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## Determination of bioactive compounds by flash chromatographic fractionation from *Tecomella undulata* (Sm.) seem and its biological applications

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### Abstract

Plant extracts always provide ample opportunities for the discovery of newer bio-compounds for various applications. The specific orientation towards the isolation; purification and identification are the foundation for various functionalities addressed in modern sciences. Since plant residue possess numerous composite and promising cure elements, the present investigates the medicinal plant *Tecomella undulata* (sm.) Seem for various biological applications. The bioactive compounds isolated and crude drugs were analyzed for anti-bacterial activity and larvicidal activity. And the isolated compound by flash chromatography envisioned the possible zone of inhibition by specific compound isolated. For the evaluation of antibacterial study, three microbes viz., *Vibrio* sp., *Staphylococcus albus* and *Proteus vulgaris* were selected. Plant crude concentrations was (1.5, 2.5 and 5 mg/disc); and Streptomycin was the positive control. Notably out of these three extracts, the antibacterial effect of *T. undulata* of the ethyl acetate extract was quite negative to certain pathogens. *Proteus vulgaris* showed 13 mm inhibition at 5 mg/disc concentration of the extract. Mosquito larvicidal effect of *T. undulata* extracts, 4<sup>th</sup> instar larvae of *Culex quinquefasciatus* was used. Three extracts were taken at three different concentrations (62.5, 125, 250 ppm). The results were given as percentage of mortality (Table 4). For the estimation of larvicidal activity using hexane extract at 62.5 ppm concentration, R1, R2 and R3 showed 10% and the cumulative percentage was 10(%) out of 100%. R1, R3 mortality was 10% and R2 was 20% mortality and it resulted cumulative percentage 13.33(%). To evaluate the antibacterial activity of ethyl acetate extract five fraction has been taken by the flash chromatography - F1, F2, F3, F4, and F5. These five fractions were treated with same three pathogens that were used in the antibacterial effect of the extracts viz., *Vibrio* sp., *Staphylococcus albus* and *Proteus vulgaris*, and streptomycin was taken as the positive control. The cumulative results indicate the probable elements of *T.undulata* to ameliorate the biomedical and pharmaceutical sector to provide a positive underlying principle for assessing *T.undulata* for the treatment against bactericidal and mosquitocidal ailments.

**Keywords:** Chromatography. concentrations. antibacterial. streptomycin. inhibition

### Introduction

The medicinal plant *Tecomella undulata* (Sm.) Seem is a pharmaceutically important plant <sup>[1]</sup>. In recent past plant based secondary metabolites and derivatives have gained attention of life sciences sector due to its effective therapeutic nature <sup>[2]</sup>. However, this plant *T.undulata* is an indigenous tree of India and belongs to family Bignoniaceae <sup>[3]</sup>. Various experimental results showed that this plant improves insulin sensitivity and have a rich therapeutic role in the prevention disease <sup>[4]</sup>. Some of the compounds isolated from this plant are stigmaterol, campesterol, Lapachol and Radermachol. More specifically the extract of this plant is used in treatment of AIDS <sup>[5]</sup>. The hepatoprotective action of *T. undulata* bark extract considered to be traditional in solving the induced liver damage <sup>[6]</sup>. The biological assay such as antimicrobial activity, analgesic and anti-inflammatory, anti-oxidant and hepatoprotective elements have been reported frequently <sup>[7-9]</sup>. The stem part of this plant is efficiently used as a blood purifier <sup>[10]</sup>. The major part of usefulness of plant relies on treating dread disease like liver and spleen diseases <sup>[11]</sup>. The report by <sup>[12]</sup> in isolation of betulinic acid and ursolic acid had a effective anti-HIV -human immunodeficiency virus that holds considerable attention earlier. There had been reports in isolation of  $\beta$ -sitosterol pharmaceutical analysis in early days <sup>[13]</sup>. However, extraction and screening of alkaloids were done in recent past for pharmaceutical analysis <sup>[14]</sup> and recently silver nanoparticles also was synthesized using same plant material <sup>[15]</sup>. Adding to it methanolic extract of *T. undulata* said to be hepatoprotective <sup>[16]</sup>. But there has been very limited reference on larvicidal activity in *T. undulata*. Therefore it is befitting to find the

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bioactive compounds from *T.undulata* using flash chromatographic technique in order to study and evaluate the composition of these fractions and determine their antibacterial and larvicidal activity for the same.

## Material and methods

### Collection of Plant material

The bark of *T. undulata* (Sm.) Seem. were collected from Srivilliputhur in Virudhunagar district. The plant was identified and authenticated.

### Extraction

The barks were chopped into small pieces, air dried, coarsely powdered with an electrical blender and stored in an airtight container. The powdered form of plant (500 g) was sequentially extracted with 3 L of hexane, ethyl acetate and ethanol for 48 hours' time period respectively for each solvent by cold percolation method. The extracts were then concentrated in a rotary vacuum evaporator at reduced pressure at 40° C. The extracts were stored at 4 °C, until used.

### Bioassay guided isolation of the active fraction

Due to the significant antibacterial efficacy of the ethyl acetate extract, it was selected for fractionation; 2.5 g. of the extract was added to acetone, and it was adsorbed over silica gel (60-120 mesh). In a flash chromatographic system (Buchi Pure C-810), the admixture was loaded and eluted through a silica gel column (50 x 9 cm; Silica gel: Acme, 230-400 mesh). The contents were eluted with hexane, mixtures of hexane and ethyl acetate and then with ethyl acetate and methanol in increasing polarity. The elutants were divided

into five fractions on the basis of its polarity and their antibacterial effect was evaluated.

### Evaluation of the antibacterial effect of *T. undulata*

Antimicrobial activities of *T.undulata* extracts were evaluated using disc diffusion method [17]. Petri plates were prepared with 20 mL of sterile Muller Hinton agar (MHA, Himedia). The selected human pathogens such as were swabbed on top of the solidified media. The antibacterial efficacies of the extracts were tested at 1.25-5.0 mg/disc concentrations; for fractions the dose was fixed as 20 µg/mL. Streptomycin (25 µg/disc) was used as the positive control.

### Anti-inflammatory effect of *T.undulata* using egg albumin denaturation assay

Protein denaturation is the cause of inflammatory and arthritic diseases. As a result, anti-inflammatory drugs can benefit from agents with the ability to denature proteins. The *in vitro* anti-inflammatory effect of *T. undulata* was tested against the denaturation of egg albumin in the percent investigation. Briefly, in 2.8 mL of pre-warmed phosphate buffered saline (PBS, pH 6.4), 0.2 mL of egg albumin was added and incubated at 37 °C for five minutes. Then 2 mL of *T. undulata* extracts at 200 µg/mL concentration was added. The tubes in control groups were treated with PBS alone; the tubes in the positive control group were treated with acetyl salicylic acid (200 µg/mL). After 15 min incubation at 37 °C, the tubes were heated at 70 °C for 5 min and the degree of protein denaturation was read at 660 nm using a spectrophotometer. The inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition of protein denaturation} = \frac{\text{Absorbance of untreated} - \text{Absorbance of treated}}{\text{Absorbance of untreated}} \times 100$$

### Evaluating mosquito larvicidal effect of *T. undulata* on *Cule quinquefasciatus* larvae

#### Larvae rearing

*Culex quinquefasciatus* larvae (fourth instar) were collected in and around Palayamkottai and were used in the study. The rearing of the same was done at laboratory with standard methods [18].

#### Larvicidal assay

The standard protocol was followed for this assay recommended by WHO. The extracts toxicity procedure was done placing 20 mosquito larvae (*Culex quinquefasciatus*) into 100 mL of water with extracts into a beaker. The extracts were diluted using DMSO as a solvent with concentrations (62.5, 125, 250 ppm). The dead larvae were counted after 24 h, and the percentage of mortality was reported from the average of three replicates. The percentage mortality was calculated using Formula (1) and the corrected percentage mortality data using standard Abbott's (1925) Formula (2).

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100 \dots \dots \dots (1)$$

$$\text{Corrected percentage of mortality} = \frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100 \dots \dots \dots (2)$$

### Statistics

One way ANOVA followed by Student's *t* test (GraphPad Prism version 8.0.2) was used to assess the significance among the variables and the level of significance was set at *P* ≤ 0.05.

## Results & Discussion

### Antibacterial assay

To evaluate the antibacterial effect of *T. undulata* extracts, three different microbes viz., *Vibrio* sp., *Staphylococcus albus* and *Proteus vulgaris* were selected. The extracts were taken in three different concentrations (1.5, 2.5 and 5 mg/disc); streptomycin was taken as the positive control. These results

the anti-bacterial assay was assessed for zone of inhibition; the high zone of inhibition showed high antimicrobial effect and vice versa (Table 3). Three different concentrations of hexane extract were taken for the evaluation against three pathogens. The hexane extract showed 10 mm inhibition against *Vibrio* sp. at 5 mg/disc concentration; at lower concentrations no activity was recorded. Result against *Staphylococcus albus* showed 8 mm inhibition against positive control, 8 mm inhibition at 1.5 mg/disc, 8 mm inhibition at 2.5 mg/disc and 11 mm inhibition at 5 mg/disc. Out of these three hexane concentrations against *Staphylococcus albus*, 5 mg/disc showed highest zone of inhibition (activity) and lower concentrations such as 1.5 and

2.5 almost showed same results. Result against *Proteus vulgaris* showed 13 mm inhibition at positive control and, 10 mm inhibition at 1.5 mg/disc, 11 mm inhibition at 2.5 mg/disc and 14 mm inhibition at 5 mg/disc; out of these three concentrations 5 mg/disc showed highest zone of inhibition (14 mm) lowest is at lower concentration 1.5 mg/disc (10 mm) and 5 mg/disc (14 mm) showed more activity than the control (13 mm). In this antibacterial activity test by using hexane extract with different concentration highest activity is against *Proteus vulgaris*; it showed 14 mm zone at 5 mg/disc concentration of the extract. Three different concentrations (1.5 mg/disc, 2.5 mg/disc, 5 mg/disc) of ethyl acetate extract were taken for the evaluation of antibacterial activity, against three pathogens. The ethyl acetate extract was treated against *Vibrio* sp. showed 7 mm inhibition at 1.5 mg/disc, 8 mm inhibition at 2.5 mg/disc and 11 mm inhibition at 5 mg/disc. Out of these three concentrations 5 mg/disc gave (11 mm) more inhibition against *Vibrio* sp. and it was higher than the activity present in the positive control. 2.5 mg/disc and control showed almost same activity. Lower concentration (1.5 mg/disc) showed low inhibition. The ethyl acetate extract was administered against *Staphylococcus albus* showed 8 mm inhibition at 1.5 mg/disc, 10 mm inhibition at 2.5 mg/disc and 11 mm inhibition at 5 mg/disc, respectively. Out of these three concentrations 5 mg/disc showed highest zone of inhibition against *Staphylococcus albus* (11 mm) and lowest inhibition was present in 1.5 mg/disc (8 mm). The ethyl acetate extract was treated against *Proteus vulgaris* showed 10 mm inhibition at 1.5 mg/disc, 11 mm inhibition at 2.5 mg/disc and 13 mm inhibition at 5 mg/disc, respectively. Out of these three concentrations 5 mg/disc showed highest inhibition against *Proteus vulgaris* (13 mm) and lowest inhibition at 1.5 mg/disc (10 mm). In this assay ethyl acetate extract showed highest inhibition against *Proteus vulgaris*; it showed 13 mm inhibition at 5 mg/disc concentration of the extract. Out of the three extracts, the ethyl acetate extract showed comparatively more activity than other two extracts (Table 3). Three different concentrations (1.5 mg/disc, 2.5 mg/disc, 5 mg/disc) of ethanol extract were taken for the evaluation of antibacterial activity, against three pathogens. According to the results, only the positive control showed the antibacterial effect, and no efficacy was found for the ethanol extract at all tested concentrations against *Vibrio* sp. Ethanol extract was treated against *Staphylococcus albus* showed 11 mm inhibition at 5 mg/disc and no activity was recorded at lower concentrations. Ethanol extract was treated against *Proteus vulgaris* showed 9 mm inhibition at 1.5 mg/disc, 11 mm inhibition at 2.5 mg/disc and 14 mm inhibition at 5 mg/disc, respectively. Out of these three concentrations, 5 mg/disc showed highest zone of inhibition (14 mm) against *Proteus vulgaris* and lowest concentration showed lowest rate of inhibition (Table 3). Out of these three extracts used for evaluation of the antibacterial effect of *Tecomella undulata*, the ethyl acetate extract was found to be more active against selected pathogens. While other two extract used for the evaluation not (hexane and ethanol) showed the result as ethyl acetate showed. Comparatively Ethyl acetate showed more activity than other extract used for the evaluation. Here a group of molecules was exhibiting the result, so we can further take this extract for fraction test to find specific molecules that having the antibacterial activity. Inhibitory effect was found in chloroform and hexane extracts [19]. Anti-microbial assay on stem bark of *T. undulata* with petroleum ether, acetone, alcohol and water extracts were used for carried out against *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S.*

*aureus* [20]. Comparing to my study the method, solvent and pathogen conducted in the study is different and the inhibition rate in my result is higher than this. On this discussion up on previous studies shows that antimicrobial study using leaf & bark extract was conducted but no one was carried out the antibacterial activity on the specific extract fraction to find the specific molecules that exhibit the antibacterial activity and it was a research gap.

### Anti- inflammatory assay

According to the study about anti- inflammatory activity of *T. undulata*, absorbance in egg albumin denaturation assay with *Tecomella undulata* extract at 200 µg/mL concentration shown significant stabilization towards the egg albumin denaturation assay by an anti-inflammatory method. Mean value of absorbance in control showed 1.842%, in hexane extract 1.629%, in ethyl acetate extract 1.597% in ethanol extract 1.751% and in acetyl salicylic acid 1.574%. Here Acetyl salicylic acid was taken as positive and out of these extract in ethyl acetate and acetyl salicylic acid extract showed the significantly low absorbance, the two values significantly varied from the negative control values and the both showed lower-level absorbance from the negative control. Here the Hexane and Ethanol extract was up to the level of negative control and they showed high absorbance and hence low activity. When the protein denaturation decreased, the turbidity will also be decreased (Figure 4). Inhibition of egg albumin denaturation by *T. undulata* extracts at 200 µg/mL concentration showed significant stabilization towards the egg albumin denaturation assay by an anti - inflammatory method. Inhibition in hexane extract showed 39.3%, in Ethyl acetate extract 45.275%, in ethanol extract 16.750% and in acetyl salicylic acid 49.390%. Here hexane and ethanol extracts showed decreased inhibition and the inhibition for the ethanol extract was the lowest. In the case of absorbance in egg albumin denaturation, it was up to the level of control, so the hexane, ethanol extracts absorbance was high and Inhibition rate is low. But in the case of ethyl acetate and acetyl salicylic acid they have high inhibition rate when comparing to the other two extracts and absorbance was low (Figure 4). In this study ethyl acetate extract and acetyl salicylic acid extracts shown anti-inflammatory activity because they are showing low absorbance and inhibition rate. The bark of *T. undulata* was identified as a pharmacological potential for chronic inflammation [21] (Figure 4). Consecutively, Methanolic extracts of whole plants of *T. undulata* was tested with mice. The results showed only analgesic property [22]. *T. undulata* is a source of chemicals such as quinonoid and iridoid glucoside [23]. Earlier reports had been documented as Undulatin, glucoside isolated [24]. Reports on Petroleum ether extract yields a compound namely radermachol, an unusual rare pigment [25]. Another compound Quercetin, which has effective role anti-cancer and isolated for the first time and reported [26].

### Larvicidal activity

To evaluate the Mosquito larvicidal effect of *T. undulata* extracts, 4<sup>th</sup> instar larvae of *Culex quinquefasciatus* was used. Three extracts were taken at three different concentrations (62.5, 125, 250 ppm). The result was given as percentage of mortality (Table 4). For the estimation of larvicidal activity using hexane extract at 62.5 ppm concentration, R1, R2 and R3 showed 10% and the cumulative percentage was 10(%) out of 100%. R1, R3 morality was 10% and R2 was 20% morality and it resulted cumulative percentage 13.33(%). Hexane fraction concentration at 250 ppm R1 and R2 morality was 10% and R3 showed no result, and it resulted cumulative percentage 6.66(%). Here by using hexane extract

for the estimation of larvicidal effect no good result is obtained (Table 4). For the estimation of larvicidal activity using ethyl acetate extract showed no results in all three concentrations (62.5, 125 and 250 ppm) that used in the estimation purpose. So, in the ethyl acetate extract no mosquito larvicidal principle was found (Table 4). For the estimation of larvicidal activity using ethanol extract at the lowest concentration 62.5 ppm, R1, R2 and R3 showed no results. Ethanol extract concentration at 125 ppm R1, R2 mortality was 10% and R3 showed no result and it resulted cumulative percentage 6.66(%). Ethanol extract concentration at 250 ppm R1, R2 and R3 showed no results (Table 4). Mosquito larvicidal effect of *T. undulata* extracts showed no good result. Every extract (Hexane, Ethyl acetate and Ethanol) used for the study with low to high concentration (62.5, 125, and 250 ppm) only showed cumulative percentage below 20%. So, the result that got from the study was not good for the further studies. No one has carried out Mosquito larvicidal effect in this *T. undulata* before so it was a research gap (Table 4). *T. undulata* ethyl acetate extract showed more activity than other extracts used for the evaluation here a group of molecules are exhibiting the result, so we further took this extract for fraction test to find specific molecules that having the antibacterial activity.

### Fractionation of biocompounds

To evaluate the antibacterial activity of ethyl acetate extract five fraction has been taken by the flash chromatography - F1, F2, F3, F4, and F5. These five fractions were treated with same three pathogens that were used in the antibacterial effect of the extracts viz., *Vibrio* sp., *Staphylococcus albus* and *Proteus vulgaris*, and streptomycin was taken as the positive control (Table 5). Five different fractions of ethyl acetate extract were taken for the evaluation of antibacterial activity, against three pathogens. Ethyl acetate fractions showed 10 mm inhibition in control, 5 mm inhibition in F2, 5 mm inhibition in F3, 4 mm inhibition in F4 and F1, F2 showed no result against *Vibrio* sp. Here F2, F3 and F4 were showed inhibition against the pathogen. Out of these three fractions F2 and F3 showed higher inhibition (Table 5). Five different Ethyl acetate fractions were taken for the evaluation of antibacterial activity, against *Staphylococcus albus*, Here the result showed as 11 mm inhibition in control, 4 mm inhibition in F1, 7mm inhibition in F2, 6 mm inhibition in F3, 6 mm inhibition in F4, and 5 mm inhibition in F5. Against *Staphylococcus albus* all five fractions has showed result and F2 showed highest inhibition. Five different ethyl acetate fractions were taken for the evaluation of antibacterial activity, against *Proteus vulgaris*, Here the result showed as 10 mm inhibition in control, F1, F2 showed no result and 5 mm inhibition in F2, 5 mm inhibition in F3, 4 mm inhibition in F4 against *Proteus vulgaris* Here F2, F3 and F4 were showed inhibition against the pathogen. Out of these three fractions F2 and F3 showed higher inhibition (Table 5). When try to compare with the previous studies, no one has taken this

specific *Tecomella undulata* extract for the evaluation. Bioassay guided isolation of the active fraction showed the significant antibacterial efficacy of the ethyl acetate extract.

### Conclusion

*T. undulata* (Bignoniaceae) is an endangered medicinal plant widely used in *Ayurveda*; it has been commonly found in Rajasthan and now planted by Tamil Nadu Forest Department in many southern districts of Tamil Nadu. This research had fulfilled the objective of antibacterial fraction of this plant, in order to evaluate its anti-inflammatory and mosquito larvicidal potential. The antibacterial effect of *T. undulata* extracts was evaluated against three different microbes (viz., *Vibrio* sp., *Staphylococcus albus* and *Proteus vulgaris*). The anti-inflammatory effects of the extracts were evaluated using egg albumin denaturation assay; mosquito larvicidal's outcome was evaluated using lab reared *Culex quinquefasciatus* larvae. Final outcome indicated that the ethyl acetate extract was more efficacious against selected pathogens; other two extracts showed comparatively lower antibacterial efficacy. Literature survey showed that antimicrobial study using leaf and bark extracts; however, no one was aimed to isolate the antibacterial principle from this plant. Since *T.undulata* ethyl acetate extract showed more activity than other extracts, it was taken it for bioassay guided fractionation. The results indicated that a group of molecules that work synergistically exhibited a profound antibacterial effect. This might be the reduced antibacterial activity of all the fractions. The ethyl acetate extract also exhibited anti-inflammatory effect. While, the extracts of *T. undulata* did not exhibit any significant mosquito larvicidal effect and the mortality was about 20%. Further studies are required to isolate and characterize the antibacterial and anti-inflammatory principle from this endangered and medicinally important plant. Further studies can be conducted in anti-inflammatory activity on ethyl acetate fraction.

### Declarations

The Ethics approval and Consent to participate  
The authors declare that we have NO conflict of Interest. This article does NOT contain any studies with human or animal subject performed by any of the authors.

**Consent for Publication:** Not Applicable

**Competing Interest:** The Authors declare that this research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of Interest.

**Availability of data and materials:** All data generated or analyzed during this study are included in this article.

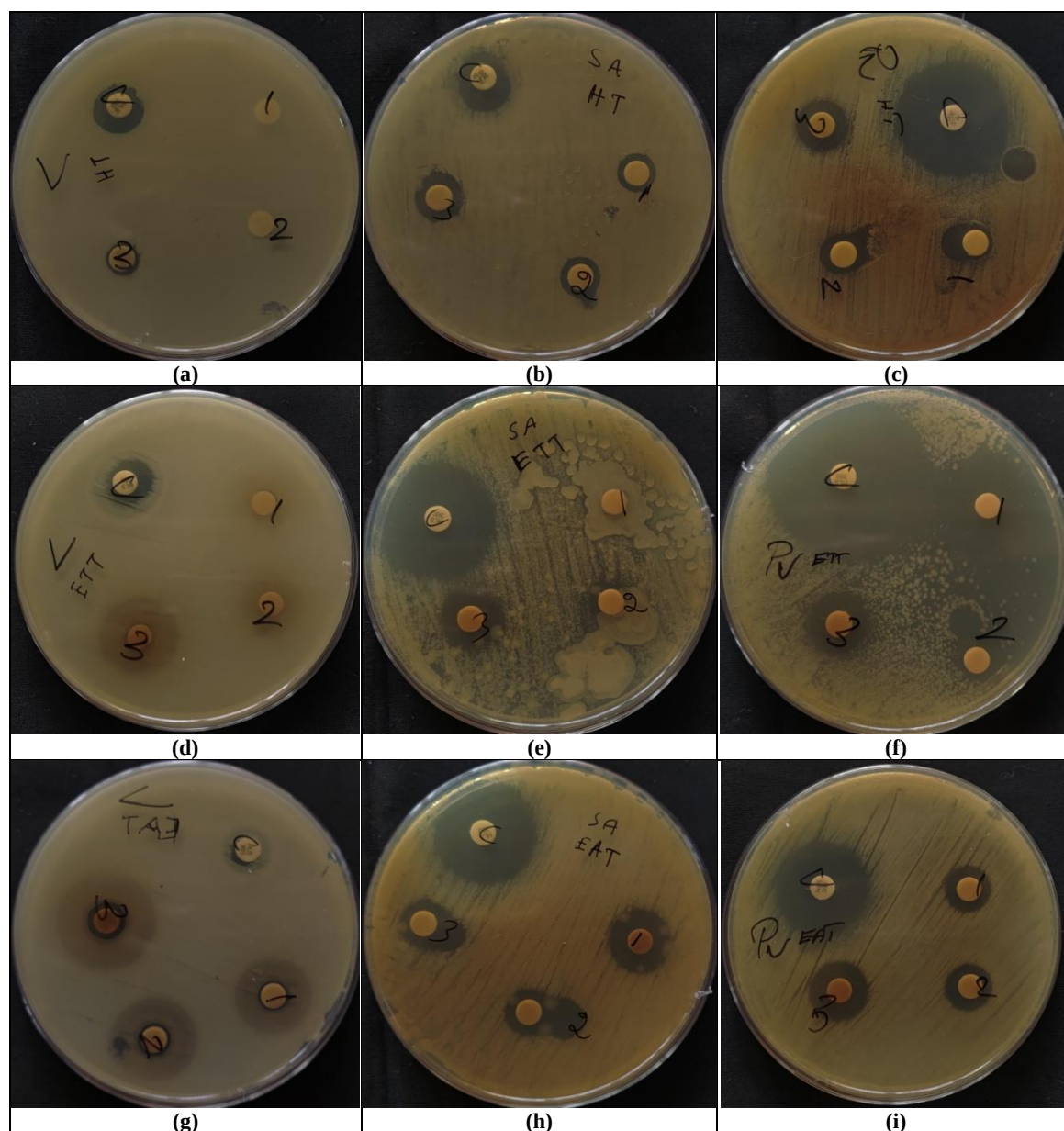
**Funding:** This research paper is a presentation of our original research work. It does NOT support by any funding.



**Fig1:** Flash chromatography



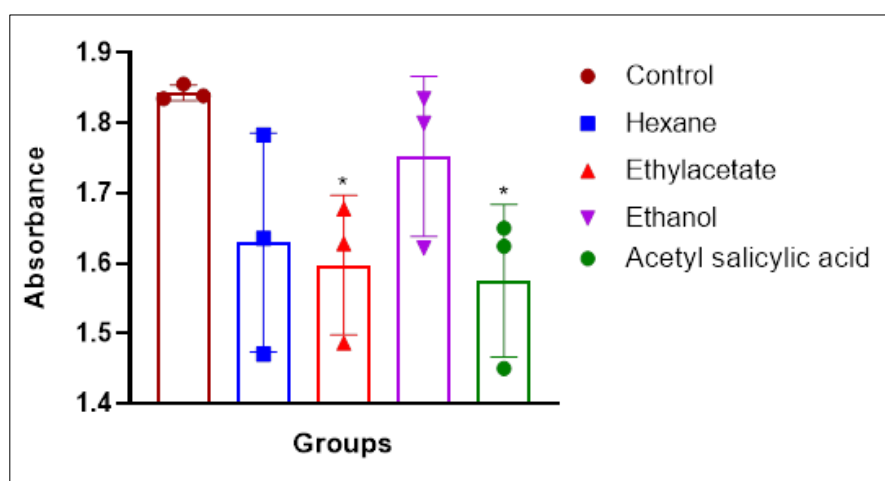
**Fig 2:** *Tecomella undulata* (Sm.) S



**Fig 3:** Representative photographs showing the antibacterial effect of *T.undulata* extracts

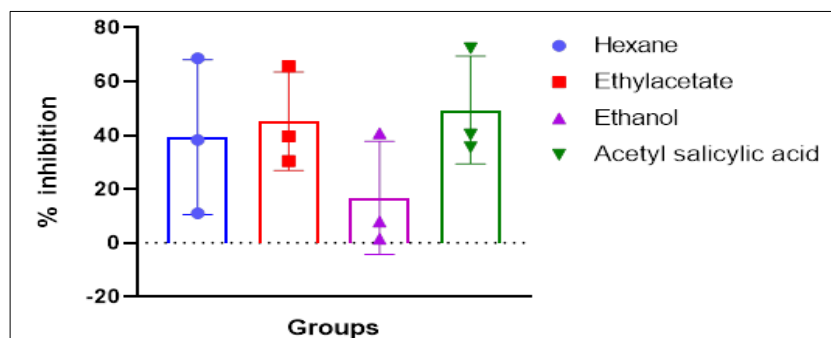
**a-c** are *Vibrio* sp., *Staphylococcus albus* and *Proteus vulgaris* treated with *Tecomella undulata* hexane extract; **d-f** are *Vibrio* sp., *Staphylococcus albus* and *Proteus vulgaris* treated

with *T. undulata* ethyl acetate extract; **g-i** are *Vibrio* sp., *Staphylococcus albus* and *Proteus vulgaris* treated with *T. undulata* ethanol extract, respectively



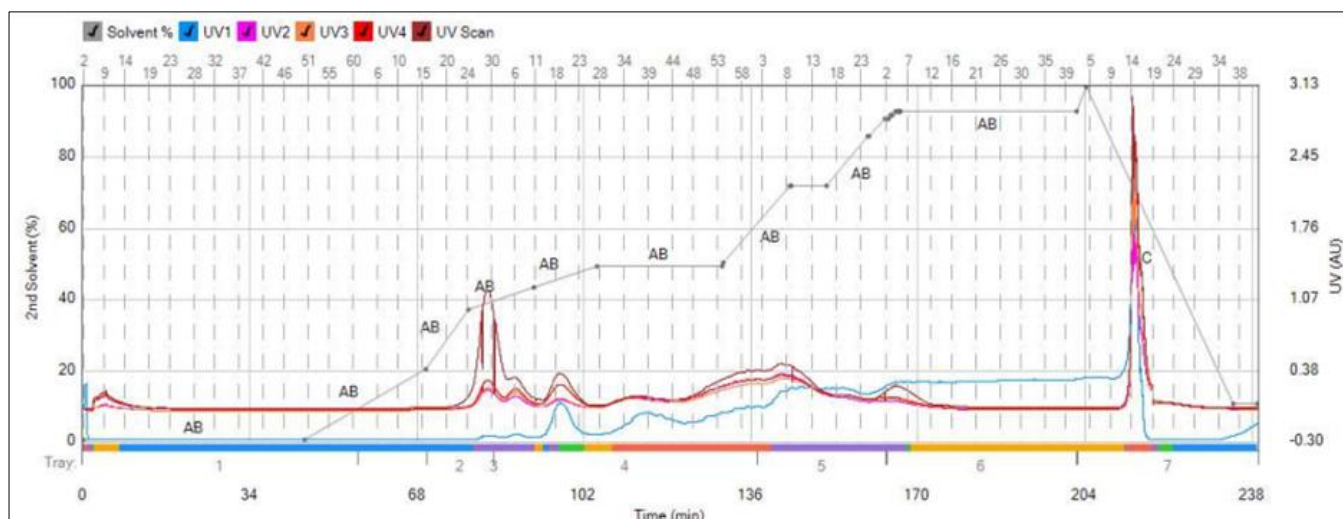
Dots indicate the individual values and the bars indicate mean  $\pm$  SD of the replicates;  
\* values vary significantly from the control values (Student's *t* test;  $P \leq 0.05$ )

**Fig 4:** Absorbance in egg albumin denaturation assay with *T. undulata* extracts at 200  $\mu$ g/mL concentration



Dots indicate the individual values and the bars indicate mean  $\pm$  SD of the replicates

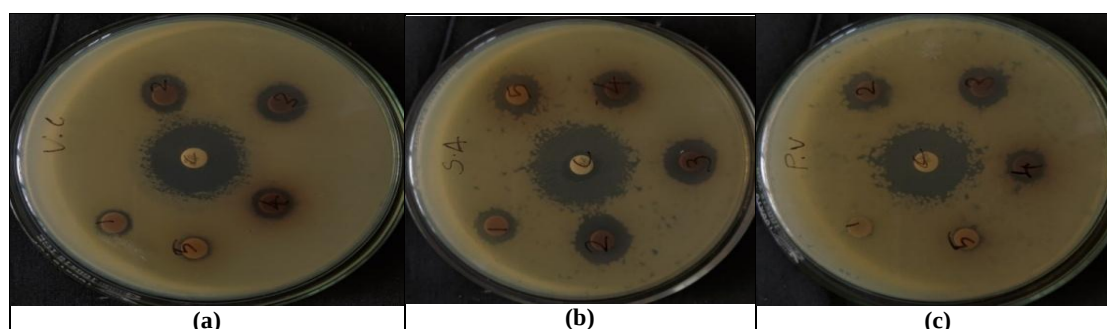
**Fig 5:** Inhibition of egg albumin denaturation by *T.undulata* extracts at 200  $\mu$ g/mL concentration 200  $\mu$ g/mL concentrations



**Fig 6:** Flash chromatographic separation of *T. undulata* ethylacetate extract

Tubes 1-50 (elutant: hexane 100%) were pooled as Fraction 1; tubes 51-120 (elutant: hexane:ethylacetate 7.5:2.5) were pooled as Fraction 2; tubes 121-155 (elutant: hexane:ethylacetate 1:1) were pooled as Fraction 3; tubes

156-204 (elutant: ethylacetate 100%) were pooled as Fraction 4 and tubes 205-238 (elutant: ethylacetate:methanol 8:2) were pooled as Fraction 5



**Figure 7:** Representative photographs showing the antibacterial effect of *T.undulata* extracts  
a) *Vibrio* sp., b) *Staphylococcus albus* and c) *Proteus vulgaris* treated with *Tecomella undulata* fraction

**Table 1:** Antibacterial effect of *T. undulata* extracts on selected pathogens

| Extract       | ID of the microbes          | Zone of inhibition (mm) |                                        |     |    |
|---------------|-----------------------------|-------------------------|----------------------------------------|-----|----|
|               |                             | Control                 | Concentration of the extract (mg/disc) |     |    |
|               |                             |                         | 1.5                                    | 2.5 | 5  |
| Hexane        | <i>Vibrio</i> sp.           | 10                      | --                                     | 7   | -- |
|               | <i>Staphylococcus albus</i> | 18                      | 8                                      | 8   | 11 |
|               | <i>Proteus vulgaris</i>     | 13                      | 10                                     | 11  | 14 |
| Ethyl acetate | <i>Vibrio</i> sp.           | 8                       | 7                                      | 8   | 11 |
|               | <i>Staphylococcus albus</i> | 14                      | 8                                      | 10  | 11 |
|               | <i>Proteus vulgaris</i>     | 16                      | 10                                     | 11  | 13 |
| Ethanol       | <i>Vibrio</i> sp.           | 8                       | --                                     | --  | -- |
|               | <i>Staphylococcus albus</i> | 16                      | --                                     | --  | 11 |
|               | <i>Proteus vulgaris</i>     | 20                      | 9                                      | 11  | 14 |

'--' No activity

**Table 2:** Mosquito larvicidal effect of *T. undulata* extracts against the 4<sup>th</sup> instar larvae of *Culex quinquefasciatus*

| Extract       | Concentration (In ppm) | % Mortality |    |    | Cumulative % Mortality |
|---------------|------------------------|-------------|----|----|------------------------|
|               |                        | R1          | R2 | R3 |                        |
| Hexane        | 62.5                   | 10          | 10 | 10 | 10                     |
|               | 125                    | 10          | 20 | 10 | 13.33                  |
|               | 250                    | 10          | 10 | 0  | 6.66                   |
| Ethyl acetate | 62.5                   | 0           | 0  | 0  | 0                      |
|               | 125                    | 0           | 0  | 0  | 0                      |
|               | 250                    | 0           | 0  | 0  | 0                      |
| Ethanol       | 62.5                   | 0           | 0  | 0  | 0                      |
|               | 125                    | 10          | 10 | 0  | 6.66                   |
|               | 250                    | 10          | 10 | 10 | 10                     |

**Table 3:** Antibacterial effect of *T. undulata* extracts on selected pathogens at 20 µg/mL concentration

| Pathogens                   | Control | F1 | F2 | F3 | F4 | F5 |
|-----------------------------|---------|----|----|----|----|----|
| <i>Vibrio</i> sp.           | 10      | -- | 5  | 5  | 4  | -- |
| <i>Staphylococcus albus</i> | 11      | 4  | 7  | 6  | 5  | 5  |
| <i>Proteus vulgaris</i>     | 10      | -- | 5  | 5  | 4  | -- |

‘--’ No activity

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