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# Antimicrobial and phytochemical screening of methanolic fruit extract of *Withania coagulans* L. Dunal for evaluating the antidiabetic activity

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**Abstract**
The *Withania coagulans* L. Dunal, a small bush, belongs to the family Solanaceae and is widely spread in India, Pakistan, Afghanistan and South Asia. It is commonly known as 'Indian cheese maker' or 'vegetable rennet' due to its milk coagulating property found especially in its leaves and fruits. In the present study phytochemical screening of methanolic fruit extract of *Withania coagulans* was performed. Fourier Transform Infrared Spectroscopy analysis was performed for screening Withanolides, having potential for treatment of diabetes in natural way. Different withanolides are responsible for different therapeutic properties. It is widely used in treating cancer, diabetes mellitus, nervous exhaustion, disability, insomnia, wasting diseases and failure to thrive in children, etc. The antibacterial activity of methanolic fruit extract of W. coagulans was tested against various bacteria (Salmonella paratyphi, Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Micrococcus luteus), highest inhibitory activity of methanolic extract was observed against Bacillus subtilis (12 mm).

**Keywords:** antibacterial, diabetes, fourier transform infrared spectroscopy, therapeutic, *withania coagulans*, withanolides

## 1. Introduction
The *Withania coagulans* L. Dunal belongs to family Solanaceae (Shahid W., *et al.,* 2013) [13]. It is most commonly known as 'Indian cheese maker' and the fruits of W. coagulans are used as the foundation of coagulating enzyme for clotting of milk which is called paneer (Gupta V. & Keshari B.B., 2013) [5]. *Withania coagulans* L. Dunal shows free radical scavanging, cardiovascular, hypoglycemic, hypolipidemic, central nervous system depressant, hepatoprotective, antiinflammatory, antitumor, immuno-suppressive, and cytotoxic, wound healing effect (Gupta V. & Keshari B.B., 2013) [5]. This shrub is widely used in the North India, Afghanistan, Pakistan and adjoining countries (Gupta V. & Keshari B.B., 2013) [5]. It is also known as Paneer Doda in Hindi. W. coagulans L. Dunal plant has shown to have profound hypoglycemic activity because it contains compounds which can utilize blood glucose and also repair the pancreatic β-cells, thus providing insulin to the body (Agarwal N., *et al.,* 2014) [1]. Various plant parts such as roots, fruits and leaves have different therapeutic effect. Antidiabetic effect is mainly observed from the fruit extracts of the plant. An effective dose of 750 mg/kg of body weight/ day of aqueous and ethanolic extracts of W. coagulans in Steptozotocin-induced diabetic rats has shown to possess antidiabetic effect, reported by (Jaiswal D., *et al.,* 2009), (Hoda *et al.,* 2010) [6] reported that at a dose of 1 g/kg for 14 days in experimental Diabetes Mellitus in rats, the antihyperglycemic and antihyperlipidemic effects of aqueous and chloroform extracts of W. coagulans were observed. The phytochemical screening of extracts shows the presence of various primary and secondary metabolites such as carbohydrates, proteins, amino acids, alkaloids, phenols, tannins, steroids, saponins, organic acids, etc. Various therapeutic activities of W. coagulans plant are due to a specific steroid derivative group, steroidal lactones known as "Withanolides" (Agarwal N., *et al.,* 2014) [1]. A variety of Withanolides such as coagulanolide, coagulin-F, coagulin-G, Withacoagulin, etc. have been isolated from the whole plant of W. coagulans (Pandey I. & Nama K.S., *et al.,* 2015).

## 2. Material and Methods
### 2.1 Collection of *W. coagulans* Sample:
*Withania coagulans* dried fruits were obtained from local Ayurvedic medical store

(Gopal Govind Lokhande - Ayurvedic Medicines) in Pune, Maharashtra with identification from Department of Botany, Modern College, Ganeshkhind, Pune–411016.

**2.2 Extraction of sample**

100g of dried fruit part of the plant was grinded in a blender and was subjected to extraction using solvent methanol (1:4) and kept at room temperature for 24-48 hours with intermittent shaking after which the extract was filtered. The filtrate was then dried using Rota evaporator (Roteva, Equitron) and kept for cooling and further evaporation on a Hotplate. After complete evaporation of the solvent, the weight of extract obtained from filtrate of solvent was determined as grams of extract / gram of dried plant powder.

**2.3 Phytochemical screening**

**2.3.1 Qualitative Analysis**

A series of qualitative tests were carried out as per the standard protocols described by Tiwari P. *et al*., 2011 [16]; Saxena M, *et al.,* 2012 [12]; and Salwaan C. *et al*., 2012 [10].

**1. Test for detection of Alkaloids:** Dilute Hydrochloric acid was added to the extracts and then the solution was filtered.

a) **Mayer's Test**: The filtrate was treated with Potassium Mercuric Iodide (Mayer's reagent). The formation of a yellow coloured precipitate indicates the presence of alkaloids in the extracts.
b) **Dragendroff's Test**: The filtrate was treated with the solution of Potassium Bismuth Iodide known as the Dragendroff's reagent. A formation of a red precipitate will indicate the presence of alkaloids.
c) **Hager's Test:** The filtrate was treated with saturated picric acid solution (Hager's reagent). Yellow colour precipitate formation confirmed the presence of alkaloids.

**2. Test for Detection of Reducing Sugar:** The extracts were dissolved in distilled water (5ml) and then the filtrates were used for carrying out the tests.

a) **Molisch's Test**: 2 drops of alcoholic α-naphthol solution was added to the filtrate. The presence of carbohydrates is indicated by the formation of a violet ring at the junction.

b) **Benedict's Test**: Five drops of filtrate was added to 2 ml of Benedict's reagent and then the solution was heated in water bath for few minutes. The formation of orange/red precipitate indicates the presence of reducing sugars.

c) **Fehling's Test**: 1 ml of Fehling's solution A and 1 ml of Fehling's solution B were added to five drops of the test filtrates. Presence of reducing sugars is indicated by the presence of red precipitate.

**3. Test for Detection of Glycosides**

**a) Borntrager's test:** 0.5 g of the plant extract was shaken with benzene and organic layer got separated and half of its own volume of 10% ammonia solution added. A pink, red or violet colouration indicated the presence of glycosides

**4. Test for Detection of Saponins**

**Foam test:** 0.5 ml of the extract was taken and mixed with 2 ml of water. The solution was shaken for a few minutes. The presence of saponins is indicated if the foam persists for more than 10 minutes.

**5. Test for Detection of Phytosterols**

**Salkowski's Test:** The extract was treated with chloroform and filtered. The filtrate was then treated with a few drops of concentrated Sulphuric acid and the solution was shaken and allowed to stand for a few minutes. The appearance of yellow golden colour indicates the presence of triterpenes.

**6. Test for Detection of Tannins**

**Gelatin Test:** 1% gelatin solution containing sodium chloride was added to the extracts. The presence of tannins is indicated by the formation of a white precipitate.

**7. Test for Detection of Flavanoids**

**Lead Acetate Test**: A few drops of lead acetate solution were added to the extract. The presence of flavonoids is indicated by the formation of yellow precipitate.

**8. Test for Detection of Phenols**

**Lead Acetate Test**: A few drops of lead acetate solution were added to the extract. The presence of phenols is indicated by the formation of yellow precipitate.

**9. Test for Detection of Proteins**

**Xanthoproteic Test:** The extract was mixed with a few drops of conc. Nitric acid solution. The presence of proteins is indicated by formation of yellow colour.

**10. Test for Detection of Diterpenes**

**Copper Acetate Test**: Water was added to the extract and then 3-4 drops of copper acetate solution were added to the solution. Formation of emerald green colour will indicate the presence of diterpenes.

**2.3.2 Quantitative Analysis**

The estimation of total contents of certain metabolites such as Flavonoids, Alkaloids, Phenols and Proteins in methanolic extract of *W. coagulans* fruit was carried using standard protocols described as follows.

**a) Total Flavonoid Estimation**

The total flavonoid content in methanolic extract was determined by following the modified spectrometric method described by Saradha M., *et al.,* 2014. Varying concentrations (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1 mg/ml) of Quercetin (standard) were prepared from 1 mg/ml stock solution. For testing the concentration of Flavonoids in the extract, 0.5 ml of extract was taken from 1mg/ml of individual stock solutions. After making volume of solution to 2.5 ml, 0.15 ml of 5% sodium nitrate solution was added and incubated at room temperature for 6 minutes. 0.15 ml of 10% aluminum chloride was added and incubated at room temperature for 6 minute. 2 ml of 4% sodium hydroxide was added and mixed thoroughly. Final volume of solution was made to 5 ml with distilled water and after mixing, incubated at room temperature for 15 minutes. The absorbance was taken at 510 nm. The concentration of flavonoids in the extract was calculated using the standard curve. The total flavonoid content in the extract was expressed as mg of flavonoid / g of extract.

**b) Total Alkaloid Estimation:** The total alkaloid content in

the extract was determined by following the UV spectrometric method described by Gundkalle MB, *et al.,* 2012. Varying concentrations (0.02 mg/ml, 0.04 mg/ml, 0.06 mg/ml, 0.08 mg/ml, 0.1 mg/ml) of Atropine (standard) were prepared from 0.1 mg/ml stock solution. For testing the concentration of Alkaloids in the extract, 1 ml of extract was taken from 1mg/ml of individual stock solutions. The solutions of the extract were transferred to different separatory funnels. 5 ml of Phosphate buffer pH 4.7 was added. 5 ml of Bromocresol Green solution was added to each separatory funnel. The mixtures were shaken by adding 1, 2, 3 and 4 ml of chloroform. The separated layer was collected in a conical flask and the volume was made up to 10ml with Chloroform. The absorbance was measured at 470 nm. The concentration of alkaloids in the extract was calculated using the standard curve. The total alkaloid content in extract was expressed as mg of alkaloid / g of extract.

**c) Total Phenol Estimation:** The total phenolic contents in extract of *W. coagulans* was determined according to the method described by Khatiwora E, *et.al.,*2010 [7] Varying concentrations (0.002 mg/ml, 0.004 mg/ml, 0.006 mg/ml, 0.008 mg/ml, 0.01 mg/ml) of Catechol (standard) were prepared from 1 mg/ml stock solution. For testing the concentration of phenols in the extract, 1 ml of extract was taken from 1mg/ml of individual stock solutions. 0.5 ml of Folin-Ciocalteu (FC) reagent was added and incubated for 3 minutes at room temperature in dark. After adding 2 ml of 20% Sodium carbonate solution, each solution was mixed thoroughly and incubated in boiling water bath for 30 minutes and cooled. Finally, the absorbance was measured at 650nm. The concentration of phenols in the extract was calculated using the standard curve. The total phenolic content in extract was expressed as mg of phenol / g of extract.

### 2.3.3 Thin Layer Chromatography

The TLC was performed using standard procedure (Dyer W.G., *et.al.,* 1963) [3] on a 20×20 cm and 0.25 mm thick plate. Slurry of Silica Gel G was prepared in the ratio 1:2 (1part Silica and 2 parts water). It was coated onto a clean grease free glass plate. The coat was approximately 0.25 mm thick. Plate was kept for air dry at RT for 10-20 minutes. Then plate was activated at 100-120 °C by placing in a hot air oven for 1hour. Meanwhile the plates get activated; the mobile phase was prepared and kept for saturation covered in a TLC solvent chamber. After activation of silica plate, methanolic extract was spotted onto the plate using a capillary. The chromatoplate was placed in saturated TLC solvent chamber and the chromatoplate was allowed to develop. The same solvent system used for Methanolic extract was Butanol: Water: Acetic Acid (7: 1: 2) (Shodhganga). The development was stopped shortly before the solvent front reached the top edge of the plate. The chromatoplate was removed and allowed to dry for 10 minutes. The spots were observed under UV light. For the spot development, the chromatoplates were sprayed with 2 separate freshly prepared detecting reagents:

- 50% Sulphuric acid in ethanol (Dyer W.G., et al., 1963 & Panchawat S., 2012) [3]
- Dragendroff `s Reagent (Atta-ur-Rahman, et al., 2002) and placed at 90 °C for charring for 15 minutes. The chromatoplates were cooled and kept at room temperature for 24 hours for spot colour development. The Retention Factor (Rf) value for each spot was determined.

### 2.3.4 Fourier Transform Infrared Spectroscopy (FTIR) Analysis of *Withania coagulans* Dunal

Dry powder of *Withania coagulans* Dunal fruit was submitted to Central Instrumentation Facility, Savitribai Phule Pune University for FT-IR analysis of the sample.
The FT-IR of various functional groups was measured by Bruker Tensor 37 FT-IR instrument, at the range of 3500-500 cm-1. Also, the chromatographic spots development on TLC plates, both sprayed with 50% Sulphuric acid as well as Dragendroff`s reagent, respectively, were scrapped out and analyzed by FT-IR for the determination of functional groups of Steroids and its derivative Steroidal lactone (Withanolide).

### 2.4. Antibacterial Activity

Pure cultures of bacteria namely, *Salmonella typhi, Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Micrococcus luteus* were obtained from Department of Biotechnology, Modern College, Ganeshkhind, Pune. Cultivation of bacteria was carried in 0.8% Nutrient Broth and pure cultures were also maintained in 0.8% Nutrient Broth medium. For investigating antibacterial properties, agar well diffusion method was adopted because of the precision. Nutrient Agar plates were made for the base and Mueller Hinton agar plates were used for the seed. The different test organisms were proceeded separately and poured with the seed agar on the Nutrient agar plates (100 µl of each test organism broth culture). Three wells on each plate were made with the help of sterilized well borer, one containing the negative control (DMSO), one with the positive control (Ampicillin) and one with the test sample extract (Methanol). The concentration of the positive control was 2 mg/ml. 20 µL of test solution was poured in respective well. The plates were then kept for cooling in the refrigerator for 30 minutes for better diffusion and then incubated at 37 °C for 24 hours. Then the zone of inhibition (Shahid W. *et al*., 2013) [13] was observed and activity index (Ranjan S., *et al.*, 2012) [9] was calculated and noted down.

## 3. Results and Discussion

### 3.1 Qualitative Analysis

The phytochemical screening results of *Withania coagulans* L. Dunal methanolic extract(fruit) contains alkaloids, reducing sugar, glycosides, flavonoids, tannins, phytosterols and proteins.

**Table 1:** Phytochemical screening of *Withania coagulans* fruit extract.

| Sr. No. | Tests/Plant extract | Methanol |
|---|---|---|
| 1. | a. Mayer's Test<br>b. Dragendroff's Test<br>c. Hager's Test | Test for alkaloids<br>+<br>+<br>+ |
| 2. | a. Molisch's Test<br>b. Benedict's Test<br>c. Fehling's Test | Test for reducing Sugar<br>+<br>+<br>+ |
| 3. | Borntrager's test | Test for Glycosides<br>+ |
| 4. | Lead Acetate Test | Test for Flavanoids<br>+ |
| 5. | Lead Acetate test | Test for Phenols<br>+ |
| 6. | Gelatin test | Test for Tannins<br>+ |
| 7. | Salkowski's test | Test for Phytosterol<br>+ |
| 8. | Foam Test | Test for Saponins<br>- |
| 9. | Xanthoproteic test | Test for proteins<br>+ |
| 10. | Copper Acetate test | Test for Diterpenes<br>- |

### 3.2 Quantitative Analysis

Quantitative evaluation of phytochemicals has roles in providing antioxidant properties to plants. The quantitative analysis of the extract in order to estimate the concentration of Flavonoids, alkaloids and phenols was performed and following observations were made. The estimated amount of each metabolite was expressed as: mg of standard equivalent/g of extract.

**Table 2:** Total phenol, flavonoid and alkaloid contents of W. coagulans.

| Extract | Flavonoids | Alkaloids | Phenols |
|---|---|---|---|
| Methanol | 5.5 | 9.5 | 5.2 |

**Flavonoid, alkaloid, phenol equivalents mg $g^{-1}$ extract**

### 3.3 Thin Layer Chromatography

Thin layer chromatography of Methanolic Extract of *Withania coagulans* L. Dunal (fruit) performed for preliminary identification of Steroids. The partial identification of steroids was done by using two different steroid detecting agents that is 50% Sulphuric acid in ethanol and Dragendroff `s reagent. A distinct spot was developed on each chromatographic plate respectively. By using the solvent system Butanol: Water: Acetic Acid (7: 1: 2), 1 spot was observed on plate after spraying with 50% Sulphuric acid in ethanol having Rf value as 0.932. Similarly, by using the same solvent system, 1 spot was observed on plate after spraying with Dragendroff ` s reagent having Rf value as 0.810. (Table 3)

**Table 3:** TLC of W. coagulans for preliminary identification of steroids.

| Sr. No. | Solvent system | Detecting reagents | D1 (cm) | D2 (cm) | Rf value |
|---|---|---|---|---|---|
| 1 | Butanol: Water: Acetic Acid (7: 1: 2) | 50% Sulphuric acid in ethanol | 11 | 11.8 | 0.932 |
| 2 | Butanol: Water: Acetic Acid (7: 1: 2) | Dragendroff`s Reagent | 9 | 11.1 | 0.810 |

### 3.4 Fourier Transform Infrared Spectroscopy (FTIR) Analysis of *Withania coagulans* L. Dunal:

The FT-IR analysis of dry powder of *W. coagulans* fruit was performed and different functional groups represented as peaks at different wavenumber cm-1 were identified and are mentioned in table 4. The FTIR spectra for dry powder of plant were shown in figure 1. The FT-IR spectra for analysis of TLC Spot developed by detecting reagent, 50% sulphuric acid in ethanol for steroids and steroidal lactones was shown in figure 2 and the spectral assignments were represented in table 5. The inspection of the spectra revealed the presence of following peaks at 878.33 cm-1 representing 4-en 3-one structure vibration; wavenumber 1115.45cm-1 representing C-O structure vibration of Alcohol and wavenumber 1449.20 cm-1 representing CH2 bending vibrations. All of these functional groups were characteristic of steroids. Wavenumber 1016.39 cm-1 representing C-O Structure vibration of β isomer of hydroxy-ketones and 1739.42 cm-1 representing vibrations of lactones structure were also found in addition to the above mentioned functional groups of steroids. Thus, indicating the presence of steroidal lactones. These steroid derivatives are the active compound group called Withanolides which include many steroidal lactones having different combinations of these functional groups and each one shows different therapeutic activity. While on the other hand, the FT-IR spectra for analysis of TLC Spot developed by detecting reagent, Dragendroff `s reagent for steroids and steroidal lactones was shown in figure 3 and the spectral assignments were represented in table 6. The inspection of the spectra revealed the presence of following peaks at wavenumber 1021.11 cm-1 representing C-O Structure vibration of β isomer of hydroxy-ketones; wavenumber 1114.66 cm-1 representing C-O structure vibration of Alcohol and wavenumber 1450.04 cm-1

representing CH2 bending vibrations. Although some of the functional groups of steroidal lactones were present in these spectra but not all, especially lactone structure vibrations were absent. Thus, indicating that in the chromatographic spot developed by using dragendroff `s reagent steroids and steroidal lactones were not isolated.

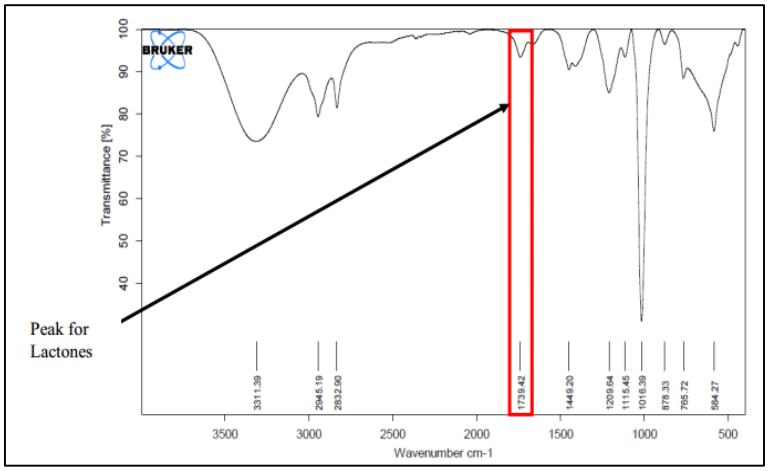


**Fig 1:** FT-IR Analysis of W. coagulans Dunal (Dry Powder)

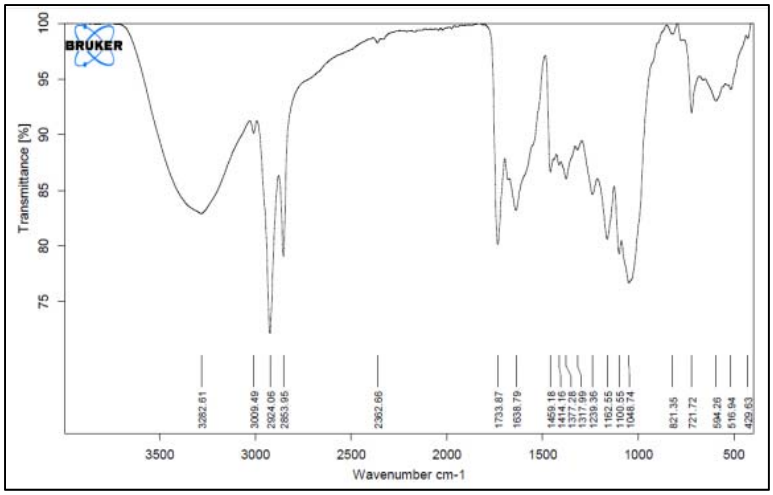


**Fig 2:** Analysis of TLC Chromatographic Spot (detected by 50% Sulphuric acid)

**Table 4:** FT-IR Results of *Withania coagulans* Dunal

| Sr. No | Wavenumber cm-1 | Assignments |
|---|---|---|
| 1 | 721.72 | Monosubstituted Aromatic Rings |
| 2 | 821.36 | Parasubstituted Aromatic Rings |
| 3 | 1048.74 | Primary Alcohols |
| 4 | 1100.55 | Secondary Alcohols |
| 5 | 1162.55 | Tertiary Alcohols |
| 6 | 1239.36 | Phenols |
| 7 | 1317.99 | Carboxylic Acid |
| 8 | 1377.28 | Alkanes (sp3 C-H bend) |
| 9 | 1414.16 | Alkanes (sp3 C-H bend) |
| 10 | 1459.18 | Aromatic Compounds (C=C stretch) |
| 11 | 1638.79 | Primary Amines |
| 12 | 1733.87 | Normal Aliphatic Esters |
| 13 | 2362.66 | Carboxylic Acids (overlaps C-H stretch) |
| 14 | 2853.95 | Aldehydes |
| 15 | 2924.08 | Alkanes (sp3 C-H stretch) |
| 16 | 3009.49 | Alkenes (sp2 C-H stretch) |
| 17 | 3282.61 | Alkynes |

**Table 5:** FT-IR Results of TLC Chromatographic Spot (detected by 50% Sulphuric acid) for Steroids and Steroidal lactones

| Sr. No | Wavenumber cm-1 | Assignments |
|---|---|---|
| 1. | 765.72 | C-H out of plane bending |
| 2. | 878.33 | 4-en 3-one |
| 3. | 1016.39 | C-O Structure of β isomer of hydroxyketones |
| 4. | 1115.45 | C-O structure of Alcohol |
| 5. | 1209.64 | C-O stretching of Phenolic Acetates |
| 6. | 1449.20 | CH2 bending |
| 7. | 1739.42 | Lactones |
| 8. | 2832.90 | O-H Structure |
| 9. | 2945.19 | O-H Structure |
| 10. | 3311.39 | Hydrogen Bonded O-H in Carboxylic Acid |

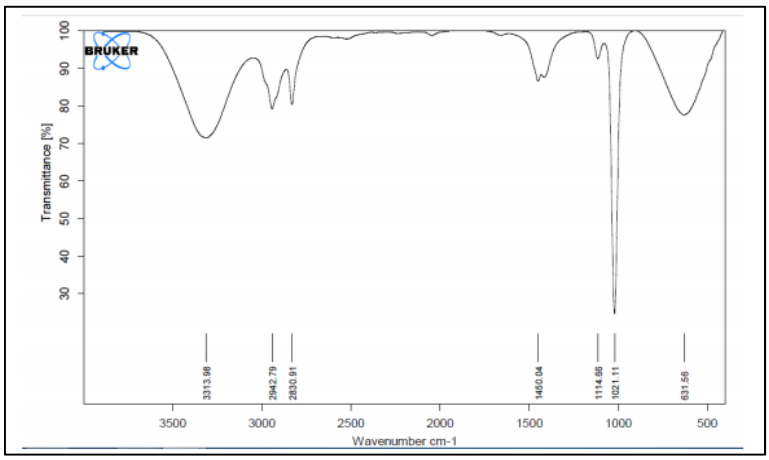


**Fig 3:** FT-IR Analysis of TLC Chromatographic Spot (detected by Dragendroff `s Reagent)

**Table 6:** FT-IR Results of TLC Chromatographic Spot (detected by Dragendroff ` Reagent) for Steroids and Steroidal lactones

| Sr. No | Wavenumber cm-1 | Assignments |
|---|---|---|
| 1. | 631.56 | C-H out of plane bending |
| 2. | 1021.11 | C-O Structure of β isomer of hydroxyketones |
| 3. | 1114.66 | C-O structure of Alcohol |
| 4. | 1450.04 | CH2 bending |
| 5. | 2832.90 | O-H Structure |
| 6. | 2945.19 | O-H Structure |
| 7. | 3311.39 | Hydrogen Bonded O-H in Carboxylic Acid |

**3.5 Antibacterial Activity:** The methanol extract was used for determining the antibacterial activity of *W. coagulans.* Antibacterial activity of methanol extract against the strains and the activity index was calculated using formula:

$$\textbf{Activity index} = \frac{\text{Zone of inhibition by sample}}{\text{Zone of inhibition by positive control}}$$

**Table 7:** Antibacterial activity of Methanolic extract of *W. coagulans*

| Sr. no. | Bacterium | Control | Zone of Inhibition (I.Z.) (mm) | Activity Index |
|---|---|---|---|---|
| 1. | *Salmonella typhi* | Sample | 10 | 0.43 |
| | | Positive | 23 | |
| | | Negative | 0 | |
| 2. | *Klebsiella pneumoniae* | Sample | 0 | 0.00 |
| | | Positive | 0 | |
| | | Negative | 0 | |
| 3. | *Escherichia coli* | Sample | 10 | 0.3 |
| | | Positive | 35 | |
| | | Negative | 0 | |
| 4. | *Bacillus subtilis* | Sample | 12 | 0.85 |
| | | Positive | 14 | |
| | | Negative | 0 | |
| 5. | *Staphylococcus aureus* | Sample | 11 | 0.73 |
| | | Positive | 15 | |
| | | Negative | 0 | |
| 6. | *Micrococcus luteus* | Sample | 11 | 0.73 |
| | | Positive | 15 | |
| | | Negative | 0 | |

The antibacterial activity of the Methanoic extract of *W. coagulans* (fruit) was determined. Water as a solvent is mainly used for extracting the active compounds form plants, but other solvents also give stable and consistent antibacterial activity is also reported earlier (Sudhanshu *et al*., 2012) [15]. Antibacterial activity of methanol against three strains of gram negative and gram positive bacteria were shown in table 7. Table 7 shows that the methanolic extract of *W. coagulans* (fruit) showed best inhibition against *Bacillus subtilis* (12mm) (figure 8), while good inhibition was seen against *Staphylococcus aureus* (11 mm) (Figure 7) and *Micrococcus luteus* (11 mm) (Figure 9). No extract exhibited inhibition was observed against *Klebsiella pneumoniae* (figure 6). Thus, from the above results it is evident that the methanol extract of *W. coagulans* (fruit) showed potential antibacterial activity.

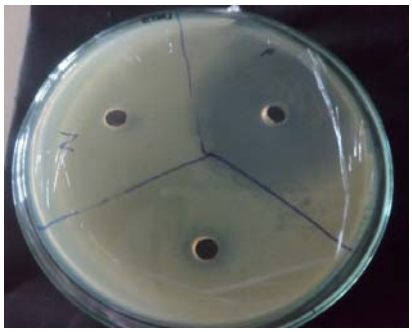

**Fig 4:** Antibacterial assay (*Salmonella typhi*)

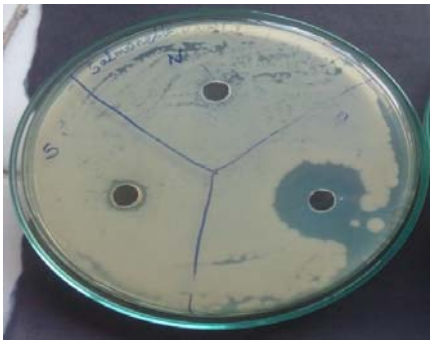

**Fig 5:** Antibacterial assay (*Escherichia coli)*

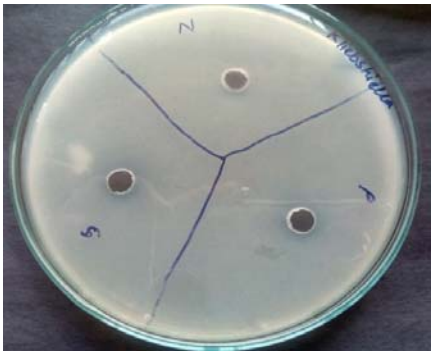

**Fig 6:** Antibacterial assay (*Klebsiella pneumoniae*)

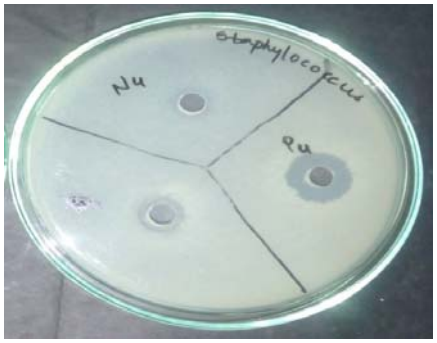

**Fig 7:** Antibacterial assay (*Staphylococcus aureus)*

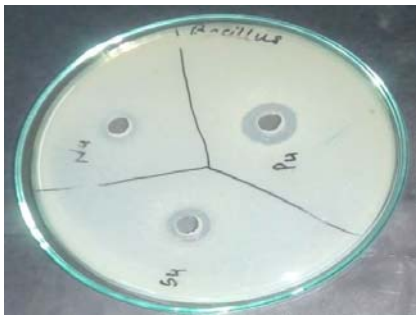

**Fig 8:** Antibacterial assay (*Bacillus subtilis)*

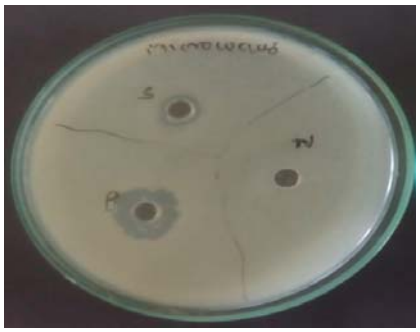

**Fig 9:** Antibacterial assay (*Micrococcus luteus*)

**P-** Positive control, N- Negative control, S- Sample (Methanolic extract)

## 4. Conclusion

The Phytochemical analysis, both qualitative and quantitative, has shown the significant presence of large number of important phytochemical in the fruits of *Withania coagulans* L. Dunal plant. Withanolide are the major biologically active group of compounds in *W. coagulans*, showing a wide range of therapeutic and antioxidant effects which can used in treatment of Diabetes.

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