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Phytochemical Profiling, Antimicrobial, and Antioxidant Activities of Tamarindus indica Pulp Extracts: A Comprehensive Evaluation

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Abstract

The study looks at the antioxidant, antibacterial, and phytochemical characteristics of pulp extracts from Tamarindus indica. Several solvent fractions were obtained from the extraction process, such as hexane, butanol, ethyl acetate, crude, and aqueous, all of which indicated the existence of primary and secondary metabolites. High amounts of flavonoids, alkaloids, tannins, saponins, and steroids were found by phytochemical screening, especially in the butanol and ethyl acetate fractions. With an inhibition zone of 17 mm against Bacillus subtilis, the ethyl acetate extract had the most excellent antibacterial activity in antimicrobial tests conducted using the cup-plate agar diffusion method. With a radical scavenging activity of 11 ± 0.1%, the crude extract's antioxidant activity was found to be modest, in contrast to the positive control's 87% activity, propyl gallate (PG). These findings show that T. indica extracts have a promising antibacterial potential, despite their still-low antioxidant efficiency. According to the research, T. indica may be a valuable source of bioactive substances for medical and pharmacological uses, especially in the treatment of infections.

Keywords: Antimicrobial properties; Antioxidant activity; Ethanolic extract; phytochemical screening; Tamarindus indica.

Abbreviations: T. indica: Tamarindus indica, DPPH: 2,2-Diphenyl-1-picrylhydrazyl, PG: Propyl Gallate, RSA: Radical Scavenging Activity, DMSO: Dimethyl Sulfoxide.

INTRODUCTION

The tropical tree Tamarindus indica L., often known as tamarind, is extensively grown throughout Africa, Asia, and South America. The edible fruit of the tree is prized for its applications in traditional medicine and cooking. For ages, people have utilized extracts from T. indica's leaves, seeds, and bark to cure a range of conditions, including fever, diarrhea, and inflammation (Aqil, F. et al., 2006; Doughari, J. H. 2006). The pharmacological potential of T. indica has been the subject of increased scientific investigation in recent decades, especially in light of its phytochemical composition, antioxidant qualities, and antibacterial activity (Nwodo et al., 2011; Saleem et al., 2022). Tamarindus indica is a rich source of primary and secondary metabolites, including flavonoids, alkaloids, tannins, and polyphenols, according to phytochemical studies (Soni et al., 2019; Baliga et al., 2011). It is well recognized that these substances support the biological functions of plants, such as their anti-inflammatory, antibacterial, and antioxidant qualities. For example, tamarind's high flavonoids and polyphenols have been linked to the ability to scavenge free radicals, making them powerful antioxidants (Ismail et al., 2022; Kumar et al., 2008). According to studies (Al Fatimi et al., 2007; Bhadoriya et al., 2012), these phytochemicals also have antibacterial properties against a range of bacterial infections. T. indica is unique among medicinal plants because of its wide range of phytochemical profiles (Tsuda et al., 1994). Isolating certain chemicals from T. indica extracts has been the subject of several investigations; these studies have demonstrated the ability to prevent the growth of microorganisms, including Staphylococcus aureus and Escherichia coli (Kidaha et al.,2023; Morton, 1987). Though these investigations have established the foundation for comprehending the plant's therapeutic qualities, T. indica's entire potential as a source of bioactive chemicals is still unrealized (Alzoreky et al., 2003). Antioxidants are essential for reducing oxidative stress, which is linked to a number of illnesses, such as cancer, heart disease, and neurological conditions (Ghaly et al.,2023; Ajayi et al.,2011). Tamarindus indica has drawn attention as a possible source of naturally occurring antioxidants due to the growing interest in natural antioxidants as alternatives to synthetic ones

(Harborne et al., 2000; Sudjaroen et al., 2005). Using assays like DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), previous research have shown the antioxidant properties of T. indica extracts (Ayala et al.,2014; Ghazanfar 1989). According to this research (Bhadoriya et al., 2012; Tsuda et al., 1994), some substances can neutralize free radicals and lessen oxidative damage in biological systems.

While T. indica has been shown to possess antioxidant qualities, nothing is known about the precise chemicals that are in charge of this action (Kidaha et al.,2023). Furthermore, the majority of research has concentrated on the flesh of the fruit, paying less attention to other plant components like the seeds and leaves, which may contain unique phytochemicals with even higher potential for antioxidant activity (Morton, 1987; Sudjaroen et al., 2005). This emphasizes the need for more investigation to isolate and identify certain antioxidant chemicals found in various T. indica sections.

The quest for novel antimicrobial agents, especially those sourced from natural sources, has become imperative due to the rise of bacteria resistant to antibiotics (Alzoreky et al., 2003; Ghaly et al., 2023). Numerous investigations have demonstrated the antibacterial activity of Tamarindus indica against both Gram-positive and Gram-negative bacteria, as well as fungi, suggesting that it has promise in this field (Ajayi et al.,2011; Harborne et al.,2000). T. indica extracts have demonstrated promising outcomes in tests conducted against common bacterial strains, including Bacillus subtilis, Pseudomonas aeruginosa, and Candida albicans (Sudjaroen et al., 2005; Ayala et al., 2014). Tamarindus indica's complex phytochemical composition—particularly the presence of tannins, saponins, and alkaloids—has been linked to its antibacterial activity (Ghazanfar, 1989). According to Morton (1987), these substances can damage microbial cell membranes and obstruct enzymatic functions, which ultimately results in cell death. Though these early results are encouraging, much more needs to be discovered regarding the methods by which T. indica carries out its antibacterial activities (Bhadoriya et al., 2012;, Tsuda et al., 1994). This work aims to assess the antioxidant capacity, antibacterial activity, and phytochemical composition of Tamarindus indica extracts, emphasizing their pharmacological potential for use in future medicine.

MATERIALS AND METHODS

Plant Material

The plant components were purchased from the Omdurman, Sudan, local market, specifically the pulp of Tamarindus indica. After being verified, the samples were kept for later examination in the Chemistry Laboratory of the Omdurman Islamic University in Khartoum, Sudan's Faculty of Science and Technology (Figure 1).

Figure 1. *Tamarindus indica*. pulp and seed.

Preparation of Extract

96 g of Tamarindus indica (pulp) were soaked in 1 liter of 80-90% ethanol for three days, followed by filtration and evaporation of the solvent at room temperature. With a calculated percentage yield of 58.8%, the T. indica pulp extract was shown to have a good extraction efficiency. After that, the extract was partitioned three times using ethyl acetate, chloroform, hexane, butanol, and water. Then, at room temperature, the solvent extracts were evaporated.

Phytochemical Screening

Primary Metabolites:

A number of tests were used in the phytochemical screening process to look for Tamarindus indica main metabolites. Using the Molisch test, which created a crimson ring when sulfuric acid was added to the alpha-naphthol-treated material, carbohydrates were identified. After heating the sample with Benedict's reagent, the Benedict's test revealed the presence of reducing sugars, giving the sample a dark red color. Barfoed's test was used to identify monosaccharides; after heating the sample in a water bath for more than four minutes with Barfoed's reagent, the sample displayed a dark red color. These findings show the presence of primary metabolites that are necessary for the plant's energy storage and metabolism, such as lowering sugars and carbohydrates (Harborne, 1998).

Secondary Metabolites:

Numerous tests were used to identify secondary metabolites. Using Wagner's and Dragendorff's assays, which produced brown and orange precipitates, respectively, alkaloids were verified. The lead acetate, KOH, and alkaline tests all yielded yellow hues or precipitates, which were used to identify flavonoids. The ferric chloride test revealed a dark green tint, indicating the presence of tannins. Saponins were detected by the

development of steady foam in the foaming test. The Salkowski test revealed a crimson ring when steroids and terpenes were present, and the ammonia layer turned red when diluted hydrochloric acid and ammonia were used to demonstrate the presence of anthraquinones. The biological properties of these secondary metabolites, such as their antibacterial and antioxidant properties (Harborne.,1998).

Antioxidant Activity:

DPPH Free Radical Scavenging Activity

With minor adjustments, the DPPH radical scavenging was calculated using the methodology of Mollyneux, P. (2004). The test samples were exposed to 2.2 Di (4-tert-octylphenyl)-1-picrylhydrazyl stable free radical (DPPH) in 96-well plates for 30 minutes at 37ºC. The DPPH concentration was maintained at 300µM. While DPPH was being produced in ethanol, the test samples were dissolved in DMSO. Using a multiplate reader spectrophotometer, the absorbance drop was recorded at 517 nm following incubation. Percentage radical scavenging activity by samples was measured in comparison with a DMSO treatedcontrol group. Every test was run three times.

Antimicrobial Activity

The antimicrobial activity was tested using the cup-plate agar diffusion method with some modifications. 20 ml aliquots were placed into sterile Petri dishes after a bacterial solution containing 108−10910^8 - 10^9108−109 CFU/ml was combined with 100 milliliters of sterile nutritional agar at 45°C. After they had hardened, sterile cork borer holes measuring 10 mm were made. After adding 0.1 ml of sample extract to each well, the plates were allowed to diffuse for two hours at room temperature. Following an 18-hour incubation period at 37°C, the zones of inhibition were quantified, and the outcomes were documented, every test was run twice.

RESULTS AND DISCUSSION

Phytochemical analysis

The result of phytochemical screening of T.indica pulp extract qualitatively is shown in Table 1, The phytochemical constituents of the extract included: Carbohydrates (Reducing Sugars, Mono saccharides), Flavnoids, Alkaloids, Saponins, Steroids and Terpenes and Anthraqunons. Tannins and Phenols were not detectable at the tested assay condition.

Table 1. Phytochemical screening of the crude and fractions of T.indica.

Phytochemical Group	Crude Extract	Hexane Extract	Chloroform Extract	Ethyl Acetate Extract	Butanol Extract	Aqueous Extract
-Carbohydrates	+++	+++	+++	+++	+++	+++
-Reducing Sugars	+++	+++	+++	+++	+++	+++
-Monosaccharides	-	-	-	+	-	+
-Flavnoids	+++	+	+	+	+	+
-Alkaloids	+	+	+	+	+	+
-Saponins	+++	+++	+++	+++	+++	+++
-Steroids and Terpenes	+++	+++	+++	+++	+++	+++
-Anthraqunons	+	-	+	+	+	+

+++ : high concentration,
+ : low concentration,
- : not detectable using the specified assay method.

Antioxidant activity (DPPH)

Figure 2 and Table 2 illustrate the pulp extract of T. indica's antioxidant activity. The percentage of DPPH inhibition (%) that resulted from the samples' exposure was used to express the results. The capacity of plant extracts to scavenge the stable free radical DPPH and transform it into Diphenyl picryl hydrazine was used to measure their radical scavenging activity and antioxidant potential. Compared with Propyl Gallate (PG), a positive control.

Table2: Antioxidant Activity of *Tamarindus indica* Compared to Positive Control (PG).

Sample	% Radical Scavenging Activity(DPPH)
T. indica	11%
PG (Control)	87%

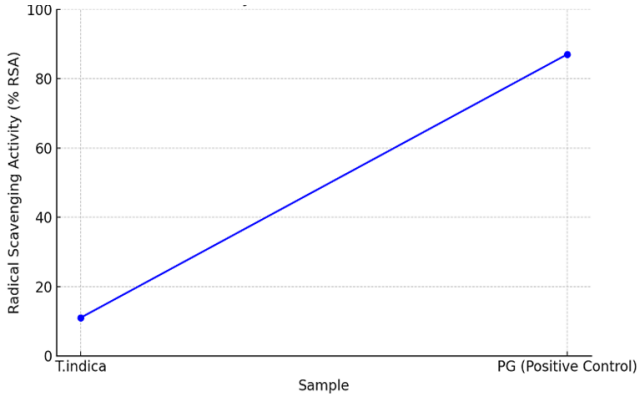

Figure 2. Antioxidant activity (%RSA) of *T.indica* and PG Control.

Antimicrobial activity

The antimicrobial activity results are presented in Figure 3 and Table 3.

Table 3: Inhibition Zones for Different Extracts of T.indica against Bacillus subtilis.

Extract	Crude	Hexane	Ethyl Acetate	Butanol	Aqueous	DMSO
Zones of inhibition (mm)	15	-	15	17	12	-

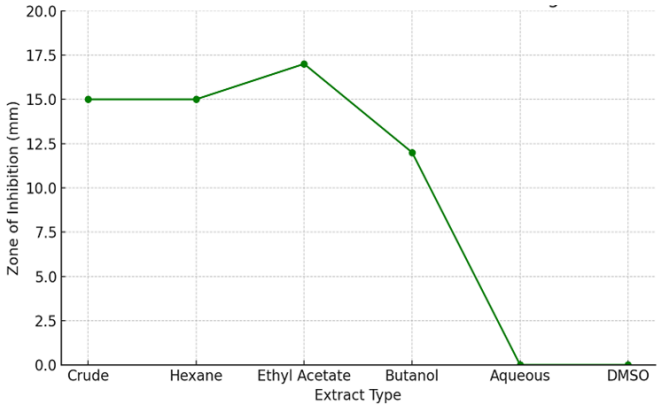

Figure 3. Zones of Inhibition for Different Extracts of T.indica against Bacillus subtilis.

Table 4. Description of T.indica plant.

Plants parts	Description
Flower	The fruit is a pod-like structure, dark and curled, bearing seeds within a sticky, delicious pulp. The little yellow blossoms have red striations.
Leaves Information	leaves are pinnately complex with many short, rectangular leaflets, bright green in hue, and finely reticulate-veined. widely available and utilized for both culinary and medicinal uses in tropical regions, particularly in Africa and India.

Note: Known for its rich content of tartaric acid and various phytochemicals beneficial in traditional medicine.

Discussion

Tamarindus indica's phytochemical screening findings show the existence of a number of primary and secondary metabolites that are essential to the biological activities of the plant. Using assays like Benedict's and Molisch's, primary metabolites, such as reducing sugars and carbs, were verified. Significant secondary metabolites, including alkaloids, flavonoids, tannins, saponins, steroids, and terpenes, were also found during screening, suggesting the plant may have antibacterial and antioxidant qualities. These data imply that T. indica possesses a rich phytochemical profile that may contribute to its therapeutic applications, warranting more study into its health benefits and practical usage.

With only 11% radical scavenging activity (RSA), the T. indica crude extract's antioxidant activity was shown to be extremely weak when tested using the DPPH radical scavenging assay. Propyl gallate (PG), the positive control, on the other hand, showed a noticeably higher RSA of 87%, demonstrating its robust antioxidant activity.The T. indica extract's low level of antioxidant activity indicates that there are not enough active antioxidant components in the crude extract to adequately neutralize free radicals in this assay. This can be the result of the extraction process or the existence of other substances that prevent the antioxidants from doing their job. On the other hand, PG is a well-known strong antioxidant, and its high RSA in this test acts as a standard for assessing how effective natural extracts are. Better outcomes from further refining the extraction procedure or isolating particular antioxidant components from T. indica could increase the plant's potential as a natural source of antioxidants.

The extracts containing ethyl acetate displayed the largest zone of inhibition (17 mm), closely trailed by the extracts containing hexane and crude, which both displayed a 15 mm zone of inhibition. Butanol showed a 12 mm inhibitory zone and moderate activity. The aqueous extract and DMSO did not exhibit any discernible antimicrobial activity, indicating that the active ingredients with antibacterial properties are more soluble in organic solvents such as hexane and ethyl

acetate. This is consistent with other research showing the effectiveness of ethyl acetate and hexane extracts in phytochemical screens, and could be caused by the presence of particular secondary metabolites that are better extracted in non-polar or semi-polar solvents. These findings imply that organic solvents—specifically, ethyl acetate—are useful for separating antimicrobial components from T. indica, which could lead to more research on antimicrobial drugs originating from plants.

CONCLUSIONS

The results of the investigation effectively established the presence of substantial antibacterial activity and noteworthy phytochemical variety in Tamarindus indica pulp extracts, especially in the ethyl acetate fraction. Important secondary metabolites with therapeutic potential, including tannins, alkaloids, flavonoids, and saponins, were found during the phytochemical screening. Against Bacillus subtilis, the antibacterial activity was especially potent, while other fractions also demonstrated some efficacy. However, the antioxidant activity of the crude extract was comparatively moderate, indicating limited radical scavenging capacity. These results imply that although T. indica is a promising strong antibacterial agent, more investigation and improvement could be required to maximize its antioxidant capacity. Overall, the research lends credence to the possible use of T. indica extracts in medicinal and pharmaceutical settings, especially in the context of antibacterial therapies.

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Competing Interests: The authors declare that there are no competing interests.

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