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Evaluation of *In vitro* antifungal activity of *Vitex negundo* against *Fusarium oxysporum* and HPTLC fingerprinting

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Abstract
Fusarium oxysporum f.sp.cumini causes cumin wilt disease all over the world. Chemical fungicides have significant environmental impacts and are hazardous to non-target organisms. Plant metabolites and plant-based pesticides appear to be among the best options since they are known to be less harmful to the environment and offer less risk to consumers than synthetic pesticides. Plant extracts of *Vitex negundo* were employed in an *in vitro* antifungal test against *Fusarium oxysporum f. sp. cumini* to create eco-friendly management. To extract the chosen plant, several solvent systems were utilised, including methanol, acetone, aqueous methanol, aqueous acetone, dichloromethanol:methanol, and water.The poison food technique was used to evaluate the antifungal activity of extracts at various concentrations (4 %, 6 %, 8 %, 10%, 12%, and 14 %) on FOC mycelial development and obtained a favourable result in the form of %inhibition. HPTLC fingerprinting was performed for some important phytochemicals like flavonoids, alkaloid and phenolic compound. This study suggests that botanical extracts could be a viable option for developing effective plant-based fungicides for the treatment of *Fusarium oxysporum f. sp. cumini* in organic farming.

Keywords: *Fusarium oxysporum f. sp. cumini*, cumin, plant metabolites, *vitex negundo*, poison food technique, organic farming

Introduction

With the increased interest in antibiotics, plants as a source of possible antimicrobial/antifungal compounds are attracting a lot of attention all over the world. The existence of naturally occurring compounds in plants with antifungal capabilities has been identified and tested against a diverse spectrum of pathogenic fungi. Many plant extracts have recently been demonstrated to have inhibitory activity against some plant pathogenic fungi as well as human pathogenic fungi. (K Dave *et al.*, 2021) [9] Some extracted components from plant extracts may be more efficient against fungus than commercial synthetic fungicides. Nowadays, various synthetic and semi-synthetic antifungal medicines are being developed, but very few of them have broad spectrum action and the majority of them are ecologically toxic.

The widespread use of agrochemicals, particularly fungicides, has resulted in a higher carcinogenic risk than other pesticides, which may result in adverse biological consequences on animals and humans. (Bose TK *et al.*, 2003) [1]

The growing public awareness about environmental concerns necessitates alternate disease control techniques that are less reliant on pesticides or are based on naturally occurring substances. The plant world is a rich source of biochemicals that may be utilised as insecticides that are less harmful to the environment than manmade poisons. (Cuthbertson AGS *et al.*, 2005) [2]

India is endowed with a plethora of medicinal plants. The Medicinal Plants have made an important contribution to the evolution of ancient Indian systems of medicine or pharmaceuticals, as well as to indigenous medicine among socioeconomic groups, etc. India is a repository for the genetic variety of medicinal plants. Plants and plant-derived compounds have a long history of being used as a source of possible chemotherapeutic drugs in the Ayurvedic and Unani medical systems. The Vitex genus is a member of the Verbenaceae family, which is also known as Nirgundi in India. Nirgundi literally means "protects our body from all ailments." (Kamlesh KB *et al.*, 2010) [3]

Vitex negundo leaf extracts have anti-oxidant and antifungal properties, as well as previously documented anthelminthic, dysmenorrheal, medicine and pain suppressing activity, anti-hyperglycemic activity, anti-filarial, anti-bacterial, and opposing plant action. (Yunos N.M *et al.*, 2005) [4]

The current study was carried out to evaluate the antifungal capabilities of *Vitex negundo* Linn against fungal pathogens responsible for wilt disease in cumin in order to develop natural fungicides and to perform HPTLC fingerprinting to determine the presence of some key phytochemicals.

Method

Plant Material

In November 2020, fresh *Vitex negundo* leaves were collected from Vijapur location in the Mehsana district. Dr.Sachin A. Punekar, Scientist & Founder President, Biospheres, Pune, Maharashtra, has authenticated the plants collected.

Processing of the Plant

The obtained healthy leaves were washed with water to eliminate any lingering unwanted particles. It is then dried. Drying is a key stage in the preparation of dried materials for further processing since it decreases the moisture content of fresh materials. Drying conditions, on the other hand, have been shown to have a significant influence on sensory quality, bioactive component stability, and activity. The plant material was dried in the shade for 7 to 15 days. The next step is to grind. Grinding is used to establish a homogeneous sample and to improve the surface contact of the sample with the solvent solution. Finally, the plant powder is kept at a lower temperature. (S. Sasidharan *et al*., 2011) [5]

Physicochemical Parameters:

The various physicochemical properties specified by The Unani Pharmacopoeia of India. The following parameters were included: odour, taste, colour, moisture content, total ash value, acid insoluble ash value, water soluble ash value, and extraction yield. (Mrunali Patel *et al*.,2021) [9]

Determination of Moisture (Loss on drying)

1.5gm powdered leaves were placed in a weighted plane and slender Porcelain dish. It was dehydrated in an oven at temperatures ranging from 100 to 105 ° C. Moisture is frequently evaluated by cooling in desiccators and monitoring weight loss.

Ash values

The values of 'total ash,' 'acid insoluble ash,' and 'water-soluble ash' of air-dried samples were determined.

Total ash value

In a formerly burnt and tarred silica crucible, weigh roughly 2 gm of the air dried material. Spread the material out evenly and gradually raise the temperature to 500 - 600°C until it is white, indicating the absence of carbon. Allow the remains to cool in a desiccator for 30 minutes before weighing with no time interval. Percentages of 'total ash' were calculated using air-dried material standards.

Total ash value of the sample15 =100(Z-x)/y %

X= 'weight of empty dish'
Y= 'weight of the drug taken'
Z= 'weight of the dish + ash (after complete incineration)'

Acid insoluble ash

Fill the crucible with 25 mL of 2N HCl, cover with a watch glass, and let it gently boil for 5 minutes. After cleaning the watch glass with 5 mL of hot water, put the liquid to the crucible. Collect ash-free filter paper that contains insoluble components and wash it in hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble particles to the first crucible, dry on a hot plate, and burn to a constant weight. Allows the residue to cool for 30 minutes in desiccators before weighing without a time gap. Calculate the acid-insoluble ash concentration in milligrammes per gramme of air-dried material.

Water soluble ash

For 5 minutes, bring 25 ml of water to a boil in the crucible containing the entire ash. Combine the insoluble materials in a sintered-glass crucible. After cleansing with hot water, ignite for 15 minutes in a crucible at 450 °C or below. Reduces the weight of this residue in milligrammes as a percentage of total ash weight. Determine the water-soluble ash content in mg per gramme of air-dried material. The percentage of water-soluble ash was estimated using an air-dried material.

Preparation of plant crude extracts

The maceration procedure was used to remove the plants. The extracts were prepared by soaking 30gm of powder plant materials in 30ml of methanol, Acetone, aqueous methanol, aqueous acetone, dichloromethane: methanole, and aqueous extractants for 6 days at room temperature. To get semisolid products, the extracts were separated and concentrated in a water bath at 70°C for the aqueous extract and 50°C for methanol, acetone, and dichloromethane. The dried extract was stored in an airtight container at 4°C for further investigation.[6]

The % extractive yield was computed using the formula shown below, and the results are shown as Table No: 1.

% 'Extractive yield (w/w) = weight of dried extract /weight of dried leave × 100'

Preparation of extracts concentrations from various extractants
The 'crude extracts' generated by the different solvents were concentrated with dimethylsulfuroxide (DMSO) to obtain concentrations of 4, 6, 8, 10, 12, 14, 16mg/ml for each extract. (Pathak D *et al*., 2019) [8]

Test organism
Fungi that cause plant disease were taken into account. The chosen fungal species was *fusarium oxysporum*, which was received from the Indian type culture collection (ITCC) in January 2021 under the ITCC number 1053. The fungal isolates were grown on potato dextrose agar at 4°C.

Antifungal activity assay of botanical extracts by using poison food technique
The experiment was repeated three times for each treatment. Plant extracts from each stock solution were administered to 10 ml of sterile potato dextrose agar in petri plates at varied concentrations of 4%, 6%, 8%, 10%, 12%, 14%, and 16%. A 5 mm diameter actively developing mycelium disc of the pathogen from a 6–7 day old culture was placed in the centre of the Petri plate. Plates with no plant extract served as negative controls. The plates were incubated at 27°C. Mycelium radial growth was assessed after seven days of incubation. The results were compared to a negative control group. The experiment was repeated three times, and the mean of the three measurements was utilised for calculation. The percentage of fungal inhibition in treatments was estimated using the formula below; (Anil Kumar *et al.*, 2015) [7]

$$L = [(C - T)/C] \times 100$$

Where, 'L is the percent inhibition'; 'C is the colony radius in control plate' and 'T is the radial growth of the pathogen in the presence of plant extracts'.[9]

Statistical analysis
Means and standard errors of the mean were calculated for the zones of inhibition examined in each case.[10]

HPTLC Analysis*:* The standard procedure outlined by Wagner *et al.* [9] was used for HPTLC experiments. A 10ul sample was put into a Silica gel TLC plate. The loaded plate was retained in a TLC twin trough developing chamber (after being saturated with solvent vapour) with the appropriate mobile phases, namely toluene-acetone-formic acid (4.5: 4.5: 1) for flavonoids and Ethyl acetate-methanol-water (10:1.35:1) for alkaloids. The plate was developed up to 90mm. The developed plate was dried with hot air to remove any remaining solvents. The plate was placed in a photo documentation chamber, and images were taken under visible light, UV 254 nm, and UV 366 nm. The peak table, peak display, and peak densitogram were noted.

Result
The initial step in this research was to collect and treat plants; next, numerous physicochemical properties of plant powder shall be observed. The following table-1 depicts the observed outcome:

Table 1: Physicochemical properties of *Ricinus cummunis* leaves)

Test parameters	***Vitex negundo***
Color	Greyish Green
Odour	Specific
Taste	Bitter
Moisture	3.95%
Total Ash	10.01%
Acid Insoluble Ash	0.71%
Water Soluble Ash	1.21%

Table 2 shows the extraction yields and physical characteristics of plant extracts. In different solvent systems, the extraction yields of *Vitex negundo* ranged from 11.03 % to 28.22 %. Extract yields vary substantially depending on the extraction solution and plant material used. Acetone extract had the lowest extraction yield, at 11.03 %. The maximum extraction yield of 50 % acetone extract was 28.22 %. The physicochemical features of plant extracts, such as colour and texture, were studied. The extract from the solvents methanol, acetone, and dichloromethane: methanol was determined to be green in colour, and the feeling of touch was discovered to be sticky. While the extract from aqueous methanol, aqueous acetone, and aqueous is brown and sticky to the touch.

Table 2: Physical characteristics and % yield of Extract: *Ricinuscommunis*)

Plant	Solvent	Colour of Extract	Sense of Touch	Amount of Extract(gm)	% Yield
Vitex negundo	Water	Brown	sticky	5.88	19.6
	100% methanol	Green	Sticky	6.83	22.766

	50% methanol	Brown	Sticky	6.61	22.033
	100% acetone	Green	Sticky	3.31	11.03
	50% acetone	Brown	Sticky	8.467	28.22
	Dichloromethane/Methanol (1:1)	Green	Sticky	5.873	19.57

Figure 1 depicts a graphical representation of the % yield comparative for all the extracts of *Vitex negundo.*

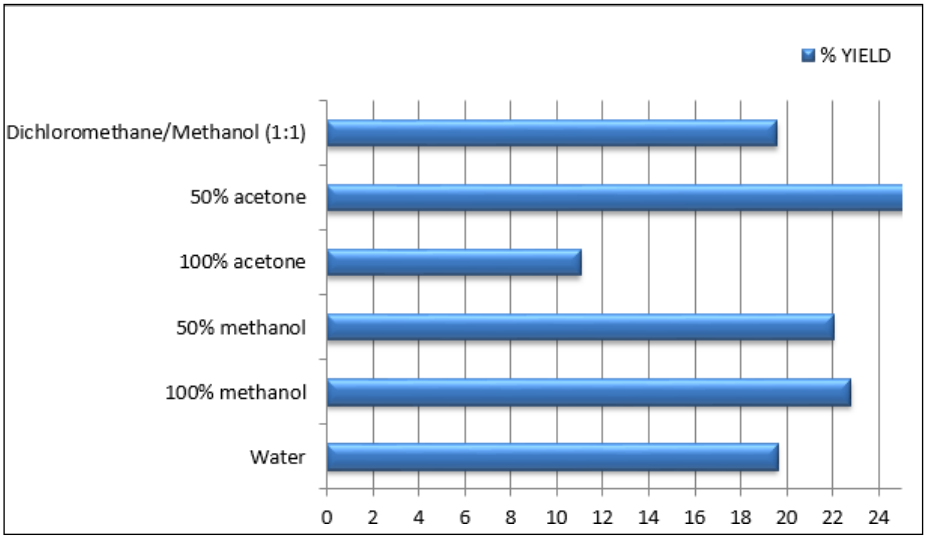
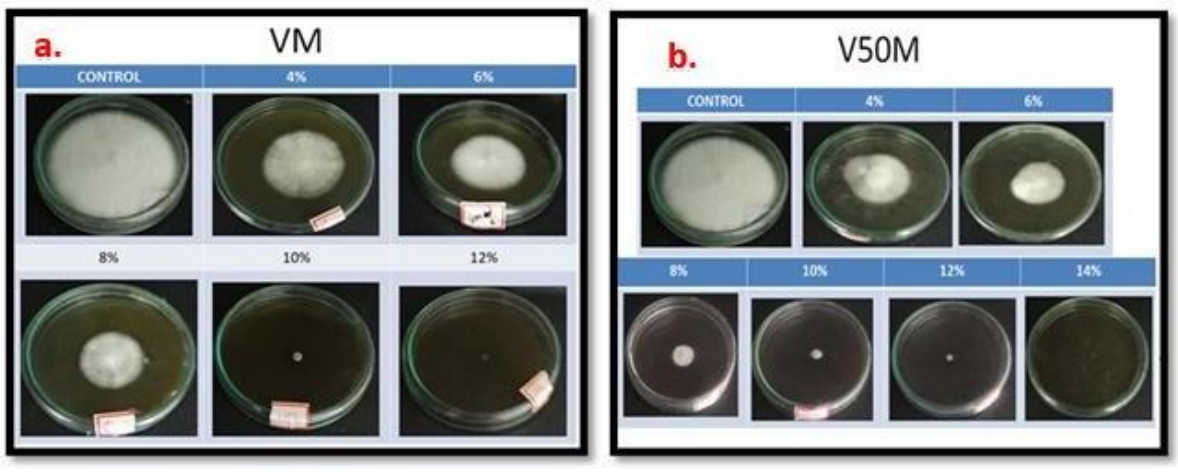


Fig 1: % Yield of extract

***In vitro* antifungal activity**

The antifungal activity of leaf extracts of selected plants (*Vitex negundo*) was examined *in vitro* against *F.oxysporum f. sp. Cumini* in concentrations of 4,6,8,10,12,14,16 % using different solvents such as methanol, 50% methanol, acetone, 50% acetone, Dichloromethane:methanol(1:1), and water. Using different extracts of selected plants, the quantity of *Fusarium oxysporum* mycelial growth suppression was determined. The results showed that all of the Bio-agents tested were effective at varying percentages in inhibiting mycelial growth of *Fusarium oxysporum f. sp. cumini in vitro*. After 8 days of incubation, the results shown in Fig.2 show that different plant extracts have an inhibitory effect on *f.oxysporum* at varied concentrations. The antifungal activity of different extract is tested at various concentrations (4,6,8,10,12,14,16%). After 8 days, mycelium development is measured for each concentration, and the radius of each concentration is compared to the radius of the control plate to determine the percent inhibition of plant extract against *F.oxysporum*. It is observed that % inhibition for methanol extract at five different concentrations (4,6,8,10,12%) are found to be 37.60%,44.80%,59.52%,97.60% and 100% respectively, for 50% methanol extract at six different concentration (4,6,8,10,12,14%) are found to be 47.75%,60.65%,84.21%,90.52%,97.46% and 100% respectively, for acetone extract at six different concentration (4,6,8,10,12,14%) are found to be 48.77%,63.68%,79.65%,92.5%,97.46% and 100% respectively, for 50% acetone extract at five different concentration (4,6,8,10,12%) are found to be 44.29%,72.34%,84.61%,88.43% and 100% respectively, for Dichloromethane: methanol extract at different concentration (4,6,8,10,12%) are found to be 50.03%,61.31%,77.25%,87.43% and 100% respectively, for water extract at different concentration (4,6,8,10,12,14,16%) are found to be 65.89%,79.53%,88.62%,94.43%,97.47%,98.87% and 100% respectively.



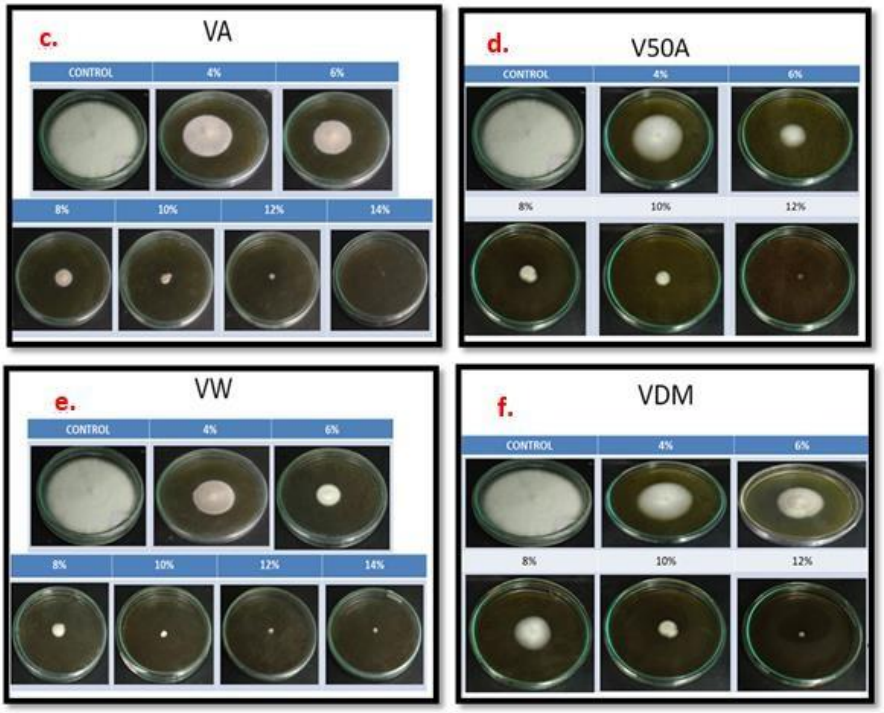


Fig 2: Efficacy study of *Vitex negundo* leaves against *fusarium oxysporum*: a. Methanol extract b.50% methanol c. Acetone extract d. 50% acetone extract e. Water extract f. Water extract Methanol: dichloromethane extract)

Means and standard errors of the mean were calculated for the zones of inhibition assessed in each case for the three sets of experiments, and the results are shown in the table: 3

Table 3: Means and standard error of the mean for plant efficacy against fungus

Vitex negundo							
	4%	6%	8%	10%	12%	14%	16%
Methanol	37.60±0.47	44.80±0.45	59.52±0.46	97.60±0.46	100±00	-	-
50 % methanol	47.75±0.56	60.65±0.45	84.21±0.40	90.52±0.41	97.46±0.31	100±00	-
Acetone	48.77±0.50	63.48±0.52	79.65±0.61	92.5±0.45	97.46±0.70	100±00	-
50% Acetone	44.29±0.58	72.34±0.49	84.61±0.50	88.43±0.59	100±00	-	-
Dichloromethane:Methanol	50.03±0.48	61.31±0.40	77.25±0.54	87.43±0.50	100±00	-	-
-Water	65.89±0.45	79.53±0.43	88.62±0.41	94.43±0.38	97.45±0.30	98.87±0.43	100±00
Control	00±00	00±00	00±00	00±00	00±00	00±00	00±00

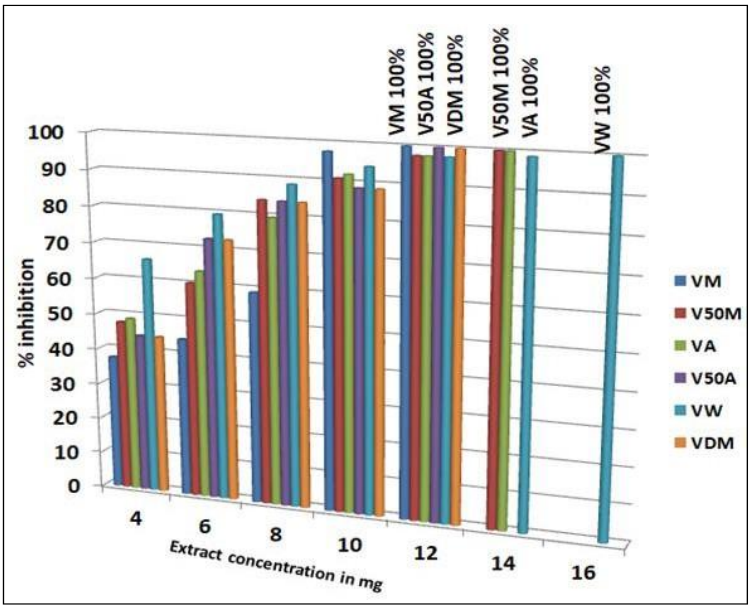


Fig 3: Effectiveness of different extract of *Vitex negundo* against *fusarium oxysporum*)

The highest inhibitory effect of methanol, 50% acetone, and dichloromethane: methanol extract was identified at 12%, whilst 50% methanol and acetone extract was found*9 5/36 at 14%, and water extract was found at 16%. By evaluating the efficiency of plant extracts against plant pathogenic fungi, we discovered three best *Vitex negundo* extracts (methanol, 50% acetone, dichloromethane: methanol) with the strongest inhibitory activity at the lowest concentration. As a result, this extract can be utilised to create natural fungicides. (Fig: 3)

The current study aims to optimise the simultaneous HPTLC fingerprint profiles of secondary metabolites in various *Vitex negundo* extracts. It reveals the presence of secondary metabolites such as alkaloids, flavonoids, and total phenolic compounds. The contour of the HPTLC at UV 254 and 366 nm, the densitogram, and the 3D display were illustrated.

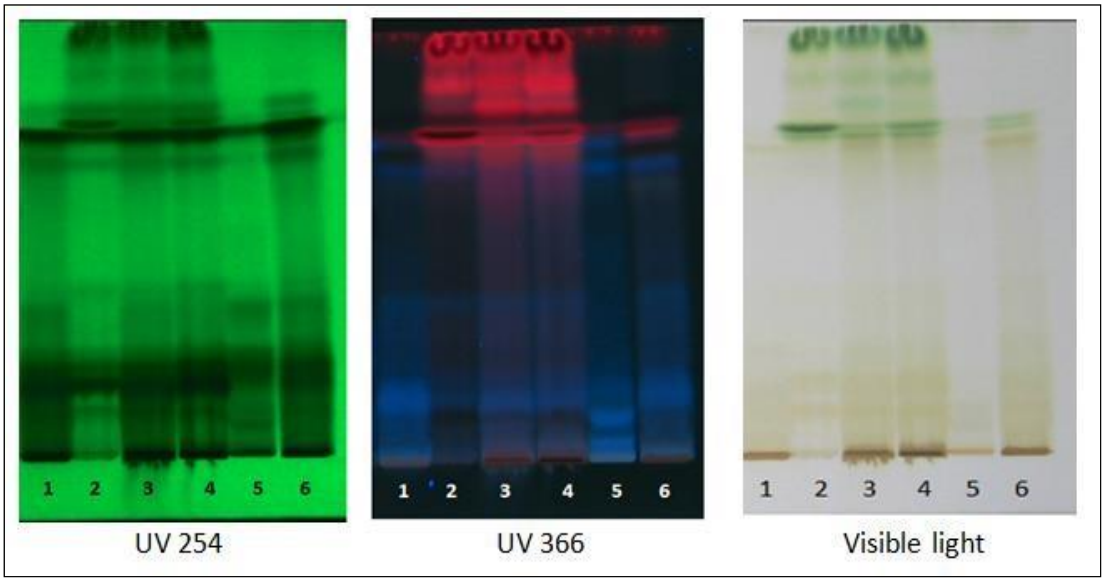


Fig 4: HPTLC Fingerprinting profile (Chromatogram) of Flavonoids for different extract of *Vitex negundo*: Track 1: 50% methanol, Track 2: Acetone, Track 3:methanol, Track 4:Dichloromethane:methanol,Track5:water and Track:6: 50%acetone)

Flavonoids Profile

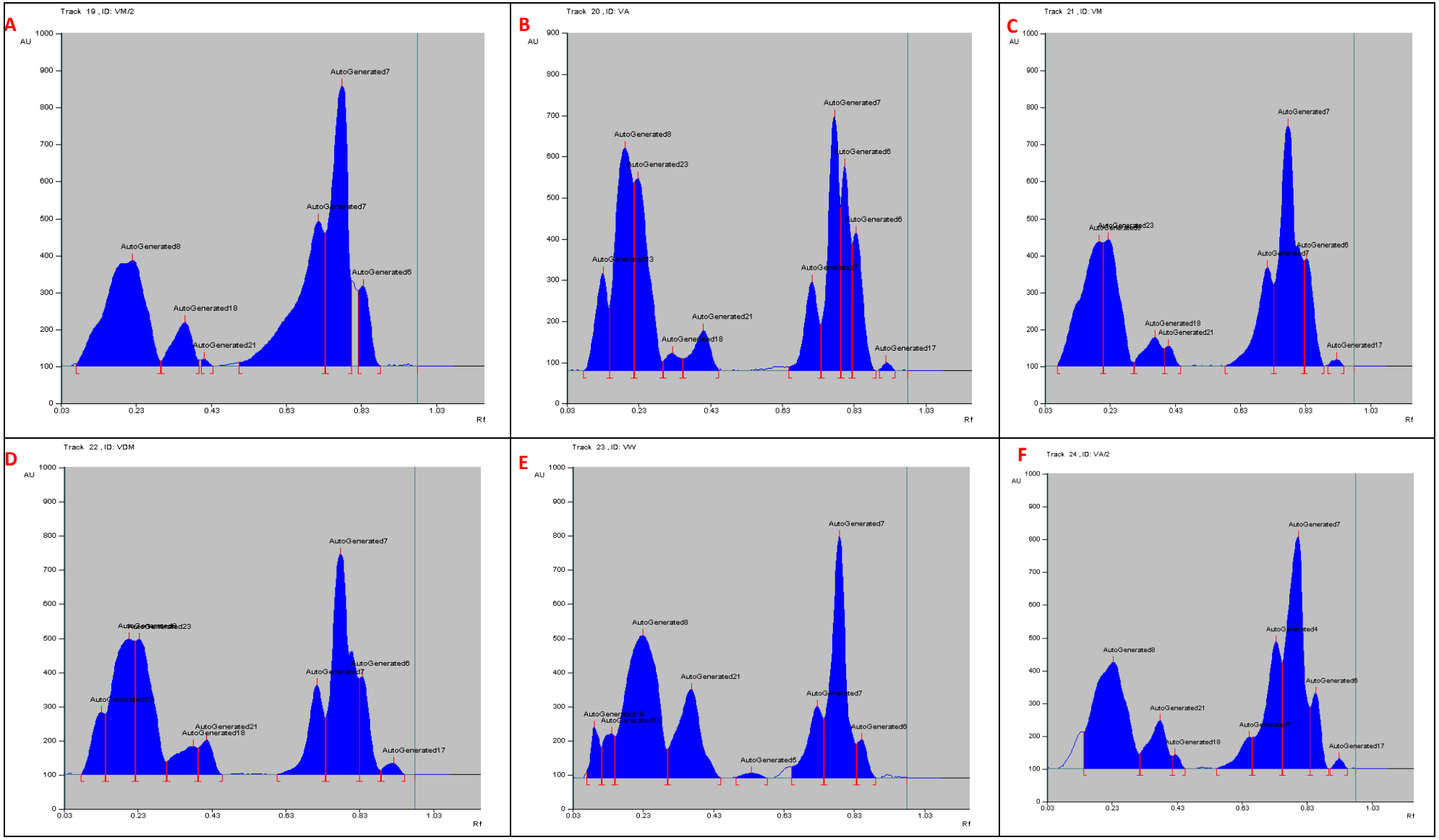
Methanol extract of *Vitex negundo* showed the presence of 8 bands and supported the existence of 1 kind of flavonoid with *Rf* values ranging from 0.07 to 0.89. 50 % methanol extracts had six bands, with one flavonoid band ranging from 0.15 to 0.9. Acetone extract exhibited ten bands and substantiated three types of flavonoids with concentrations ranging from 0.08 to 0.8.

In 50 % acetone, 8 bands were discovered, with 2 bands of flavonoid ranging from 0.15 to 0.9. Dichloromethane:methanol extract had two flavonoid bands ranging from 0.08 to 0.83 out of a total of nine bands. Water extract exhibited the existence of 8 bands and proved the presence of two kinds of flavonoids ranging from 0.07 to 0.84. The flavonoid bands with Rf values 0.3, 0.15, 0.23,0.3, 0.36, 0.58 and 0.66 displayed their unique presence in *Vitex negundo.* The HPLC analysis confirmed the flavonoid existence in all extract of *Vitex negundo*. (Table 4)

Table 4: HPTLC *Rf* value of different plant extract of *Vitex negundo* for flavonoids)

V50M		**VA**		**VM**		**VDM**		**VW**		**V50A**	
Rf value	Assign substance	*Rf* value	Assign substance	*Rf* value	Assign substance	*Rf* value	Assign substance	*Rf* value	Assign substance	*Rf* value	Assign substance
0.07	Unknown	0.08	Unknown	0.07	Unknown	0.08	Unknown	0.07	Unknown	0.15	Rutin
0.3	Flavonoid	0.15	Rutin	0.21	Unknown	0.15	Rutin	0.11	Unknown	0.32	Unknown
0.4	Unknown	0.22	Unknown	0.31	Unknown	0.23	Flavonoid	0.15	Rutin	0.42	Unknown
0.51	Unknown	0.3	Flavonoid	0.4	Unknown	0.31	Unknown	0.3	Flavonoid	0.55	Unknown
0.74	Unknown	0.36	Flavonoid	0.58	Flavonoid	0.4	Unknown	0.5	Unknown	0.66	Flavonoid
0.82	Unknown	0.65	Unknown	0.74	Unknown	0.61	Unknown	0.65	Unknown	0.76	Unknown
		0.74	Unknown	0.83	Unknown	0.74	Unknown	0.75	Unknown	0.84	Unknown
		0.8	Unknown	0.89	Unknown	0.83	Unknown	0.84	Unknown	0.9	Unknown
		0.83	Unknown			0.89	Unknown				
		0.9	Unknown								

Table 5: Peak densitogram of flavonoid for different extract of *Vitex negundo*:a. 50% methanol, b.Acetone, c. methanol, d. Dichloromethane:methanol,e. water f. 50%acetone)



Alkaloid Profile: The alkaloid chromatogram from HPTLC can be identified at UV 254 nm and 366 nm. The methanolic extract revealed 10 bands ranging from 0.08 to 0.95, including two bands containing alkaloid. The 50 % methanol extract included six bands, with one band of alkaloid ranging from 0.07 to 0.74. In all, 11 bands were found in acetone extract, including one alkaloid band ranging from 0.05 to 0.96. The 50 percent acetone extract had 7 bands, with one band of alkaloid ranging from 0.07 to 0.67. One band of alkaloid was detected in a dichloromethane:methanol extract out of ten bands with values ranging from 0.07 to 0.92. Water extract exhibited 9 bands with values ranging from 0.1 to 0.89, with 2 bands indicating the presence of alkaloid.

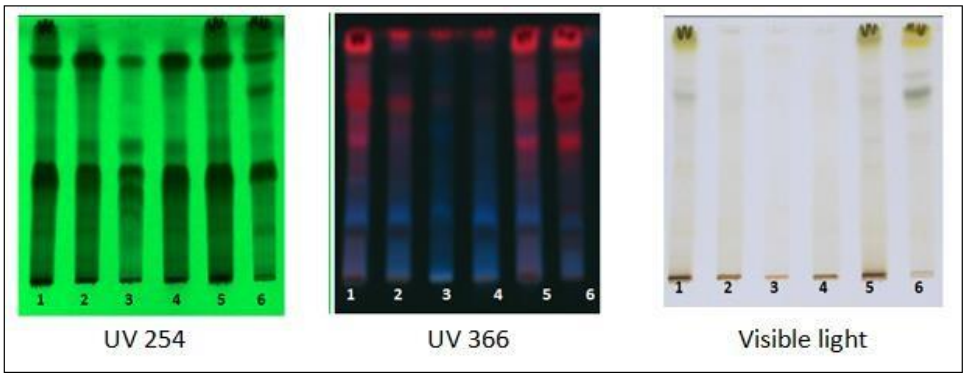


Fig 5: HPTLC Fingerprinting profile (Chromatogram) of Alkaloid for different extract of *Vitex negundo*: Track 1: Dichloromethane:methanol, Track 2: 50%acetone,Track 3: water, Track 4: 50% methanol,Track5: methanol and Track:6: Acetone)

Table 6: HPTLC *Rf* value of different plant extract of *Vitex negundo* for Alkaloid)

VDM		V50A		VW		V50M		VM		VA	
***Rf* value**	**Assign substance**	***Rf* value**	**Assign substance**	***Rf* value**	**Assign substance**	***Rf* value**	**Assign substance**	***Rf* value**	**Assign substance**	***Rf* value**	**Assign substance**
0.07	Unknown	0.07	Unknown	0.1	Nicotine	0.07	Unknown	0.08	Unknown	0.05	Unknown
0.15	Unknown	0.15	Unknown	0.2	Unknown	0.13	Unknown	0.16	Unknown	0.1	Nicotine
0.22	Colchicine	0.23	Unknown	0.3	Strychnine	0.23	Unknown	0.22	Colchicine	0.16	Unknown
0.28	Unknown	0.29	Unknown	0.39	Chelidonine	0.48	Alkaloid	0.28	Unknown	0.25	Unknown
0.5	Unknown	0.48	Alkaloid	0.47	Unknown	0.59	Unknown	0.48	Alkaloid 1	0.29	Unknown
0.6	Unknown	0.6	Unknown	0.59	Unknown	0.74	Unknown	0.6	Unknown	0.52	Unknown
0.69	Unknown	0.67	Unknown	0.66	Unknown			0.68	Unknown	0.61	Unknown
0.75	Unknown			0.76	Unknown			0.75	Unknown	0.67	Unknown
0.8	Unknown			0.89	Unknown			0.79	Unknown	0.82	Unknown
0.93	Unknown							0.95	Unknown	0.89	Unknown
										0.96	Unknown

Phenolics Profile: In the examined *Vitex negundo*, HPTLC separation of phenolics revealed good resolution and repeatable peaks. The existence of several types of phenolic bands was established by *Rf* values of 0.04,0.24,0.25,0.27,0.42,0.49,0.57,0.58,0.72 and 0.73. Acetone extract contains the greatest number of phenolic bands (4). In water extract of *Vitex negundo*, a minimal band (1) of phenolic compound has been found.

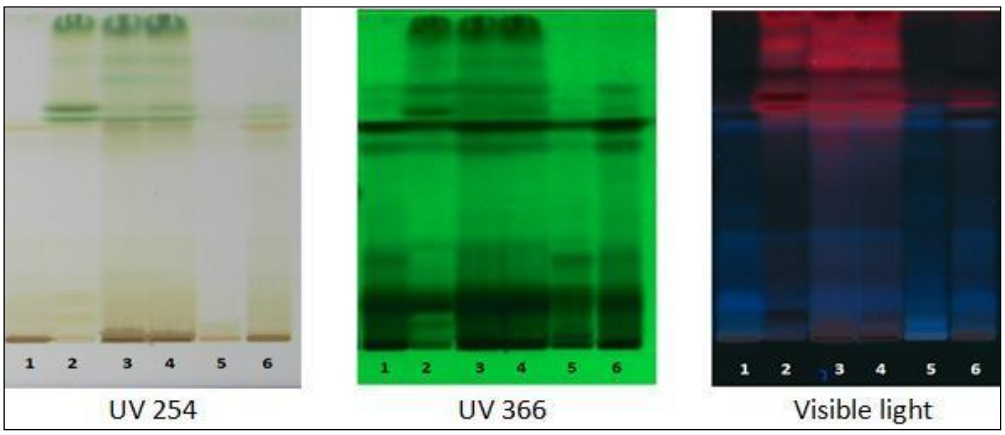


Fig 6: HPTLC Fingerprinting profile (Chromatogram) of Total phenolic compound for different extract of *Vitex negundo*: Track 1: 50% methanol, Track 2: Acetone, Track 3:methanol, Track 4:Dichloromethane:methanol,Track5:water and Track:6: 50%acetone)

Table 7: Peak densitogram of flavonoid for different extract of *Vitex negundo* a. Dichloromethane:methanol, b. 50%acetone,c. water, d. 50% methanol, e. methanol f. Acetone)

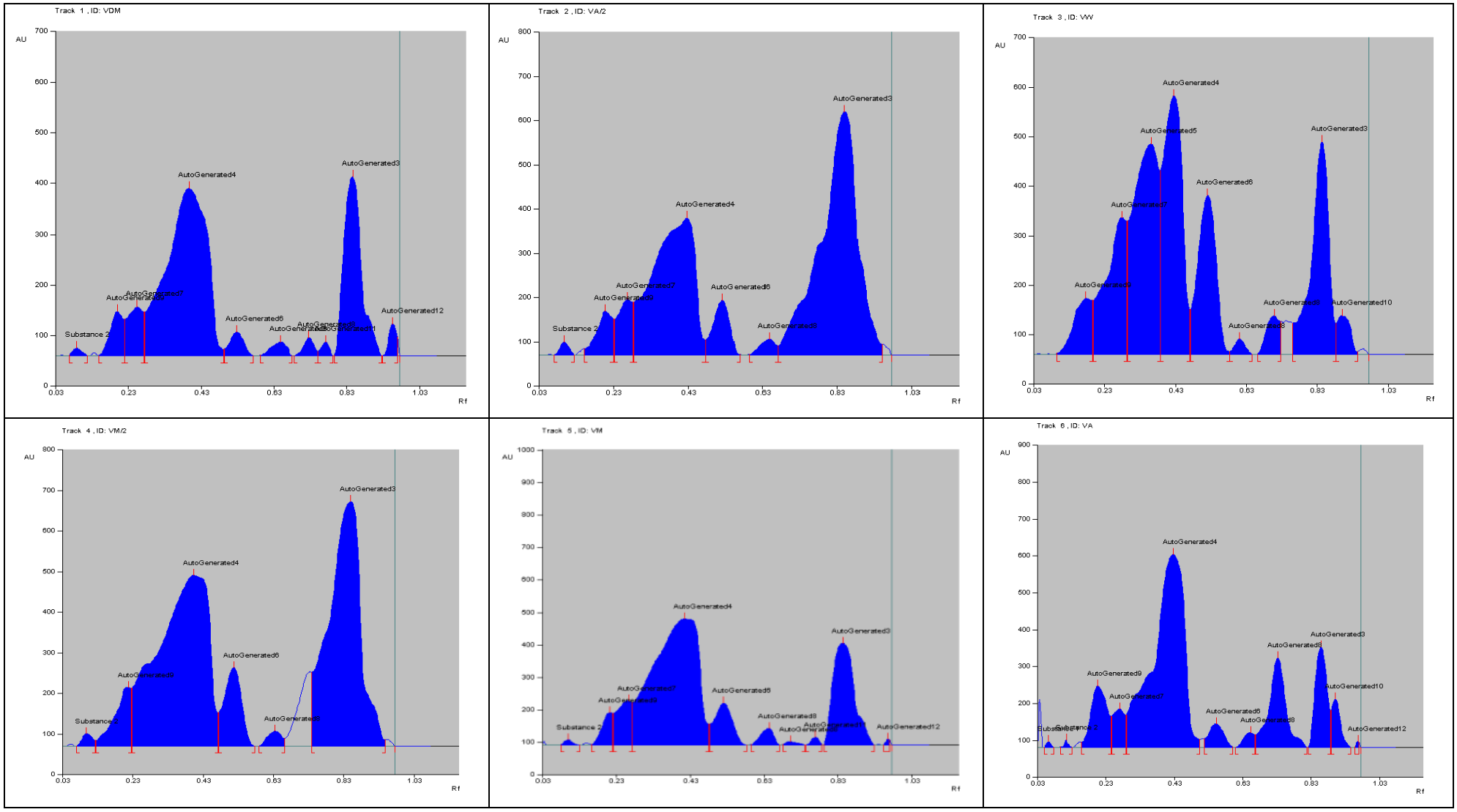


Table 8: Peak densitogram of total phenolic compound for different extract of *Vitex negundo*:a. 50% methanol, b.Acetone, c. methanol, d. Dichloromethane:methanol,e. water f. 50%acetone)

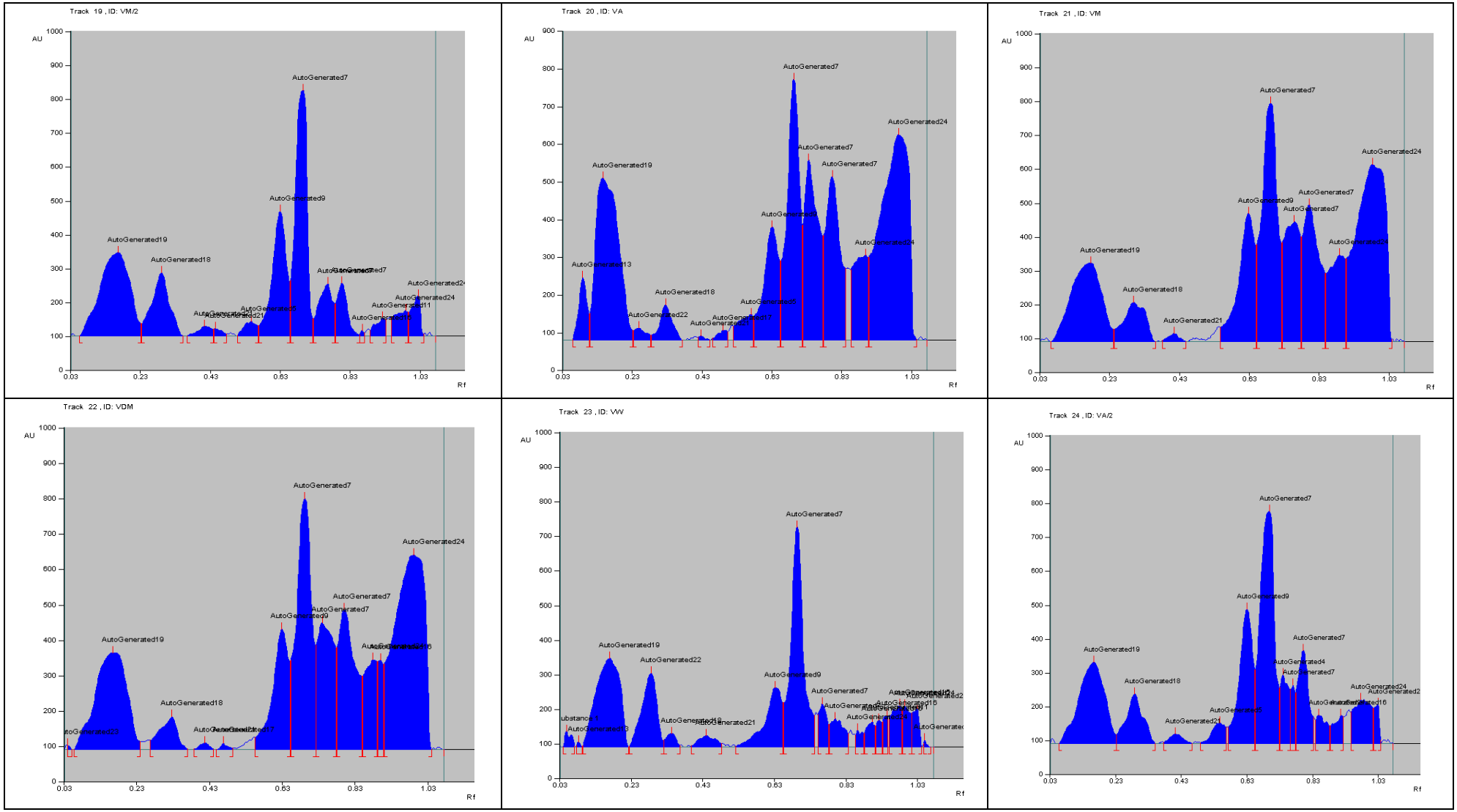


Table 9: HPTLC *Rf* value of different plant extract of *Vitex negundo* for total phenolic compound)

V50M		VA		VM		VDM		VW		V50A	
***Rf* value**	**Assign substance**	***Rf* value**	**Assign substance**	***Rf* value**	**Assign substance**	***Rf* value**	**Assign substance**	***Rf* value**	**Assign substance**	**V50A**	**Assign substance**
0.06	Unknown	0.06	Unknown	0.06	Unknown	0.04	Phenolics	0.04	Phenolics	0.06	Unknown
0.24	Phenolics	0.11	Unknown	0.25	Phenolics	0.06	Unknown	0.06	Unknown	0.24	Unknown
0.37	Unknown	0.24	Phenolics	0.38	Unknown	0.27	Catechin	0.1	Unknown	0.38	Unknown
0.44	Unknown	0.29	Unknown	0.55	Unknown	0.39	Unknown	0.23	Unknown	0.49	Phenolics
0.51	Unknown	0.42	Phenolics	0.65	Unknown	0.45	Unknown	0.32	Unknown	0.57	Phenolics
0.57	Phenolics	0.46	Unknown	0.73	Quercetin	0.56	Unknown	0.4	Unknown	0.66	Unknown
0.66	Unknown	0.52	Unknown	0.78	Unknown	0.65	Unknown	0.52	Unknown	0.73	Quercetin
0.73	Quercetin	0.58	Phenolics	0.85	Unknown	0.73	Quercetin	0.66	Unknown	0.76	Unknown
0.79	Unknown	0.66	Unknown	0.91	Unknown	0.78	Unknown	0.75	Unknown	0.78	Unknown
0.86	Unknown	0.72	Phenolics			0.85	Unknown	0.79	Unknown	0.84	Unknown
0.89	Unknown	0.76	Unknown			0.9	Unknown	0.86	Unknown	0.89	Unknown
0.95	Unknown	0.86	Unknown			0.91	Unknown	0.88	Unknown	0.95	Unknown
1	Unknown	0.91	Unknown					0.91	Unknown	1.02	Unknown
								0.93	Unknown		
								0.95	Unknown		
								0.99	Unknown		
								1.02	Unknown		
								1.05	Unknown		

Conclusion

To conclude, *Vitex negundo* demonstrated a broad spectrum of action against fungal strains. To design realistic management approach, it is necessary to understand the compatibility of bio-control agents with other components of the production system. *F. oxysporum f. sp. Cumini* disease can yield significant losses in cumin. With preliminary testing and HPTLC results, the current study demonstrated the existence of essential phytochemicals in *Vitex negundo*, as well as the inhibitory activity of plant extract against plant pathogenic fungi. According to current research, certain botanical extracts are a source of cost-effective and non-hazardous fungicides against FOC, as well as they do not have any human or environmental, health hazard or implications, so same plant extracts such as *Vitex negundo* could be a good antifungal efficacy, which may be used for formulating new, safer, and ecofriendly fungicides.

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