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REGULAR ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *LANTANA CAMARA* LEAVES AGAINST BACTERIA ISOLATED FROM EPICARP OF *SOLANUM MELONGENA* (GARDEN EGG)

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ABSTRACT

Certain plants and herbs with potencies in healing diseases are termed as medicinal or herbal plants, the efficiency they have in curing diseases are believed to be as a result of an active biochemical compounds present in known as phytochemicals, these bioactive compounds vary in compositions and types and are presence in a wide arrays of plants. This research was aimed at testing the antibacterial properties of *Lantana camara* plant leaves extracts against the bacteria isolated from the epicarp of an unwashed garden egg *Solanum melongena* fruit. Qualitative phytochemical screening of the plant leaves shows that the plant possesses Tannin, Saponin, Glycosides and Reducing sugar, while Anthraquinones, Alkaloid were absent in all the various extracts used. Three different types of bacteria were isolated from the epicarp of the unwashed fruit sample; they include *Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus spp*. The minimum inhibitory concentration of the leaf extracts on *Pseudomonas aeruginosa* were 12 mg/ml for the methanolic extract and 48 mg/ml for the ethanolic extract. The minimum inhibitory concentration on *Streptococcus spp.* was 8 mg/ml for the methanolic extract and 4 mg/ml for ethanolic extract, while the minimum inhibitory concentration on *Staphylococcus aureus* was 25 mg/ml for the methanolic extract and 7 mg/ml for the ethanolic extract. Methanolic extracts have the highest activity on *Staphylococcus aureus* at 8 mg/ml, followed by *Pseudomonas aeruginosa* at12 mg/ml and the least was on *Streptococcus spp.* at 25 mg/ml. While with ethanolic extracts, the highest activity was on *Streptococcus spp.* at 4 mg/ml followed by *Staphylococcus aureus* at 7 mg/ml and the least was on *Pseudomonas aeruginosa* at 48 mg/ml. Therefore, *Lantana camara* leaves contain bioactive compounds believe to have a bactericidal effect at various concentrations species wise.

Keywords: *Lantana camara, Solanum melongena*, Epicarp, Bacteria, Phytochemicals

INTRODUCTION

Medicinal plants are those plants which have a potential to act on disease causing agents, or ameliorate disease agents and thus can be used for treating various ailments [1]. Many natural compounds have been isolated from plants and which made the basis of many drug inventions [2, 3]. Most of the modern medicines are derived from plant based compounds and secondary metabolites from plants [4]. Reports show that more than 80% of the population in Arab and African continents still depends on plant based medicines [5]. Consumption of fresh fruits and vegetables gives potential disease resisting capacity to human [6, 7].

Deterioration of foods generally is attributed to two main causes which are natural degradation due to activities of enzymes and growth of microorganisms (bacteria, molds and yeasts). These microorganisms can result in useful products through their activities particularly during fermentation of foods such as wine and cheese. The negative effects of these microbial activities result in decay, rotting of food and food poisoning, hence the basis of microbial food spoilage occurs when these microorganisms release their own enzymes into the foods and absorb the nutrients thereby changing the physical and chemical states of the foods thus lowering the nutritional value. Bacteria and fungi may also produce waste products which act as poisons or toxins, thus causing the renowned ill-effects [6].

Pathogenic bacteria such as *Streptococcus a*nd *Pseudomonas,* cause many types of diseases in human and which are very difficult to treat or prevent [8].

Lantana camara is a shrub with flowers and strong shoot and well developed root system. The plant has been used in various purposes [9]. The present study aims to screen the phytochemicals and antibacterial activity of *Lantana camara* leaves against bacteria isolated from epicarp of *Solanum melongena* (garden egg).

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MATERIALS AND METHODS

Sample collection

Fresh leaves of *Lantana camara* were collected on 9/8/2016 at Kano-Zoological and Botanical garden which was later identified and authenticated at the Department of Biological Sciences, Yusuf Maitama Sule University, Kano state, while the voucher specimens were preserved for future reference.

Sample preparation

The leaves were washed with water and dried under shade at room temperature for 7 d. The dried leaves were pulverized with the aid of mortar and pestle, and packed in a clean dried container.

Sample extraction

The extraction was conducted according to the methods of Abdulmutalib and Shah, [10]. 60g of the dried pulverized leaves were weighed and suspended in a conical flask and soaked in 600 ml of methanol and ethanol respectively as solvents for 72 h with a periodic shaking. The Soaked sample was then filtered using Whatman's no. 1 filter paper and the filtrate was collected and stored for analysis.

Qualitative phytochemical screening

The phytochemical screening was carried out based on the standard procedure [21].

Bacterial isolation

Bacterial was isolated by washing the garden egg *Solanum melongena* with 100 ml of sterilized distilled water [11].

Serial dilution

1 ml of the stock solution was serially diluted as described previously [11].

Media preparation

The media (nutrient agar) was prepared according to the manufacturer's instruction and autoclaved at 121 °c for 15 min.

Culturing

The stock samples were inoculated on prepared Nutrient Agar media and incubated for 18-24 h at 37 °C. Growth was observed the following day as described earlier [12].

Colony identification and counting

Colonies grown were identified based on their morphological appearances like color, size, shape and elevation and were counted to the nearest CFU unit which were later sub-cultured [13].

Gram staining

Gram staining was carried out according to the procedure described earlier [14].

Biochemical tests

Bacterial isolates were biochemically screened by IMViC biochemical tests.

Antibacterial sensitivity test

Stock concentration of the extract was prepared by dissolving several concentration of the extract (25 mg, 50 mg, 75 mg, 100 mg, and 125 mg) in 1 ml of dimethyl sulfurdeoxide (DMSO).

Sensitivity test

Antibiotic sensitivity test was carried out according to Kirby Bauer's disc diffusion method [15].

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration test was carried out according to the standard method [15].

RESULTS

Qualitative phytochemical screening of the plant extract shows the presence of tannin, saponin, glycosides, reducing sugar, while Anthraquinones, alkaloid were absent in all extracts and in both the solvents (table 3.1). Saponin test shows the formation of foam that indicate the presence of saponin, alkaloid test shows red colour precipitate that indicates the presence of alkaloid, steroid was indicated by reddish brown colour, anthraquinone was absence with milkish precipitation, carbohydrates show yellow color precipitate that indicates the presence of carbohydrates.

The morphological appearances of isolated bacterial colonies were identified in which the sizes, shapes, elevation, pigmentation and appearances were identified (fig. 3.2), the isolated bacteria show different pigmentation on the media in which the staphylococcus shows yellow pigmentation, it's colony appears to be shiny with convex elevation. The second isolate was found to be greenish with convex elevation, and shiny in appearance which was assumed to be *Pseudomonas aeruginosa,* while the third isolate was found to show no pigmented coloration but with convex elevation and which was suspected to be *Streptococcus* spp. Grams staining result shows that, *Pseudomonas aeruginosa* was found to be gram negative, while the *Staphylococcus aureus* and *Streptococcusspp*. were found to be gram positive (table 3.2).

Biochemical tests resultconfirmed that, the isolated bacteria were *Pseudomonas aeruginosa, Staphylococcusaureus, Streptococcus species* in which *Pseudomonas aeruginosa* was found to be indole negative with no change of colour and as well methyl red test while *Staphylococcusaureus* was found to be coagulase positive and catalase positive but *Streptococcus specie* was found to be negative to both coagulase and catalase tests (table 3.3).

The results of antibacterial sensitivity tests against the isolated bacteria revealed that, methanolic extract at higher concentration of (75 mg/ml, 100 mg/ml, 125 mg/ml) have shown the highest activities on *Pseudomonas aeruginosa* with no activity recorded at 25 mg/ml and 50 mg/ml (fig. 3.1). The zone of inhibition of *Pseudomonas aeruginosa* for the ethanolic extract was 6 mm at 25 mg/ml and 12 mm at 125 mg/ml (table 3.4, fig. 3.2). Methanolic extract at all concentrations (25 mg/ml, 50 mg/ml, 75 mg/ml, 100 mg/ml and 125 mg/ml) were found to show excellent activities on *Streptococcus spp*. (fig. 3.3). The zones of inhibition on *Streptococcus spp*. for the ethanolic extract were 12 mm at 25 mg/ml and 17 mm at 125 mg/ml (table 3.5, fig. 3.4). Methanolic extract at all concentrations (25 mg/ml, 50 mg/ml, 75 mg/ml, 100 mg/ml, 125 mg/ml) were also found to show good activity on *Staphylococcus aureus* (fig. 3.5). The zones of inhibition on *Staphylococcus aureus* for the ethanolic extract were 8 mm at 25 mg/ml and 15 mm at 125 mg/ml (table 3.6, fig. 3.6).

The minimum inhibitory concentrations of various extracts on the isolated bacteria were revealed. The minimum inhibitory concentration on *Pseudomonas aeruginosa* was 12 mg/ml for the methanolic extract and 48 mg/ml for the ethanolic extract. The minimum inhibitory concentrations on *Streptococcus spp.* were 8 mg/ml for methanolic extract and 4 mg/ml for the ethanolic extract, while the minimum inhibitory concentrations on *Staphylococcus aureus* were 25 mg/ml for the methanolic extract and 7 mg/ml for ethanolic extract (table 3.7 and fig. 3.7, 3.8).

The overall minimum inhibitory concentrations of the two extracts on the isolated bacteria were that, the methanolic extract has the highest activity on *Staphylococcus aureus* at 8 mg/ml, followed by *Pseudomonas aeruginosa* at12 mg/ml and the least was on *Streptococcus spp.* at 25 mg/ml (Figure3.7). With the ethanolic extract, the highest activity was on *Streptococcus spp.* at 4 mg/ml followed by *Staphylococcus aureus* at 7 mg/ml and the least was on *Pseudomonas aeruginosa* at 48 mg/ml.

Table 3.1: Qualitative phytochemicals screening of *Lantana camara* leaves using methanol and ethanol as solvents

S. No.	Phytochemicals screened	Methanolic extract	Ethanolic extract
1	Tannins	+	+
2	Saponins	+	+
3	Carbohydrates	+	-
4	Reducing sugar	+	+
5	Anthraquinones	-	-
6	Glycosides	+	+
7	Alkaloids	-	-

Key: Presence: +Absence: -

Table 3.2: Result for Morphological appearance and gram staining of isolated bacterial colonies

S. No.	Size	Shape	Color	Appearance	Elevation	Gram staining	Inference
1	0.2-0.4 mm	Circular	Greenish	Shiny	Convex	_	*Pseudomonas spp.*
2	0.1-0.3 mm	Circular	Nill	Shiny	Convex	+	*Streptococcus spp.*
3	0.2-0.4 mm	Circular	Yellow	Shiny	Convex	+	*Staphylococcus spp.*

Table 3.3: Result for the biochemical characterization of bacterial isolates

S. No.	Indole	Methyl red	Catalase	Coagulase	Inference
1	-	-			*Pseudomonas spp.*
2			-	-	*Streptococcus spp.*
3			+	+	*Staphylococcus spp.*

Key: Positive: +Negative: -

Table 3.4: Result for the antibacterial sensitivity tests (Zones of Inhibitions on *Pseudomonas aeruginosa*)

Methanolic extract (mg/ml)	Zone of inhibition (mm)	Ethanolic extract (mg/ml)	Zone of inhibition (mm)
25	0	25	6
50	0	50	7
75	8	75	9
100	12	100	10
125	16	125	12

Table 3.5: Result for the antibacterial sensitivity test (Zones of Inhibitions on *Streptococcus* spp.)

Methanolic extract (mg/ml)	Zone of inhibition (mm)	Ethanolic extract (mg/ml)	Zone of inhibition (mm)
25	12	25	12
50	13	50	13
75	14	75	13
100	15	100	15
125	16	125	17

Table 3.6: Result for the antibacterial sensitivity test (Zones of Inhibitions on *Staphylococcus aureus)*

Methanolic extract (mg/ml)	Zone of inhibition (mm)	Ethanolic extract (mg/ml)	Zone of inhibition (mm)
25	6	25	8
50	8	50	9
75	9	75	11
100	10	100	13
125	13	125	15

Table 3.7: Result for the minimum inhibitory concentrations of various extracts on the isolated bacteria

Organisms	Methanolic extract (mg/ml) Minimum inhibitory conc.	Ethanolic extract (mg/ml) Minimum inhibitory conc.
Pseudomonas aeruginosa	12	48
Staphylococcus aureus	25	7
Streptococcus spp.	8	4

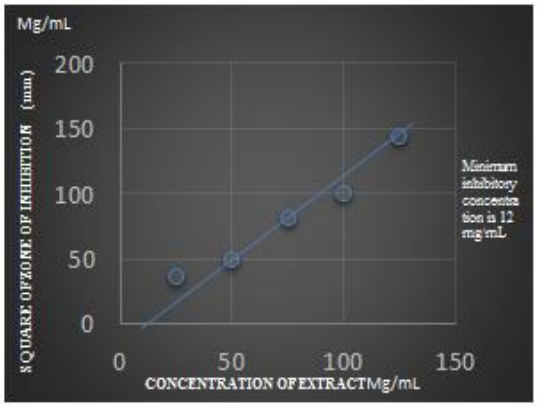

Fig. 3.1: Methanolic extract inhibition on *P. aeruginosa*

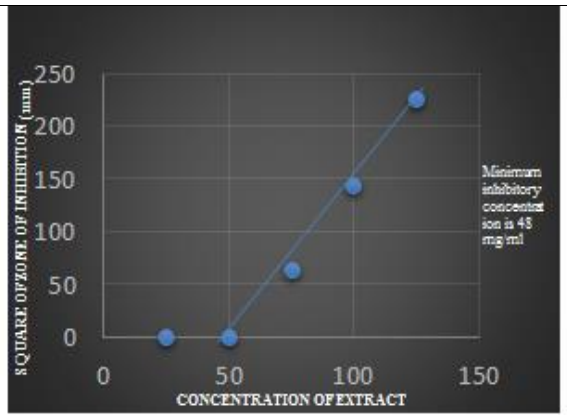

Fig. 3.2: Ethanolic extract inhibition on *P. aeruginosa*

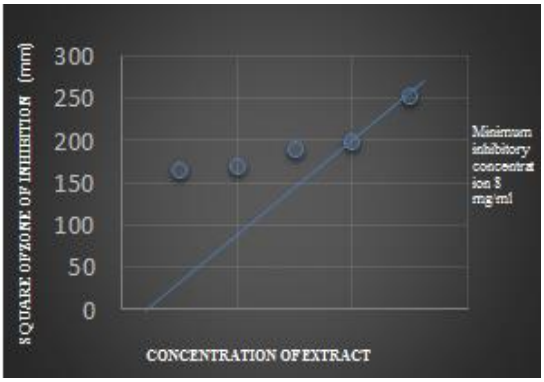

Fig. 3.3: Methanolic extract inhibition on *Streptococcus* spp

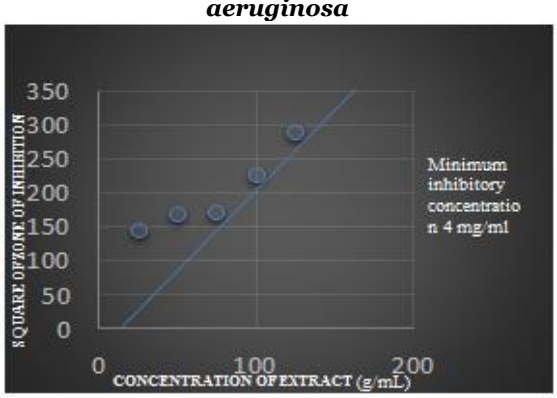

Fig. 3.4: Ethanolic extract inhibition on *Streptococcus* spp

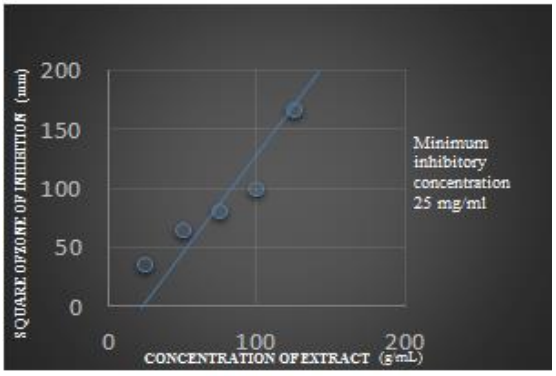

Fig. 3.5: Methanolic extract inhibition on *Staphylococcus* spp

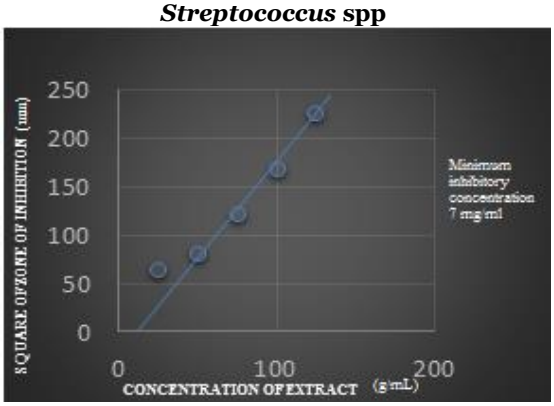

Fig. 3.6: Ethanolic extract inhibition on *Staphylococcus* spp

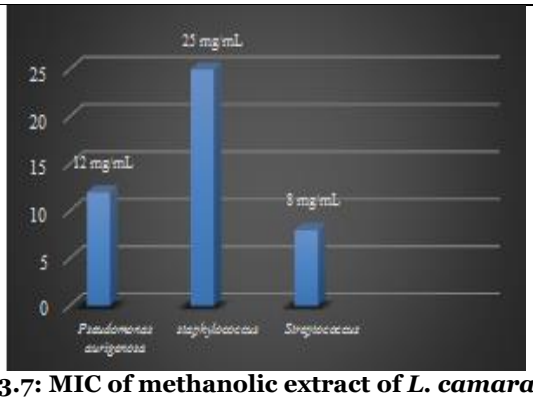

Fig. 3.7: MIC of methanolic extract of *L. camara* on bacterial isolates

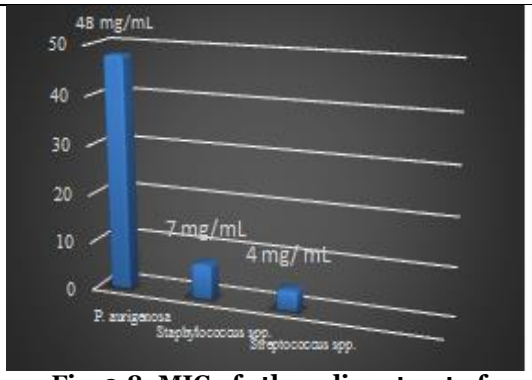

Fig. 3.8: MIC of ethanolic extract of *L. camara* on bacterial isolates

DISCUSSION

This research revealed the present of some secondary metabolites which is similar to the findings of Rabia *et al.* [16] in which the plant *Lantana camara* was found to possess same bioactive compounds phytochemicals.

This research also revealed that there are bacteria associated with the surface of unwashed garden egg. Among the bacteria isolated were *Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus spp*. the antibacterial activities of the extracts against these isolates vary with the solvents used during crude extracts extraction from the leaves and it also varies with the concentrations of the extracts in such a way that, the higher the concentration the higher the activities recorded. According to this research, the gram positive bacteria shows more activity with the extracts in which the minimum inhibitory concentrations of methanolic extract on the isolated bacteria was 8 mg/ml while that of ethanol was 4 mg/ml. Our results are in agreement with previous reports [17-20].

CONCLUSION

Conclusively, From the Phytochemical screening of the leaves of *Lantana camara,* it was found that, tannins, saponins, reducing sugar, glycosides and carbohydrates were present in both the used solvents (methanol and ethanol) respectively. It can also be concluded that pathogenic bacteria can be found on an unwashed garden egg. After performing the antibacterial activity using Kirby Bauer disc diffusion method, it was found that both the extract has excellent antibacterial activities against all the isolated bacterial strains i.e. *Pseudomonas aeruginosa, Streptococcus spp. and Staphylococcus aureus* at various concentrations. The antibacterial activity of the leaf extracts may be due to the presence of various bioactive constituents in the leaves known as phytochemicals whilethe antibacterial bioactivity of the extract is more on Gram positive bacteria than the Gram negative ones.

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