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IN-VITRO EFFECT OF *LAWSONIA INERMIS* L. (HENNA) LEAVE EXTRACT ON *LYCOPERSICON ESCULENTUM* VAR. (TOMATO) PHYTOPATHOGENIC BACTERIA *RALSTONIA SOLANACEARUM* (SMITH*)*

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ABSTRACT

Lycopersicon esculentum (Tomatoes), *is one of the most important vegetable fruits worldwide which constitute commercially and nutritionally indispensible food commodity. Ralstonia solanacearum is a relevant and widespread Phytopathogenic bacterium that causes a wilt disease which deadly affects this economically important crop. Antibacterial activity of Lawsonia inermis leaves were evaluated using three fractions of extract namely; methanolic, chloroform and aqueous against this plant pathogenic bacterium (R. solanacearum) in-vitro. Prior to antibacterial assay, the extract fractions were subjected to preliminary phytochemical screening, which revealed the present of alkaloids, terpenoides and saponins in all, while methanolic extract shows the additional flavonoids, tannins, amino acid, triterpenoids and reducing sugar, the chloroform extract showed additional anthraquinone. Nevertheless, the aqueous extract shows the additional triterpenoids, steroids and reducing sugar. Among the three fractions the methanolic extract proved to be the promising extract for management/treatment of tomato wilt caused by R. solanacearum. All the methanolic extract concentrations (1000, 2000, 3000 and 4000 µg/ml respectively) showed effects on the test organism. The (SPSS) software was used to analyse the significant differences between each concentrations, using one way Analysis of variance (ANOVA) at critical value ($p \geq 0.05$).*

Keywords: Lawsonia inermis, Lycopersicon esculentum, Phytochemical, Phytopathogenic bacterium, Ralstonia solanacearum.

Contribution/ Originality

This study contributes in the existing literature. The study also is one of very few studies which have investigated the phytochemical constituents of different fractions of *Lawsonia inermis* leaves extracts and its effect against phytopathogenic bacteria *Ralstonia solanacearum.*

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1. INTRODUCTION

Lawsonia inermis L. (Henna):- is medium sized shrub that sometimes takes a tree like shape and growth. It has many angled branches with opposite sharp pointed leaves. The leaves are simple, opposite, entire, and lanceolate, with short petals. Flowers are white numerous, fragrant seen in terminal panicle cymes. Fruits are globose capsules, with persistent calyx. Seeds are numerous, smooth and pyramidal. *Ralstonia solanacearum* is a Gram-negative, rod-shaped bacterium [1-3]. It is a widely distributed and economically important plant pathogen that invades the roots of diverse Solanaceous plant hosts from the soil and colonizes the xylem vessels, causing a lethal wilting known as bacterial wilt disease [2, 3]. On *Lycopersicon esculentum* (tomatoes) in particular, the youngest leaves are the first to be affected and have a flabby appearance, usually at the warmest time of day. Wilting of the whole plant may follow rapidly if environmental conditions are favourable for the pathogen. Under less favourable conditions, the disease develops less rapidly, stunting may occur and large numbers of adventitious roots are produced on the stem. The vascular tissues of the stem show a brown discoloration and, if the stem is cut crosswise, drops of white or yellowish bacterial ooze may be visible [4].

R. solanacearum constitutes a serious obstacle to the culture of many solanaceous plants such as (Tomatoes) in both tropical and temperate regions. The greatest economic damage has been reported on potatoes, tobacco and tomatoes in the south-eastern USA, Indonesia, Brazil, Colombia and South Africa. In the Philippines, in 1966-1968, there were average losses of 15% in tomatoes, 10% in *Capsicum*, and 2-5% in tobacco [5]. In India, there are sometimes total losses in tomato crops [6]. The focus of this research work is to evaluate the activities of three fractions of extracts namely, (aqueous, chloroform and methanolic) of *Lawsonia inermis* (henna) leaves against phytopathogenic bacteria *R. solanacearum*.

2. MATERIAL AND METHODS

2.1. Collection of the Plant Material

The leaves of the plant were collected from Dambatta Local Government area, Kano, Nigeria; the area was located in North-West Nigeria, lies between latitude 13^0N and 11^0N and longitude 8^0W and 10^0E, in the east. The plant was identified and authenticated by Prof. B.S. Aliyu of the Department of Botany (formally Biological Sciences Department) Bayero University Kano, Nigeria. The leaves were washed with distilled water to remove dirt, dust particles and dried in the laboratory at 65^0C using (Memarts, D-91126. Germany) to ensure a constant weight, then grinded to powdered form and stored until required for use.

2.2. Phytochemical Screening

Phytochemical tests were carried out on both extract fractions using standard procedures to identify the possible constituents as described by [7-9].

2.3. Test for Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered. The filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

2.4. Test for Saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

2.5. Test for Steroids

0.2 ml of Concentrated H_2SO_4 was added to about the same volume of each of the test extracts in a test tube separately. A red colour indicates the presence of steroidal ring.

2.6. Test for Glycosides

5 ml of H_2SO_4 was added to each of the test extracts in a separate test tubes. The mixture was heated in boiling water for 15 minutes. Fehling's solution was then added and the resulting mixture was heated to boiling. A brick-red precipitate indicates the presence of glycosides.

2.7. Test for Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride was added and observed for brownish green or a blue-black colouration.

2.8. Test for Anthraquinones

Borntrager's Test: 0.2g of extracts was shaken with 10ml of benzene and then filtered, 0.5 ml of 1% ammonia solution was then added to the filtrate after shaken. Appearance of a pink, red or violet colour in the ammonical (lower phase) was taken as the presence of free anthraquinones.

2.9. Test for Terpenoids

0.5ml of acetic anhydride was mixed with 1ml of each sample extracts and a few drops of concentrated H_2SO_4 was added to each. A bluish green precipitate indicates the presence of terpenoids.

2.10. Test for Steroids and Triterpenoids

Salkowski test: Each fraction of the Crude extracts were mixed with chloroform and a few drops of conc. H_2SO_4 were added with vigorous Shake and allowed to stand for 5minutes.

Appearance of Red colour at the lower layer indicated the presence of steroids and formation of yellow coloured layer indicated the presence of triterpenoids.

2.11. Test for Flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

2.12. Test for Reducing Sugar

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of reducing sugars using Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

3. PREPARATION OF THE EXTRACTS

Three different solvents were used for the extraction namely; Chloroform, Methanol and Distilled Water, using percolation method. 50g of the fine powdered was taken in a conical flask containing 500 ml of chloroform and extracted for 120hrs with regular shaking at interval, after which the residue was filtered using Whatman NO. 1 filter paper and the filtrate was evaporated to dryness at room temperature to remove the solvent, the dried filtrate was stored in the refrigerator until needed. Methanolic extract was prepared as above. Aqueous extract was prepared by mixing 50g of the leaf powder with 500ml of sterile distilled water and extracted for 72hrs with regular shaking at interval, it was also filtered and treated as above [10].

4. PREPARATION OF TEST CONCENTRATIONS

Four different test concentrations (4000, 3000, 2000 and 1000 µg/ml) were prepared. Stock solution was prepared by dissolving 8mg of each extract in 2ml of Dimethyl sulfoxide (DMSO) to give 4mg/ml (4000µg/ml) from which 1ml was taken and serially diluted with 1ml of DMSO to arrive at 2000 µg/ml and 1000 µg/ml, and 3000 µg/ml was prepared by dissolving 3mg in 1ml of DMSO. All the concentrations were sterilized using (simplepure, pes 0.22µm USA) filter membrane before used to test their effect on test isolate.

5. TEST ISOLATE

The isolate was sourced from the Department of crop production Ahmadu Bello University Zaria, Nigeria on a nutrient agar slant and maintained in sterile distilled water [11]. A selective media Tetrazolium chloride Agar (Sigma Aldrich) was used to resuscitate and confirmed the isolate prior to use.

6. BIOASSAY

Bioassay was conducted using agar well diffusion according to the method of Perez, et al. [12], with minor modification. The turbidity of the bacterial suspensions were serially diluted, and the absorbance of 1.00 was used spectrophotometrically using (Cecil, C-7500, Cambridge England) at wave length of 600nm with approximate cell count Density x10^6cells [13]. The isolate was seeded on to the entire surface of a nutrient agar plate. Wells were made in the agar plates using sterile cork borer of 6mm diameter and the prepared dilution of the extracts aseptically placed in various well. Dimethyl sulfoxide (DMSO) was used as a negative control. The plates were incubated at 30°C for 24hrs after which the activities of the extracts were recorded according to the zones of inhibition observed.

7. RESULTS

The antimicrobial susceptibility pattern of the different solvent extracts, (Aqueous, Chloroform and Methanol) were tested against the plant pathogenic bacteria *R. solanacearum* and physical properties of the extracts were determined and presented. The extracts were also under go preliminary phytochemical screening to determine the various leave active constituents present.

From the result, it can be seen that the color of the extracts range from brown to green and the texture was either gummy or jelly as presented on (table 1 below). Methanolic and aqueous extracts were gummy in nature whereas, chloroform extract was jelly like. The result shows that only methanolic extract inhibited the growth of the pathogen compared with the aqueous and chloroform extracts. This may be due to the presence of phytochemicals that possessed antimicrobial properties viz; alkaloids, tannins, terpenes and flavonoids which was found in the methanolic extract shows in (Table 2). Hence methanolic extract proved to be the best candidate for the management /control of this phytopathogenic bacteria compared to chloroform and aqueous extracts. At 1000 µg/ml of the methanolic extract the zone of inhibition was 14.7 mm and increases to 15 mm when the concentration was increased to 2000 µg/ml. By further increase of the concentration to 3000 µg/ml and 4000 µg/ml, the zone of inhibition increases to 15.3 mm and 16.3 mm respectibly. Aqueous and chloroform extract presented no activity at the concentrations tested against the pathogen , except at 4000 µg/ml of chloroform extract mild activity was observed as indicated in (tables 3). The control, DMSO also shows no zone of inhibition on the test isolate.

The extracts were subjected to preliminary phytochemical tests to determine the groups of metabolite presents and the result is shown in (table 5 below). The result shows that methanolic extract contains most of the phytochemical constituents tested compared with other solvents used (i.e. chloroform and water), which include: alkaloids, terpenes and saponins, flavonoids, tannins, Amino acid, triterpenoids and reducing sugar, the antimicrobial properties of alkaloids, flavonoids, terpenes and tannins are clearly pronounced by various researchers [11]. The

chloroform extract showed additional Anthraquinone, and Amino acid, while the Aqueous extract shows the additional triterpenoids, steroids and reducing sugar.

8. DISCUSSION

The aqueous, chloroform and methanol leave extracts of *L. inermis* were phytochemically screened and subjected to antibacterial testing against Phytopathogenic bacteria *R. solanacearum*. The results show that the methanol extract shows inhibitory effect on the test organism, where as aqueous and chloroform extract shows very slight or no activity. This indicated that the active ingredients of the plant parts are better extracted with methanol than aqueous and chloroform. Cowan [14], found that methanol was more efficient than other solvent such as acetone in extracting phytochemicals from plant materials. The absence of antibacterial activity of chloroform and aqueous extracts of *L. inermis* indicate the insolubility of the active ingredients in these solvents [15]. This is in contrast with the result of Sukanya, et al. [15] which found no activity in methanolic extract of *L. inermis*.

The result of the antibacterial activity of the methanolic extract (Table 2) shows that the activity of the extract is dose dependant, the higher the concentration of the extract, the larger the zone of inhibition [16]. Aqueous and chloroform extract presented no activity on the test organism and this is in line with the work of other authors which shows that aqueous and chloroform extract of *L. inermis* had no activity on *R. solanacearum* [15]; however the aqueous extract of other plant such as Datura, Garlic and Nerium activity were found [17]. This might be due the differences of their phytochemical constituents. Result of the phytochemical investigation of the extracts revealed that only methanolic extract contains flavonoids which have been shown to have antibacterial activity on bacterial agents [18]. In other investigation essential oils of some plants such as *Coriandrum sativum*, *Thymus vulgaris*, *Cuminum cyminum*, *Rosmarinus officinalis* and *Eucalyptus globulus* were found to have activity on this test pathogen [19].

9. CONCLUSION

Results obtained from this preliminary study have indicated that methanolic extract from *Lawsonia inermis* leaves could play a very important role in the control/management of plant pathogens such as *R. solanacearum*. The in-vitro test of the methanolic extract being investigated ascertain the effectiveness of the extract on this phytopathogenic bacteria.

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Table-1. Physical properties of the plants extract

S/no.	Solvents	Color	Texture
1	Methanol	Dark brown	Gummy
2	Chloroform	Dark green	Jelly
3	Aqueous	Dark brown	Gummy

Table-2. Antibacterial Activity of Methanolic Extract against *Ralstonia solanacearum*

Concentrations in µg/ml	Zone of inhibition in (mm)			Mean±S.D
	R_1	R_2	R_3	
1000	14	15	15	14.7±0.58
2000	14	16	15	15.0±1.00
3000	15	16	15	15.3±0.58
4000	16	17	16	16.3±0.58
DMSO	0	0	0	0

Table-3. Antibacterial Activity of Chloroform Extract against *R. solanacearum*

Concentrations in µg/ml	Zone of inhibition in (mm)			Mean
	R_1	R_2	R_3	
1000	0	0	0	0
2000	0	0	0	0
3000	0	0	0	0
4000	8	7	7	7.3
DMSO	0	0	0	0

Table-4. Antibacterial Activity of Aqueous Extract against *R. solanacearum*

Concentrations in µg/ml	Zone of inhibition in (mm)			Mean
	R_1	R_2	R_3	
1000	0	0	0	0
2000	0	0	0	0
3000	0	0	0	0
4000	0	0	0	0
DMSO	0	0	0	0

Key:-DMSO; Dimethylsulfoxide

µg/ml; Microgram/milliliter

Table-5. Phytochemical Screening of the Extracts

S/NO.	Bioactive compounds	Methanolic Extracts	Chloroform Extract	Aqueous Extract
1	Alkaloids	+	+	+
2	Flavonoids	+	-	-
3	Tannins	+	-	-
4	Glycosides	-	-	-
5	Saponins	+	+	+
6	Terpenoides	+	+	+
7	Steriods	+	-	+
8	Anthraquinone	-	+	-
9	Reducing sugar	+	-	+
10	Triterpenoid	+	-	+

Key: + = Present, - = Absent

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