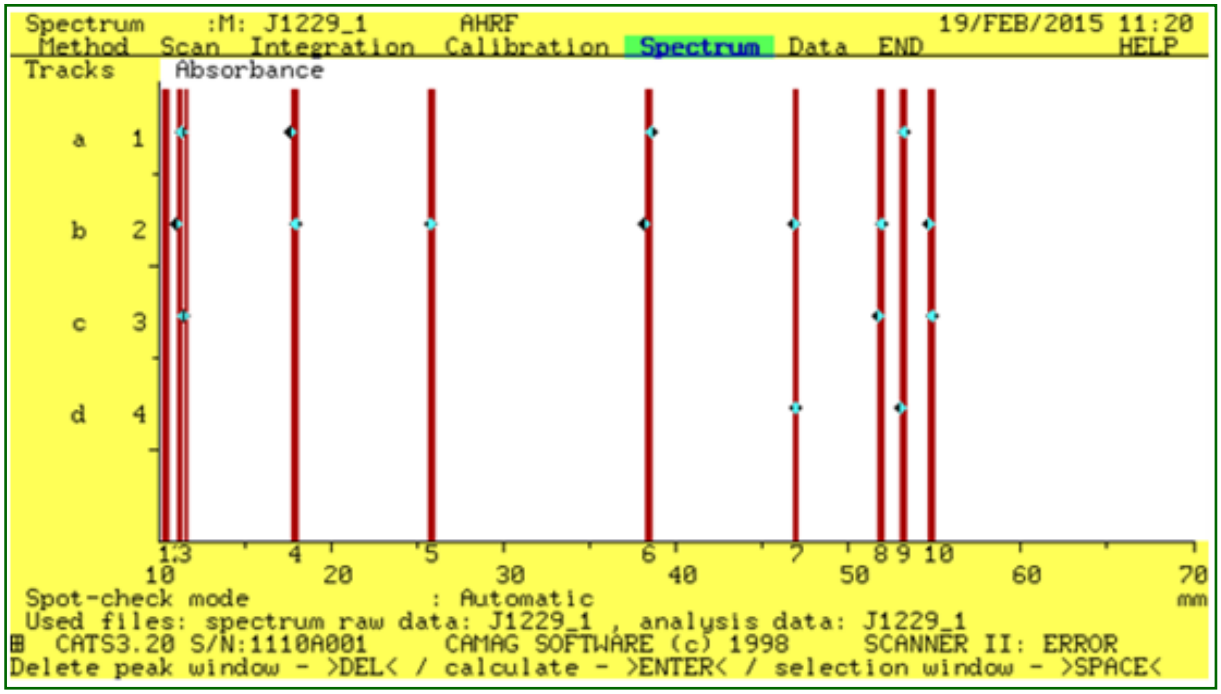


Fig.5. HPTLC Histogram of DEPNVC with ingredients

A – Curcuma; B – Neem; C - Coconut vinegar; D – Dhathri vegetable cleaning solution

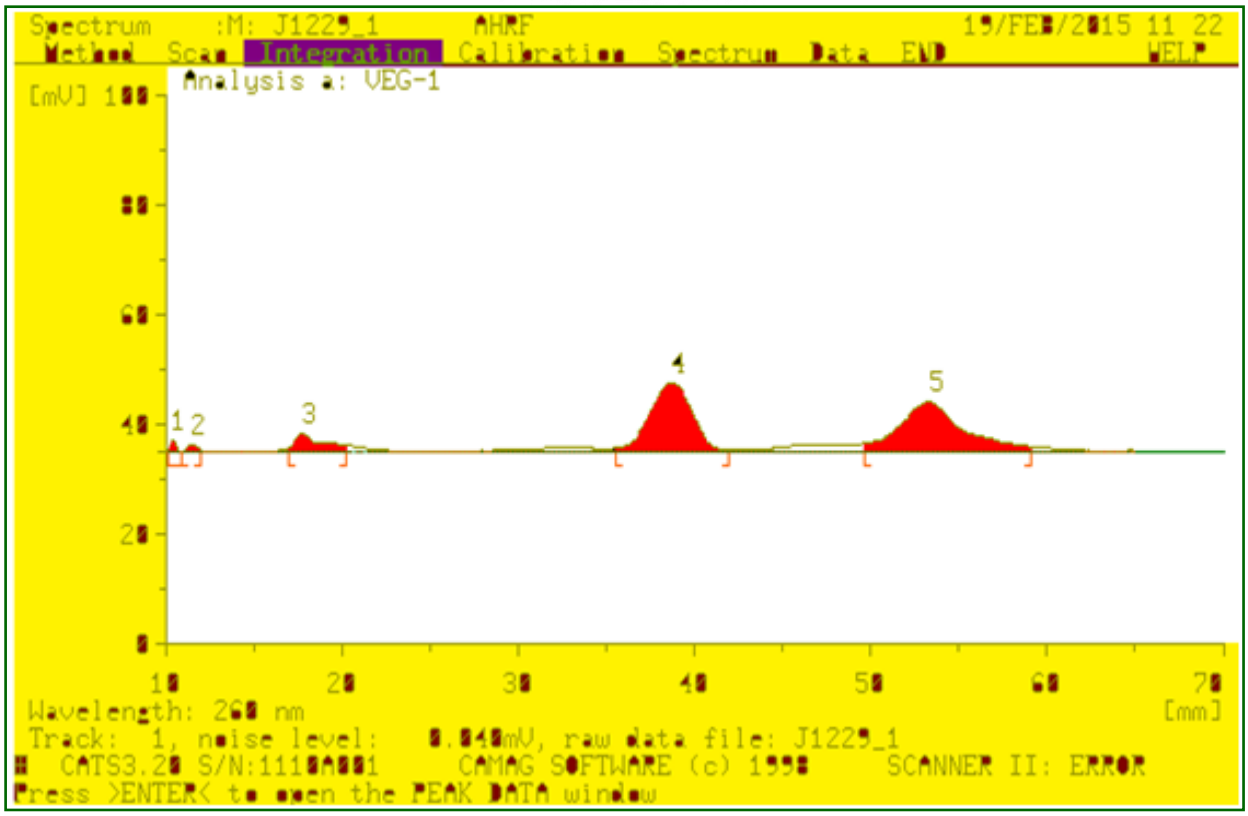


Fig.6. HPTLC Chromatogram of Curcumin

S.No	Rf	Height	Area	Lambda max
1	0.10	2.00	6.70	311
2	0.11	1.40	9.30	200
3	0.17	3.50	63.40	276
4	0.38	12.60	353.50	276
5	0.53	9.10	388.50	276

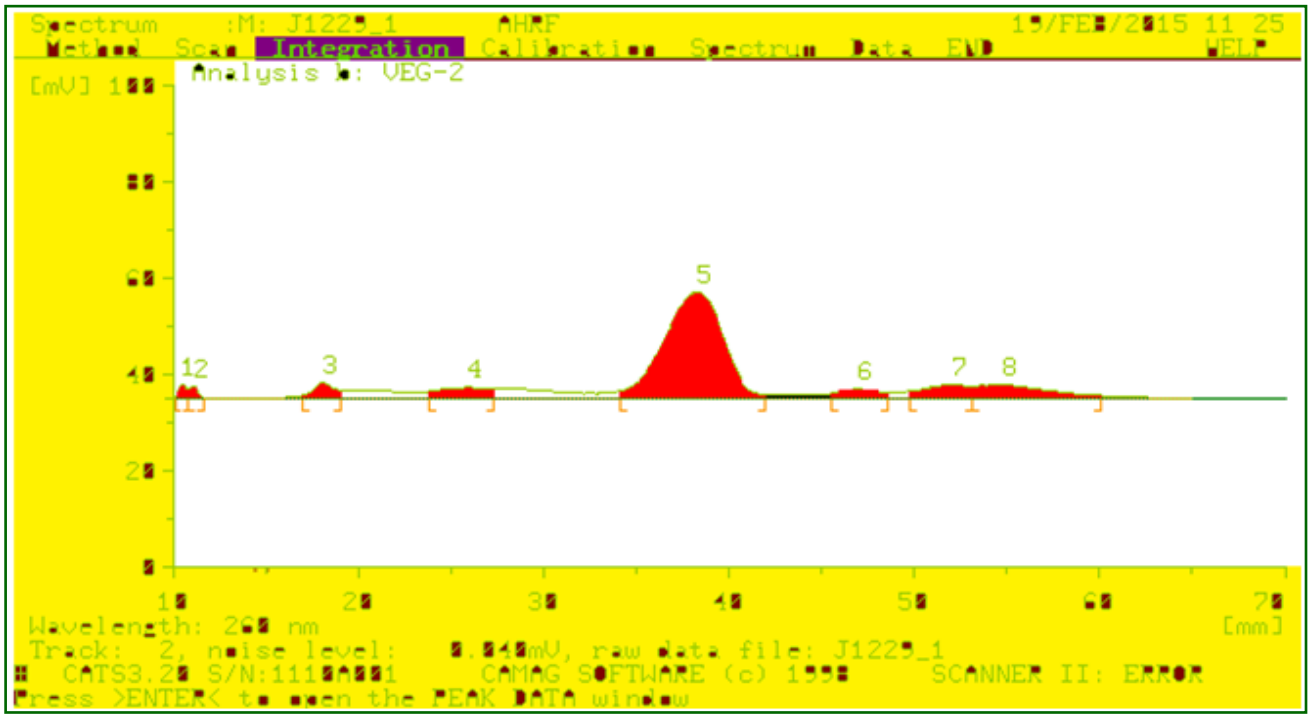


Fig.7. HPTLC Chromatogram of Neem

.No	Rf	Height	Area	Lambda max
1	0.10	3.00	13.10	221
2	0.11	2.60	15.3	209
3	0.18	3.20	44.5	276
4	0.25	2.30	70.5	276
5	0.38	22.2	823.4	251
6	0.46	2.00	49.8	276
7	0.52	3.00	81.3	276
8	0.54	2.80	134.5	276

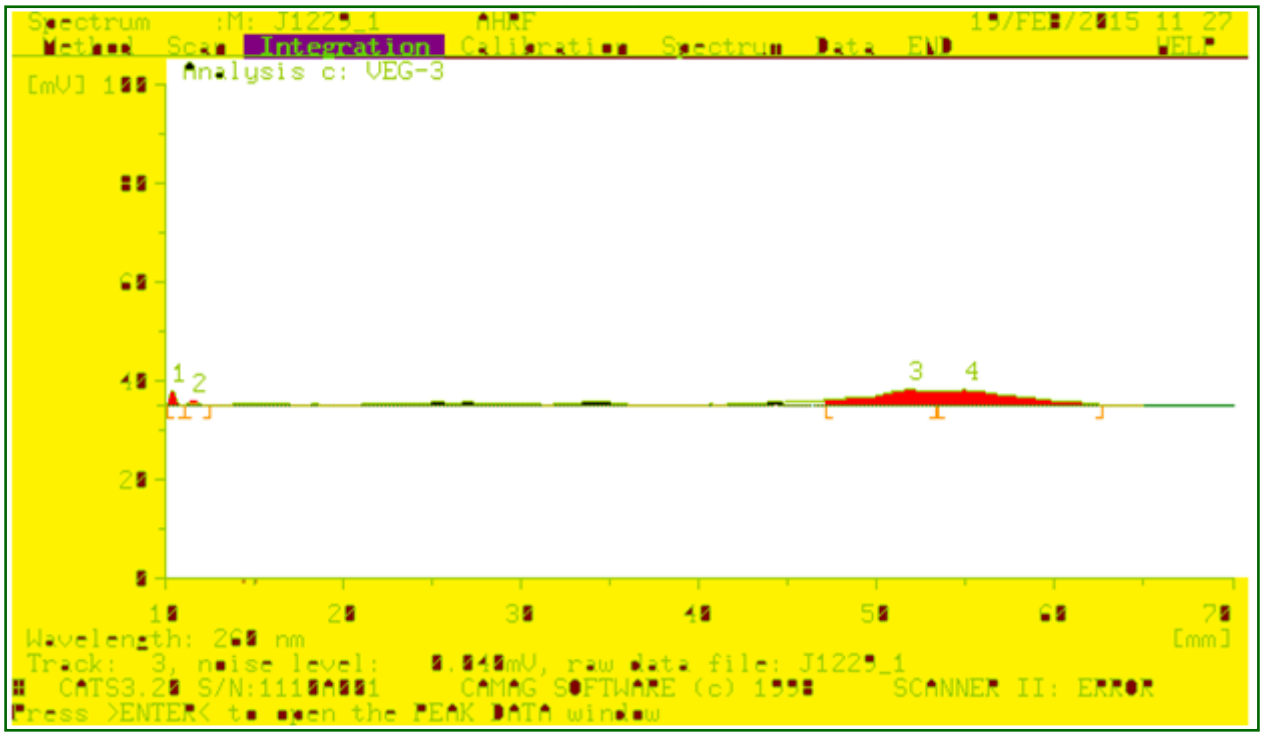


Fig.8. HPTLC Chromatogram of Coconut vinegar

S.No	Rf	Height	Area	Lambda max
1	0.10	3.2	12.3	232
2	0.47	1.6	54.6	276
3	0.53	3.1	224.5	276

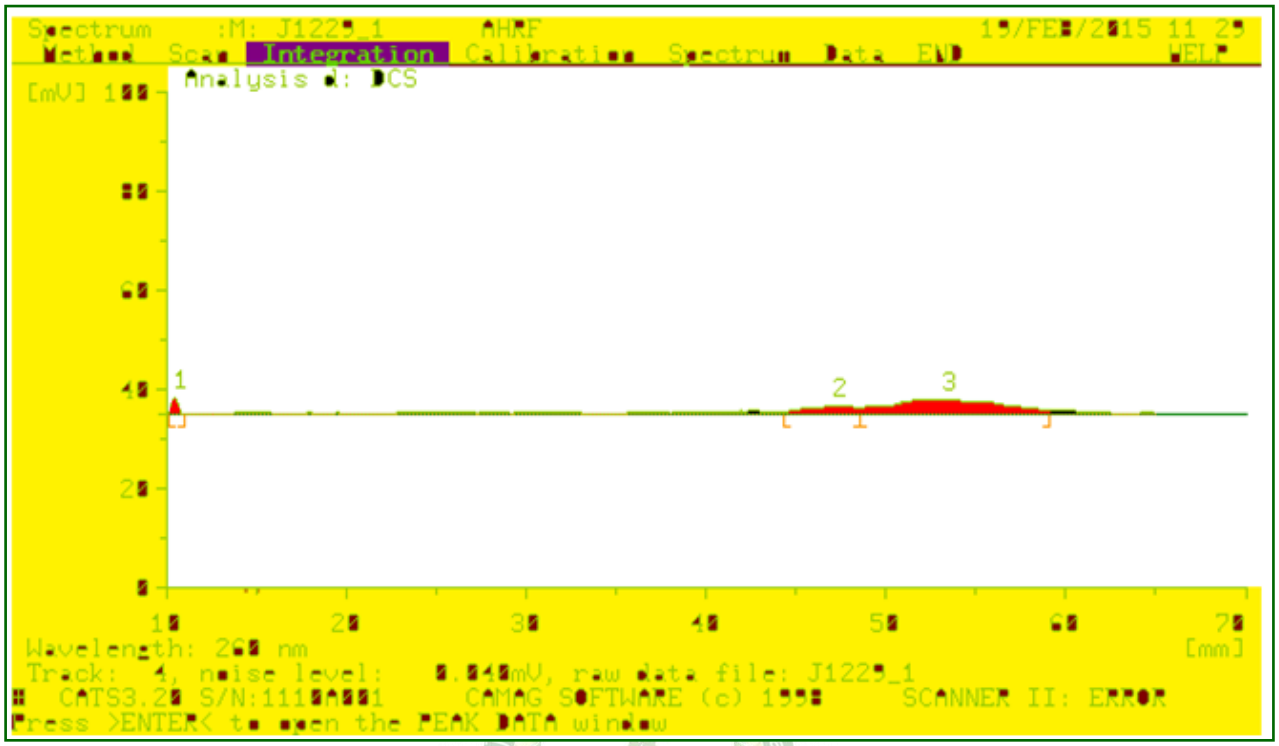


Fig.9. HPTLC Chromatogram of DEPNVC

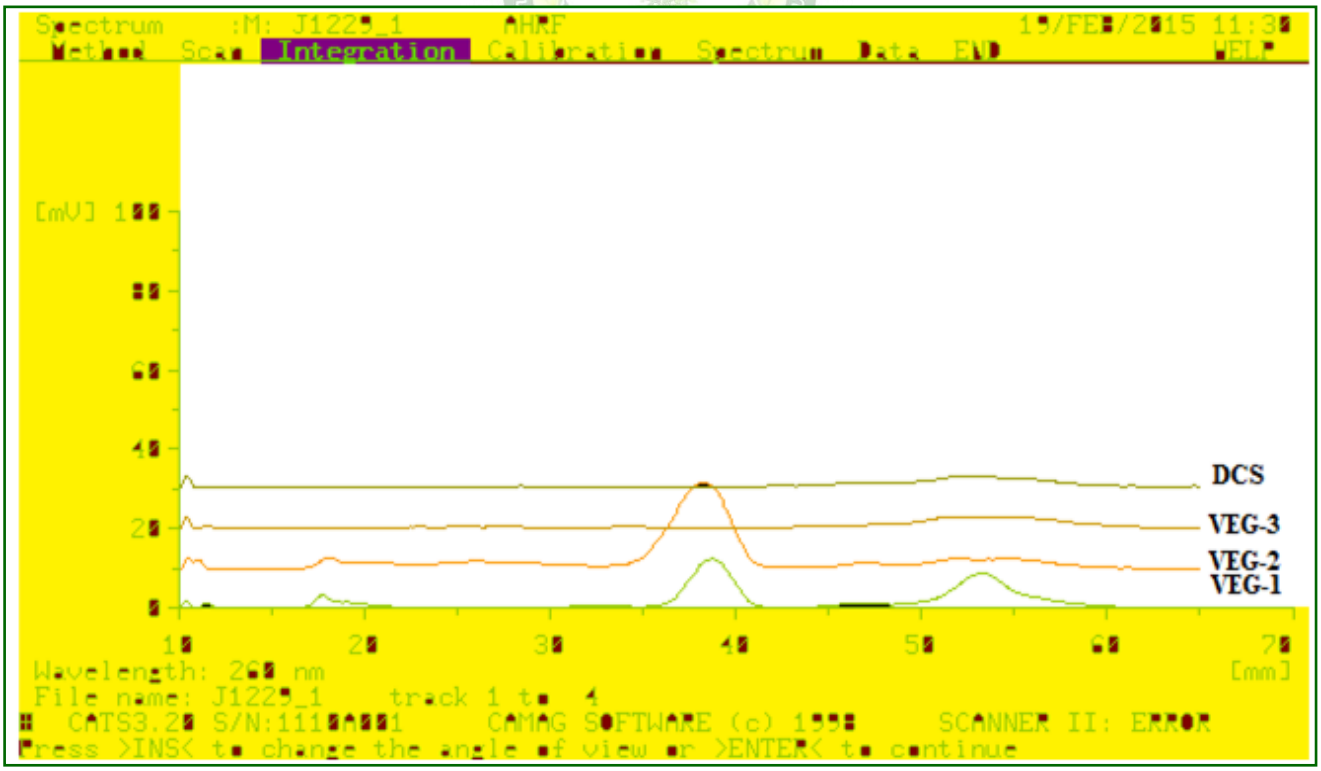


Fig.10. HPTLC Overlay DEPNVC and its ingredients

CONCLUSION

Antimicrobial efficacy

The results of antimicrobial efficacy of Dhathri Eat Pure Natural Vegetable Cleaner were shown in Table.1 and Plate 1-3. The dose dependent antimicrobial activity was observed for the cleaning solution with average zone of inhibition 11.63 ± 3.03 mm.

Chemical analysis of solution

Dhathri Veg. cleaning solution contains herbal and edible ingredients (natural) which removes the pathogenic microbes and harmful pesticides from the vegetables in more efficiently.

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