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Antimicrobial and antioxidant attributes of the ethanolic root extract of *Tamarindus indica* L.

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Abstract

The antimicrobial activities and the antioxidant properties of the ethanolic root extract of *Tamarindus indica* L., were carefully analyzed using standard methods. The antimicrobial activities of ethanolic root extract of *T. indica* L., was assessed on some bacterial isolates such as Escherichia coli, Staphylococcus aureus, Proteus mirabilis and Pseudomonas aeruginosa to estimate the inhibition zone in mm, using agar diffusion method. The Total antioxidant capacity of the ethanolic root extract of *Tamarindus indica* L., were carefully analyzed using the standards of Vitamin C Equivalent (VCE) and Gallic Acid Equivalent (GAE). The result revealed that ethanolic root extract of *Tamarindus indica* L., has antimicrobial properties as shown by its activity on test bacterial strains. The activity of the plant extract varied with concentration (mg/ml) of the ethanolic extract of *T. indica*. At 5.00 mg/ml of extract, S. aureus was the most susceptible to the ethanol extract of *T. indica*, while the least susceptible was E. coli. The Total antioxidant capacity (mg VCE) revealed a maximum and minimum value of 109.57 and 109.17, Total phenolic content (GAE100g-1) 64.04 and 61.84, and Total Ascorbic content (mg/kg) 278.64 and 278.04 respectively. The result revealed that ethanol root extract had antimicrobial properties as shown by its activity on test bacterial strains. The total antioxidant capacity detected were within a reasonable range. The presence of the secondary metabolites in the root extract of *T. indica* L., is perhaps the reason for its medicinal properties.

Keywords: *Tamarindus indica* L.; Ethanol extract; Root; Antimicrobial and antioxidant; Vitamin C Equivalent (VCE) and Gallic Acid Equivalent (GAE)

1. Introduction

Medicinal plants have over the years formed a vital part in the treatment of various kinds of sicknesses in man and animals globally. Medicinal plants are vital constituents of both traditional and modern system of medicine [23]. Research has it that *Tamarindus indicia* L., belongs to the Dicotyledonous family: Caesalpiniaceae, which is the third largest family of flowering plants with a total of 727 genera and 19, 327 species [10]. The tamarind (*Tamarindus indica* L.) is a tree-like plant. *Tamarindus indica* L. is known to be indigenous to tropical Africa. However, it has become established in North and South America; precisely from Florida to Brazil, and is also propagated in some subtropical countries such as China, India, Pakistan, Philippines, Java and Spain[9]. It is a dicotyledonous plant [11]**.** Leaf contains about 13 constituents; limonene and benzyl benzoate are prevalent over others[21].It has been reported that leaves can reduce inflammatory swelling, tumours, ring worm, diseases of blood, small pox, ophthalmia and other eye diseases, ear ache, snake bite[3]. The unsaponifiable matter from the seed oil contains: β-amyrin, compesterol, β-sitosterol [27], [26], [15] [16] and [2]. Unripe fruit pulp is an astringent, to the bowel and cure “vata”, bark heals ulcer, liver complaints [3].

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[8] reported the bioactive compounds (+)-Pinitol present in the root bark of *T. indica* for the first time. The composition of various essential oils was carried out by [14]. In Zimbabwe, the leaves are added to soup and the flowers are prepared as salads [25]. The aim and objective of this study was to ascertain the antimicrobial and antioxidant attributes of the ethanolic root extract of *Tamarindus indica* L.

2. Material and methods

The root of *Tamarindus indica* L. was collected from a live plant in Bauchi, northern part of Nigeria. The plant was identified by Prof. MacDonald Idu of the Department of Plant Biology and Biotechnology, University of Benin, Benin City and deposited at the Department of Plant Biology and Biotechnology, University of Benin, Herbarium, with a Voucher No. - UBHf0292.

The fresh roots of *Tamarindus indica* were cut from the main plant, rinsed in water and spread on laboratory tables where they were dried under room temperature. It was reduced to fine powder with the aid of mechanical grinder. The mass of the granulated sample was 750 g. The sample was then macerated using ethanol to get a percentage yield of 98.68 %.

2.1. Standardization and preparation of the microbial innocula

Standardization and preparation of the microbial innocula. The test organisms (clinical bacterial isolates) used for the study were *Escherichia coli, Staphylococcus aureus, Proteus mirabilis* and *Pseudomonas aeruginosa.* The microbial isolates were obtained from the laboratory stock of the Department of Microbiology, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria where they were also identified. 2 ml of Dimethyl Sulphoxide (DMSO) was added to 3.5 g, 2.5 g, 1.5 g and 0.5 g of ethanolic extract and made up to the mark with water to obtain a concentration of 35 mg/ml, 25 mg/ml, 15 mg/ml and 5 mg/ml respectively. 16 plates were poured; 12 MIC and 4 sensitivity. 0.5 ml solution of various concentration of extract, were poured into the agar wells (bored holes of 6 mm in diameter on the agar), using a sterile syringe. It was then allowed to diffuse for 1 hour, and then incubated at 37 ^{o}C for 24 hours (the 12 plates for MIC). 1 g positive disc placed on a plate containing gram positive bacteria. While three (3) gram negative disc placed on a plate containing *E. coli, P. aeruginosa* and *Proteus mirabilis* respectively.

2.2. Extract Dilution Method

2 ml of Dimethyl Sulphoxide (DMSO) was added to 3.5 g, 2.5 g, 1.5 g and 0.5 g of ethanolic extract and made up to the mark with water to obtain a concentration of 35 mg/ml, 25 mg/ml, 15 mg/ml and 5 mg/ml respectively. 16 plates were poured; 12 MIC and 4 sensitivity. 0.5 ml solution of various concentration of extract, were poured into the agar wells (bored holes of 6 mm in diameter on the agar), using a sterile syringe. It was then allowed to diffuse for 1 hour, and then incubated at 37 0C for 24 hours (the 12 plates for MIC). 1 g positive disc placed on a plate containing gram positive bacteria. While 3 gram negative disc placed on a plate containing *E. coli*, *P. aeruginosa* and Proteus mirabilis respectively.

2.3. Microbial Inocula Preparation for Susceptibility Testing

The inocula of the bacterial isolates were prepared by growing each pure isolate in nutrient broth at 37 ^{o}C for 24 hours. After incubation, 1 ml of the diluted cultures of the microbial isolates in normal saline solution, was inoculated unto the solidified nutrient agar at 45 ^{o}C using a Pasteur pipette.

2.4. Susceptibility Testing

The test organisms (clinical bacterial isolates) used for the study were *Escherichia coli, Staphylococcus aureus, Proteus mirabilis* and *Pseudomonas aeruginosa.* The microbial isolates were obtained from the laboratory stock of the Department of Microbiology, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria where they were also identified.

2.5. Extract Dilution Method

2ml of Dimethyl Sulphoxide (DMSO) was added to 3.5g, 2.5g, 1.5g and 0.5g of ethanolic extract to obtain a concentration of 35 mg/ml, 25 mg/ml, 15 mg/ml and 5 mg/ml respectively. 16 plates were poured; 12 MIC and 4 sensitivity. 0.5ml solution of various concentration of extract, were poured into the agar wells (bored holes of 6 mm in diameter on the agar), using a sterile syringe. It was then allowed to diffuse for 1 hour, and then incubated at 37 ^{0}C for 24 hours (the 12 plates for MIC). 1g positive disc placed on a plate containing gram positive bacteria. While 3 gram negative disc placed on a plate containing *E. coli, P. aeruginosa* and *Proteus mirabilis* respectively. The innocula of the bacterial isolates were prepared by growing each pure isolate in nutrient broth at 37 ^{o}C for 24 hours. After incubation, 1 ml of the diluted

cultures of the microbial isolates in normal saline solution, was inoculated unto the solidified nutrient agar at 45 °C using a Pasteur pipette.

2.6. Test for antimicrobial activity

The agar-well diffusion method, suitably modified was adopted for the susceptibility studies. The media used was nutrient agar to test the susceptibility of bacteria. The media was prepared according to the manufacturer's guide and sterilized in an autoclave at 121 °C for 15minutes after which they were poured into Petri dishes and allowed to set. The plates were inoculated with the respective test organism in triplicate culture plates. Using a sterile cork borer of 6 mm diameter, four (4) adequately spaced wells per plate were made into the culture agar plates. Varying concentrations of 35 mg/ml, 25 mg/ml, 15 mg/ml and 5 mg/ml of the plant extracts were poured into the four holes that have been labeled previously to correspond to each of the concentrations of the extract. The plates were left standing on the work bench for 30-40minutes to allow pre-diffusion time. The bacterial inoculated plates were incubated at 37 °C for 24 hours. Zones of inhibition around the wells indicated antimicrobial activity of the extracts against the test organisms. The diameters of these zones were measured diagonally in millimeter with a ruler and the mean value for each organism from the triplicate cultured plates were recorded. Using the agar-well diffusion technique, an already made gram positive and gram negative (Asodisks Atlas Diagnostics, Enugu, Nigeria) standard antibiotic sensitivity disc bought from a laboratory chemical equipment store in Benin City was used as positive control for the bacteria isolates. Distilled water was used as negative control for all the test isolates. All the plates used for control were incubated at 37 ° C for 24 hours. The zones of inhibition were measured after incubation and recorded.

2.7. Determination of Minimum Inhibitory Concentrations (MICs).

The standard agar dilution protocol with doubling dilution was used to determine the MICs of the extracts [18**]**. A 100 mg/ml concentration of each of the extract was prepared in sterile distilled water, and diluted to achieve a decreasing concentration of 35, 25, 15 and 5 mg/ml respectively. Each dilution was introduced into nutrient agar plates and potato dextrose agar plates already seeded with the respective test organism. All plates were incubated at 37 °C for 24hours. The minimum inhibitory concentration (MIC) of extracts for each test organism was regarded as the agar plate with the lowest concentration without growth.

2.8. Minimum bactericidal concentration (MBC) determination

Samples were taken from the MIC test bacteria plates with no visible growth. These were sub-cultured unto freshly prepared nutrient agar plates and incubated at 37 °C for 24 hours. MBC was taken as the concentration of the extract that did not show any growth on the new set of agar plate.

3. Results

The test microbial isolates showed varying degrees of response towards various concentrations of ethanolic root extract of *T. indica* L. as shown in the figures below.

Table 1 Minimum inhibitory concentration and minimum bactericidal concentration of ethanolic extract of *T. indica.*

Bacterial Isolates	MIC (mg/ml)	MBC (mg/ml)
E. coli	25.00	44.20
	50.00	-
P. aeruginosa	25.00	44.00
	50.00	44.00

= Not bactericidal.

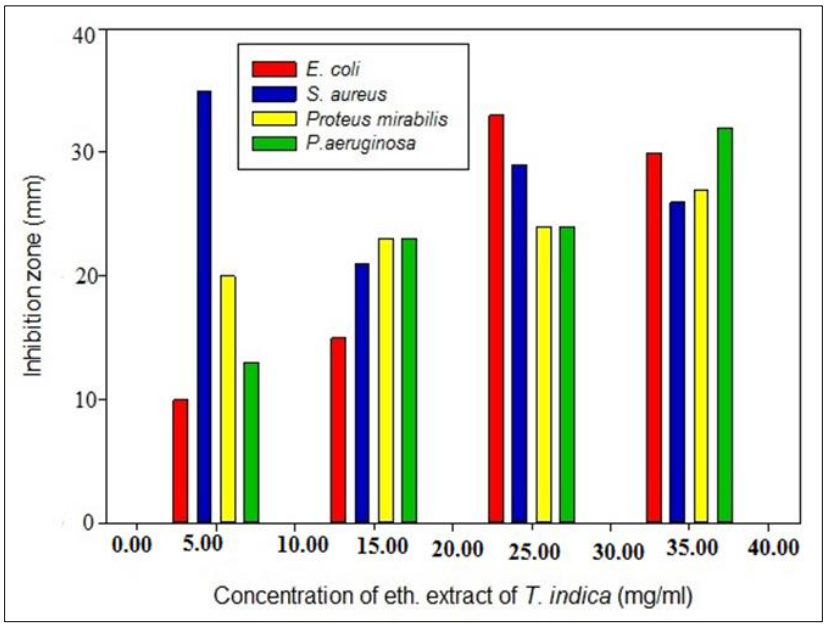

Figure 1 The antimicrobial activity of ethanolic extract of *T. indica* being assessed on *E. coli, S. aureus, P. mirabilis* and *P. aeruginosa*(bacteria)

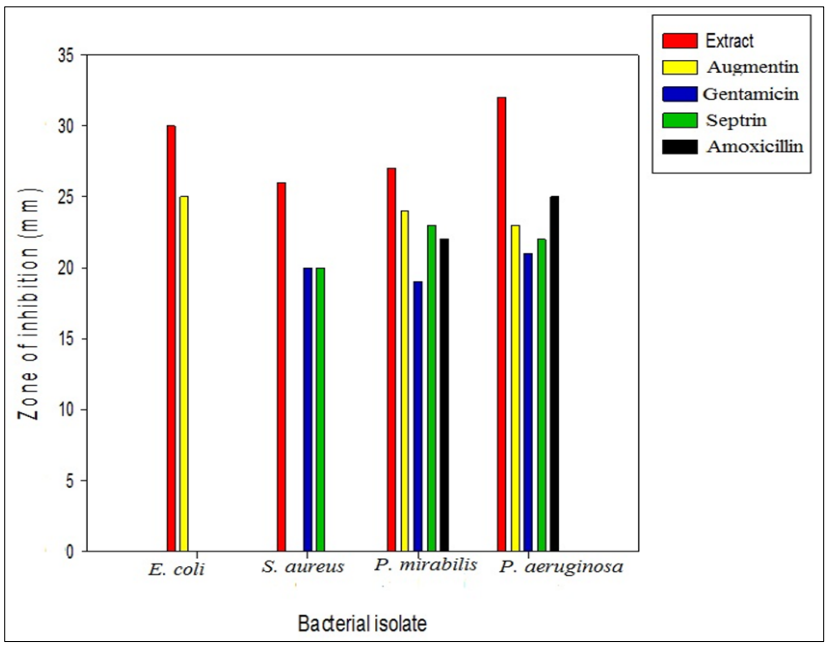

Figure 2a The antibacterial effect of ethanolic extract of *T. indica* root being compared to selected antibiotics

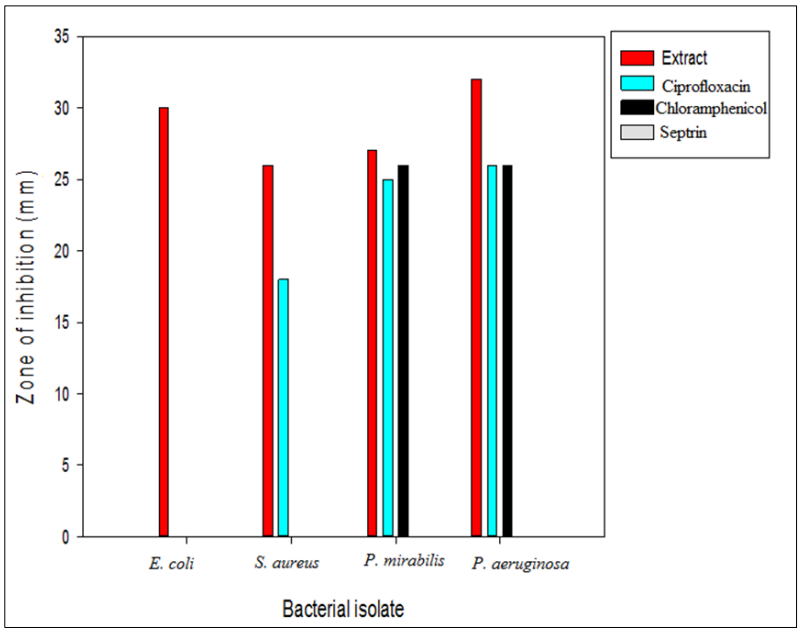

Figure 2b The antibacterial effect of ethanol extract of *T. indica* root (at a concentration of 100 mg/ml) being compared to selected antibiotics (at 5 mg/ml)

3.1. Antioxidant capacity of ethanolic extract of *T. indica.*

The Antioxidant capacity of the ethanolic extract of *T. indica* revealed three major constituents. These are; Total antioxidant capacity (mg VCE), Total phenolic content (GAE100g^{-1}) and Total Ascorbic content (mg/kg), as shown in table 2 below.

Table 2 Antioxidant Capacity of the plant extract

Antioxidants	**X±S.E**	**Max**	**Min**
Total antioxidant capacity (mg VCE)	109.37 ± 0.115	109.57	109.17
Total phenolic content (GAE100g^{-1})	62.94 ± 0.635	64.04	61.84
Total Ascorbic content (mg/kg)	278.34 ± 0.173	278.64	278.04

VCE = Vitamin C Equivalent; GAE = Gallic Acid Equivalent

4. Discussion

The antimicrobial activity of ethanolic extract of *T. indica* was assessed on some bacterial isolates such as *Escherichia coli*, *Staphylococcus aureus, Proteus mirabilis* and *Pseudomonas aeruginosa* to estimate the inhibition zone in mm. The activity of the plant extract varied with concentration (mg/ml) of the ethanolic extract of *T. indica* (Fig. 2). At 5.00mg/ml of extract, *S. aureus* was the most susceptible to the ethanolic extract of *T. indica*, while the least susceptible was *E*. coli. At 15.00mg/ml, *Proteus mirabilis* and *Pseudomonas aeruginosa* were the most susceptible, while at 25.00 and 35.00 mg/ml, *E. coli* and *P. aeruginosa* were the most susceptible respectively (figure 1).The MIC of the of the ethanolic extract of the root of *T. indica* showed that when the concentration increased, the zone of inhibition increased, this could be as a result of increase in dose leading to increasing effect. As could be seen in figure 1.

The antimicrobial activity of ethanolic root extract of *T. indica* was compared with some regularly used antibiotics to determine the potency of *T. indica*. The result revealed *T. indica* ethanolic extract at 35.00 mg/ml to be more potent, when compared with other antibiotics such as augmentin, gentamicin, streptomycin, amoxicillin, chloramphenicol, ciprofloxacin and septrin respectively, as all the bacterial isolates were susceptible to the ethanolic extract of the root of *T. indica* (Fig. 2a and b).*Escherichia coli* was resistant to all the antibiotics except augmentin, *Staphylococcus aureus* was resistant to augmentin, *Proteus mirabilis* and *Pseudomonas aeruginosa* were not resistant to any of the antibiotics under comparison (figure 2a).

The use of ethanol as a solvent for extraction is remarkable, as [6] reported that among six different extracts of *Tamarindus indica* leaves, ethanol extract had considerable activity against both Gram negative and positive bacteria and fungi.

In another study carried out on the ethanolic extract of *T. indica* seeds, by [17], it was revealed that the *T. indica* plant had profound antibacterial effects and could have some applications in medicine. It was observed that crude ethanolic seeds extract of *T. indica* possess very remarkable antibacterial effects against gram positive bacteria such as *Staphylococcus aureus* (17.25 mm/disc) and gram negative bacteria like *Shigella dysenteriae* (18 mm/disc). While other gram positive and gram negative bacteria showed moderate to good antibacterial activities ranging from (12 – 13 mm/disc) and (13 – 14 mm/disc) respectively. This corresponds to previous research by [19].In a related study, it was observed that the methanolic extract of *Tamarindus indica* L fruit pulp showed the presence of antibacterial properties against most tested bacterial strains when compared with the antibiotic chloramphenicol [5]. The various zone of inhibition in a decreasing order of sensitivity were; *Pseudomonas aeruginosa* (12.00 mm), *Escherichia coli* (11.60 mm), *Staphylococcus aureus* (11.30 mm), *Proteus vulgaris* (11.00 mm), *Bacillus cereus* (11.00 mm), and *Staphylococcus epidermidis* (10.60 mm). Accordingly, the results obtained from the current study were *Escherichia coli* (30.00 mm/disc), *Staphylococcus aureus* (26.00 mm/disc), *Proteus mirabilis* (27.00 mm/disc), *and Pseudomonas aeruginosa* (32.00 mm /disc).These results of the crude extract are promising when compared with the other compared antibiotics (augmentin, gentamicin, streptomycin, amoxicillin, ciprofloxacin and chloramphenicol) which is in pure form. Zone of inhibition above 10 mm is considered as good antibacterial activity [1]. Similarly, [7] revealed the antimicrobial action of the ethanolic root extract of *Tamarindus indica* L. The high antioxidant activity in plants is often associated with the presence of phenolic compounds [24].[4] also revealed that the ethanol extract of *T. indica* L. fruit pulp possess strong antibacterial activity against *E. coli, Klebsiella pneumoniae, Salmonella paratyphi* A and *Pseudomonas aeruginosa*, while aqueous extract possess weak antibacterial activity excluding *Pseudomonas aeruginosa*.

The Antioxidant capacity of the ethanolic root extract of *T. indica* revealed three major constituents. These are; Total antioxidant capacity (mg VCE) with a maximum value of 109.57 and minimum value of 109.17, Total phenolic content ($GAE100g^{-1}$) with a maximum value of 64.04 and minimum value of 61.84 and Total Ascorbic content (mg/kg) with a maximum value of 278.64 and minimum value of 278.04, as well as the mean content values, as shown in table 2.

The antioxidant properties of the ethanol root extract of *T. indica* could be attributed to the afore-mentioned antioxidant capacity. In a related study, it was reported that the antioxidant activity/attribute (% scavenging property) of tamarind pulp extract mixed nano emulsions and coded as F1-3.3TE and F1-6.6TE were assessed by considering the outcome of currently prepared formulations and those subjected to stress conditions. The quantity of these two formulations were rationed into various concentrations by mixing with concentrated ethanol and distilled water in a ratio of 1:1 by volume. The result showed that % scavenging activity (antioxidant property) was a direct function of the concentrations of the tamarind pulp extract [22]**.**While the pulp and fruit extract of *T. indica* showed antioxidant activities on rats fed with cholesterol rich diet [12]. Also, the hydro alcoholic and aqueous extracts of *T. indica* leaves possess antioxidant activity like Fe^{+3} reducing potential, NO·, OH· and DPPH· radical scavenging potential [13]. The Minimum inhibitory concentration and minimum bactericidal concentration of the ethanolic root extract of T. indica L. were remarkable compared to [20].

5. Conclusion

This research work has revealed the antimicrobial action of the ethanol extract of the root of*Tamarindus indica* L., on the test microbial isolates (both with thick and thin peptidoglycan cell wall). Hence, it could perhaps be seen as a potential source of antimicrobial agent. It has also shown the antioxidant capacity of the plant under study. I would recommend further pharmacological evaluations and toxicological studies on the root of*Tamarindus indica* L

Compliance with ethical standards

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Disclosure of conflict of interest

The authors emphatically declare that no conflict of interest capable of influencing this research was recorded in this study.

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