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(RESEARCH ARTICLE)

Investigating the nutritional, antioxidant and antimicrobial properties of Tamarindus indica linn seed and pulp

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Abstract

This study seeks to investigate the nutritional (i.e proximate composition), antioxidant and anti-microbial properties of *Tamarindus indica* Linn seed and pulp. The proximate composition estimated during the study was analyzed by using standard AOAC methods. Ethanolic, aqueous and ethyl acetate extracts were prepared from seed and pulp powders of Tamarind. The tamarind seed was found to have a substantial protein value (17.51%) which means that it can be processed and used as a food supplement. Phytochemical screening of the samples revealed the presence of flavonoids and tannins in pulp and saponins in the seed which might validate the traditional uses of the plant in the treatment of various diseases. The antimicrobial activity of the extracts was evaluated using agar well diffusion and broth dilution assay. Ethanol, aqueous and ethyl acetate extracts of tamarind fruit pulp at different concentrations (100ppm, 50ppm, 25ppm, 12.5ppm) were used to scavenge DPPH free radicals. The standards: ascorbic acid and gallic acid were found to possess high antioxidant activity than the extracts in all the various concentrations for the DPPH assay and hydrogen peroxide assay respectively. 2, 2- diphenyl -1- picrylhydrazyl (DPPH) radical and hydrogen peroxide scavenging assays were used to investigate the tamarind seed' and the fruit pulp. The results from the antioxidant assays show good antioxidant activities for the various extracts which might confirm the use of the plant in folkloric medicine.

Keywords: Proximate composition; Phytocompounds; Broth dilution; Medicinal properties

1. Introduction

Tamarindus indica L., also called tamarind, is a member of the Fabaceae family and belongs to the subfamily Caesalpinioideae. It is botanically identified as *Tamarindus indica* Linn. *Tamarindus indica* is generally referred to as tamarind and locally referred to as "Samia" in Ghana and "Imli" in Hindi. The plant is determined mainly in Africa and Asia but is said to be indigenous to tropical West Africa where the primary belt runs from, Sierra Leone, Liberia, Ivory Coast and Ghana, at some point to the equatorial regions of Cameroun, the Republic of Congo and Kenya [1]. The tamarind tree produces bean-like pods, packed with seeds and surrounded by a fibrous pulp as fruit. The fruit pulp possesses a sweet acidic taste. This is attributed to the presence of excessive quantities of reducing sugars and tartaric acid. The importance of *Tamarindus indica* L. is highlighted using the fact that nearly every part of the plant has some use in the pharmaceutical, meals, chemical and textile industries. The fruit pulp is used to flavour confections, curries and sauces, as a seasoning in prepared ingredients, and as a significant component in juices and other liquids [2]. In conventional medication, the seeds of tamarind are used in the remedy of chronic diarrhoea and dysentery. Investigations into the materials of the plant have revealed the presence of phytochemicals which include alkaloids, sesquiterpenes, saponins, tannins, flavonoids and phlobatamins. Phenolic compounds consisting of catechin, epicatechin and procyanidin B had been proven to be gifts in the plant. These phytochemicals positively influence human health and indicate high antioxidant activity. Hence, it is considered crucial to increase the antioxidant intake in the human diet and one way of achieving this is by enriching food products with seeds rich in phytochemicals.

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Tamarind seeds are traditionally used to treat diabetes, fevers and intestinal infections. They are also used in the treatment of diarrhoea and as a laxative. Tamarind is suited for dry tropical conditions and grows well in several wet tropical areas with seasonally high rain. *T. indica* is rich in nutrients and plays an important role in human nutrition, mainly in developing countries. It contains a high protein level with many essential amino acids, which help build strong and efficient muscles. Tamarind pulp has been reported to be used in the treatment of several ailments, including the alleviation of sunstroke and the intoxicating effects of alcohol and cannabis [3]. Also, a large amount of seed waste is discarded from the tamarind industry, so the utilization of tamarind seed is interesting from the perspective of the possible utilization of this waste. In the present study, the nutritional, antioxidant and anti-microbial properties of tamarind seed powder and fruit pulp were analyzed.

2. Materials and methods

2.1. Materials

Ethanol, hydrogen peroxide, Ethyl acetate, aqueous (water), nutrient agar, sodium carbonate, 1,10-Phenanthroline, Ferrous ammonium sulphate, methanol, Folin-Ciocalteu reagent, gallic acid, aluminium chloride, quercetin, tannic acid, UV/VIS spectrophotometer, analytical balance, micropipette, measuring cylinder, oven, titre plates, centrifuge tubes, rotary evaporator, vacuum pump, Oven (Thermo Scientific, model PR395220G, USA).

2.2. Methods

- SEED COLLECTION**:** A single lot of tamarind seeds were purchased from the Ayigya market in Kumasi. They were identified and verified by Mr. Asare, a botanist from Kwame Nkrumah University of Science and Technology's Department of Herbal Medicine and Faculty of Pharmacy and Pharmaceutical Sciences.
- SEED PROCESSING: The fruit pulp was cleaned off the seeds. To get rid of rotten seeds, debris, stones, and dirt, all of the seeds were sorted, and the good seeds were kept. By soaking the seeds overnight and manually removing the seed coat from each seed, the seed coat of the roasted tamarind kernel was separated. The pulp was oven-dried for three hours at 40 °C while the seeds were washed and sun-dried for three days. After that, the seeds were ground up, and the resulting tamarind kernel flour was passed through a 60-mesh sieve before being packaged in airtight plastic containers for further nutritional analysis.
- EXTRACTION: Cold maceration was used as the extraction technique. The pulp sample and the pulverized tamarind seed were both subjected to extraction. Ethanol, water, and ethyl acetate were the solvents used for the extractions. As a result, six extracts were made in all.
- PROXIMATE COMPOSITION: For the proximate analyses of crude fibre, moisture, protein, fat, and ash, three replicates were used. The amount of moisture in the kernels was determined by weighing them before and after three hours of drying in an oven at 105 °C. The crude protein was calculated by multiplying the N value by 6.25, and the nitrogen content was estimated using the micro-Kjeldahl methods [4]. For the fat content, the sample was extracted for 16 hours with ether in a Soxhlet apparatus. The ash content was determined using the AOAC-approved method. The number of carbohydrates was determined by dividing the ratio of total carbohydrates to crude protein, crude fibre, crude fat, ash, and moisture equal to one hundred times the amount of total carbohydrates. The weight of the analyzed samples was expressed as a percentage for each proximate result.
- Moisture content (HOT-AIR OVEN METHOD): 5g of the sample was added to a dish that had already been weighed and dried. After five hours at a controlled temperature of 105 °C, the dish was removed from the oven and placed in a desiccator to cool to room temperature before being weighed.

$$\% \text{ Moisture } = \frac{\text{weight of wet sample} - \text{weight of dry sample}}{\text{Weight of wet sample}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots \text{Eq.1}$$

- Ash content; 5g of sample was weighed into a tarred crucible and ignited for 2 hours at 600°C. Crucibles were made to cool and later weighed.

$$\% \text{ ash } = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq.2}$$

- Crude fat: 5g of dried sample was weighed into a paper thimble. 150 ml of petroleum ether was added to the round bottom flask, and the apparatus was assembled and made to reflux. Distillation was used to recover solvent and remove the thimble. To get rid of the solvent, the oil flask was heated to about 103 °C. The contents were cooled to room temperature to determine the amount of oil collected and the flask was weighed [5].

$$\% \text{ Crude Fat} = (W2 - W1) \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq 3}$$

- Crude Protein (Kjeldahl method): 2g of sample and a small amount of selenium-based catalyst were placed in the digestion flask. 25 ml of concentrated sulphuric acid was added and shaken well for the entire sample to be wet. Flask was placed on a digestion burner and heated slowly until boiling ceased and a clear solution was formed. It was made to cool to room temperature, transferred to a 100 ml volumetric flask and topped up to the mark. The apparatus was flushed by boiling distilled water and making it circulate through all the connections of the distillation apparatus for 10 minutes. Two drops of the indicator were added to 25 ml of 2% boric acid and pipetted into a 250 ml conical flask. 10 ml of the digested sample was added to the decomposition flask of the Kjeldahl unit, along with an excess 20 ml of 40% NaOH. The ammonia produced in the collection flask was distilled until a volume of 150 ml was collected [6]. 0.1N HCl solution was used for the titration of the distillate. The solution was made colourless by adding the acid. The quantity of nitrogen was then determined.

$$\% \text{ Total nitrogen } = \frac{100\times(Va-Vb)\times NA\times 0.01401}{W\times 100}\times 100 \ldots\ldots\ldots\ldots\ldots\ldots \text{Eq.4}$$

Nitrogen free extract (NFE) NFE (%) = 100 - (%moisture + %fat + %fibre + %protein + %ash) … Eq.5

- Crude fibre: 2g of defatted sample was weighed into a 750 ml Erlenmeyer flask. A 200ml of 1.25% H_2SO_4 was added and the flask was set on a plate and connected to a condenser. After 30 minutes, the flask was removed and the contents were filtered and rinsed with lots of water. The filtrate was washed back into the flask with NaOH. Flask was connected to a condenser and made to boil for another 30 minutes. The solution was filtered and rinsed thoroughly with water. The crucible and its contents were dried for 2 hours at 105 °C cooled and weighed.

$$\% \text{ Crude fibre } = \frac{\text{Weight of crude fibre}}{\text{Weight of sample}}\times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq.6}$$

- Total carbohydrate: The amount of total carbohydrate was calculated from the sum of moisture, crude protein, crude fat, ash and crude fibre and lastly subtracted from 100.

Carbohydrate (percent) = 100 – (% moisture + % ash + % fat + % fiber + % protein) ….. Eq. 7

The following factors were used to determine the total energy value:

The powdered seed materials were screened for the presence of some secondary metabolites (phytochemicals) using equation 7: energy value = (% crude protein x 4) + (% carbohydrate x 4) + (% crude fat x 9)……Eq.8

Test for tannins (Ferric chloric test): 10 ml of boiling, filtered water was used to treat a small number of crude extracts and crude samples in each test tube. The filtrate had a few drops of ferric chloride at 1% added to it. The presence of tannins is confirmed by the appearance of a brownish-green or blue-black colour [7].

Test for saponins (Foam test): A small amount of the crude extracts and crude samples were treated with 20 ml of distilled water. After that, the test tubes were manually shaken for fifteen minutes. The formation of foam on top of the test tube shows the presence of saponins.

Test for flavonoids: Add magnesium ribbon chips to 1 ml of powdered seed and extract to test for flavonoids. Conc. drops of hydrochloric acid were added. The absence of red colouration indicated the absence of flavonoids.

Test for alkaloids: The powdered seed and extract (1g) were heated for five minutes in a water bath with 2 ml of diluted sulfuric acid. 3 ml of the filtrate was taken from the mixture after it was filtered. Draggendorff's reagent was added in a few drops. The formation of an orange-red precipitate indicated the presence of alkaloids.

Triterpenoids test (Liebermann-Burchard test). The Liebermann-Burchard test was used to screen for triterpenoids on powdered seed and extract, with slight modifications made by adding 15 ml of chloroform to 1 gram of the samples. After being heated for five minutes in a water bath, the mixture was filtered and cooled. Along the walls of the chloroform extract-filled test tubes, concentrated sulphuric acid was added. Triterpenoids can be identified by the formation of a brown ring.

Test for phenol; Ferric chloride test. In each test tube, a small amount of the crude extracts and the crude sample were treated with three to four drops of ferric chloride solution. Phenols are indicated by the formation of a bluish colour.

2.3. Antioxidant Assays

DPPH free radical scavenging activity: Preparation of solutions and reagents 20 mg/L DPPH solution was prepared by weighing 0.002 g of DPPH and dissolving it in 100 ml of methanol. This confirmed the presence of steroids in the plant extract. It was kept in an amber glass bottle until it was ready to be used to prevent photodecomposition.

Methanol was used to prepare various concentrations of the ascorbic acid extract and fractions, ranging from 100 to 12.5 g/ml, which served as the standard.

The standard drug of reference is ascorbic acid.

The free radical scavenging activity was measured by mixing 50 ml of the various concentrations of the prepared extract and the standard with 150 ml of 0.02 mg/ml DPPH solution. The reaction mixture was kept in the dark for 30 minutes at 25°C. For ascorbic acid and fractions of varying concentrations, the procedure was repeated. Using a UV-visible spectrophotometer, the residual DPPH's absorbance at 517 nm was measured [8]. The following equation was used to calculate the DPPH radical scavenging activity:

$$(\text{Abs of control} - \text{Abs of sample})/(\text{Abs of control}) \times 100 \quad \ldots\ldots\ldots \text{Eq. 9}$$

Hydrogen peroxide radical scavenging: Weighing 0.02841g of ferrous ammonium sulphate and dissolving it in 100 ml of distilled water produced 1 mM of ferrous ammonium sulphate. By weighing 0.04955g of 1,10-phenanthroline, a small volume of ethanol was used to dissolve it, and distilled water was added until the 250 ml volumetric flask mark was reached. After pipetting 5 µL of hydrogen peroxide into a 100 ml container, distilled water was added to the top to make 5 mM hydrogen peroxide. In addition to preparing various concentrations of the ascorbic acid that would serve as a standard, various concentrations of the seed extract and fractions were also prepared. 3 ml of extract prepared in 5% DMSO and 0.13 ml of 5 mM H_2O_2 were added to 0.5 ml of ferrous ammonium sulphate (1 mM) solution, and the samples were incubated for 5 minutes at room temperature away from light. After that, each tube received 3 ml of 1,10-phenanthroline (1 mM), was thoroughly mixed, and was once more incubated for 10 minutes at room temperature [9]. Using a UV-Vis spectrophotometer, the absorbance of each reaction mixture was measured at 510 nm.

$$\%\ \text{Hydrogen peroxide scavenged} = A_{\text{test}} / A_{\text{control}} \times 100 \quad \ldots\ldots\ldots \text{Eq. 10}$$

2.3.1. Determination of Total Phenol Content (Folin-Ciocalteau Method)

The total phenol content of the extract was measured spectrophotometrically using the Folin-Ciocalteau method. 10% Folin-Ciocalteau reagent was prepared by diluting the Folin-Ciocalteau ten times with distilled water.

By weighing 3.75g of sodium carbonate and dissolving it in 50 ml of distilled water, an aqueous sodium carbonate solution containing 75 mg/ml was made.

To serve as a standard, various concentrations of gallic acid (100-12.5 g/ml) and the seed extract and fractions (100-12.5 g/ml) were prepared.

The Folin-Ciocalteau reagent was added to each of the various extract solutions (0.5 ml) that were measured in test tubes. Each was given 2 ml of the 75 mg/ml aqueous sodium carbonate solution. After 10 minutes in the oven at 50 °C, a UV-visible spectrophotometer was used to measure the absorbances at 760 nm. Using the same method, the solutions and fractions of gallic acid were used to draw a calibration curve. With some modifications, the phenolic content was expressed in gallic acid equivalents (mg GAE/g extract) [10].

2.3.2. Determination of total flavonoid content

The aluminium chloride colourimetric method was used to determine the total flavonoid content. The standard calibration curve was created with quercetin. The stock quercetin solution was made by dissolving 5 mg of quercetin in 10 ml of methanol. The standard quercetin solutions were made by dilutions in series with methanol ranging from 5 to 200 µg/ml Separately, 0.6 ml of 2% aluminium chloride and diluted standard quercetin solutions and extracts were combined [11].

After being mixed, the solution was left to stand at room temperature for 60 minutes. At 420 nm, the absorbance of the reaction mixtures was compared to a blank. The calibration plot was used to calculate the test samples' total flavonoid

content, which was expressed as mg quercetin equivalent (QE)/g of dried seed material. Triplicate testing was used for every determination.

2.3.3. Determination of Total tannins:

Total tannins were determined by preparing an acetone-based stock solution containing 1000 g/ml of the extract. The standard tannic acid solution consisted of 5ppm, 10ppm, 20ppm, 40ppm, and 80ppm of 70 per cent acetone. From 0.5 ml of the test solution, 0.5 ml of water, 0.5 ml of Folin-Ciocalteu, and 2.5 ml of 20% NaCl, a reaction mixture with a total volume of 4 ml was made. After that, the test tubes were covered with parafilm and shaken for 40 minutes with a vortex. The absorbances were measured at 725 nm after it was refrigerated [12].

2.4. Anti-microbial assay

The antimicrobial activity of the seed extract was tested on six (6) different microbes. These microbes were chosen because they affected the majority of infections. The Pharmaceutical Microbiology Section of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Science, KNUST, Kumasi, provided the microbial strains. Two gram-positive bacteria (S. aureus and S. pneumoniae), two gram-negative bacteria (E. coli and P. aeruginosa), and two fungi (C. Albicans and two T. corporis) are among these microbes. The fungus was cultured overnight at 37 °C in Sabouraud dextrose agar and the bacterial strains were cultured overnight at 37 °C in nutrient broth.

2.5. Broth micro-dilution

One method for determining microbial susceptibility is broth micro-dilution. The appropriate MIC values can be determined using this method. The antimicrobial agent's MIC is the lowest concentration that, when observed by an unaided eye, completely inhibits the growth of the organism in tubes or microdilution. Glass Petri dishes were sterilized in a hot air oven at 200 °C for two hours before being allowed to sufficiently cool in their respective canisters.

Agar preparation: A 28g powdered nutrient agar sample was suspended in one litre of distilled water. To completely dissolve the solution, it was brought to a boil. After that, the solution was sterilized by autoclaving it for 45 minutes at 121 °C.

Preparation of nutrient broth: A beaker was filled with 13 grams of nutrient broth powder. It was mixed with about 500ml of distilled water to dissolve. 1000 ml of freshly prepared distilled water were added. Test tubes were stuffed with cotton wool and filled with 10ml of the mixture. After that, they were heated for 15 minutes at 121°C in an autoclave to sterilize them.

PREPARING STOCK SOLUTION: The 12th was filled with 100 L of the growth control and 100 L of the ciprofloxacin. (NB: this served as the benchmark). At 37 °C, the titer plates were left to incubate for 24 hours. MTT staining was used to estimate MIC values. By adding 20 L of a solution of tetrazolium salt (3- (4, 5 dimethylimidazole-2yl-2, 5 diphenyl tetrazolium bromide) (MTT) and incubating for an additional 30 minutes, the microorganisms' growth was measured. As the dehydrogenase enzymes in the live bacteria react to form a dark complex with the tetrazolium salt, dark purple wells indicate the presence of microorganisms. DMSO served as a negative control, and ciprofloxacin served as a positive control. Each experiment was carried out twice.

3. Results and discussion

The purpose of the study was to evaluate the tamarind pulp, seed, and its ethanolic, aqueous, and ethyl acetate extract's nutritional, antioxidant, and antimicrobial properties.

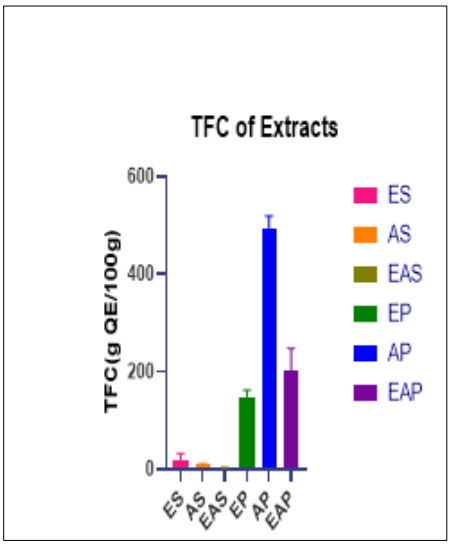

Figure 1 Total flavonoid content of extracts

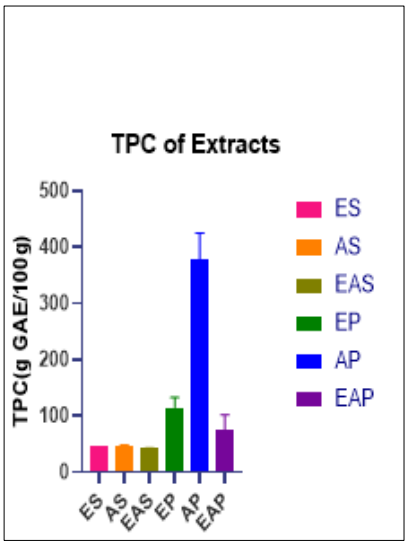

Figure 2 Total Phenolic content

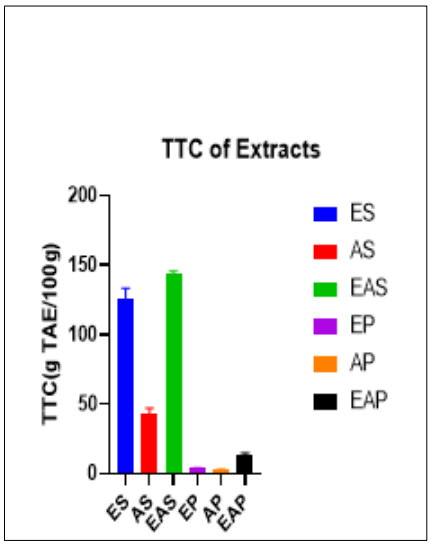

Figure 3 Total tannins content of extracts

KEY: Ethanol seed (ES), Aqueous seed (AS), Ethyl acetate seed (EAS), Ethanol pulp (EP), Aqueous pulp (AP) and Ethyl acetate pulp (EAS) extracts

3.1. Results of antioxidant assays

In the DPPH free radical scavenging assay and hydrogen peroxide scavenging assay; the extract and standards (Ascorbic acid and Gallic acid respectively) showed a concentration-dependent radical scavenging effect.

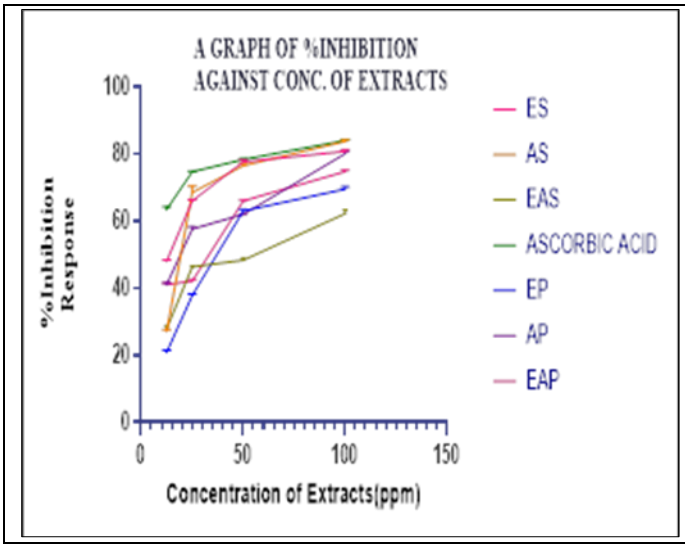

Figure 4 DPPH free radical scavenging assay

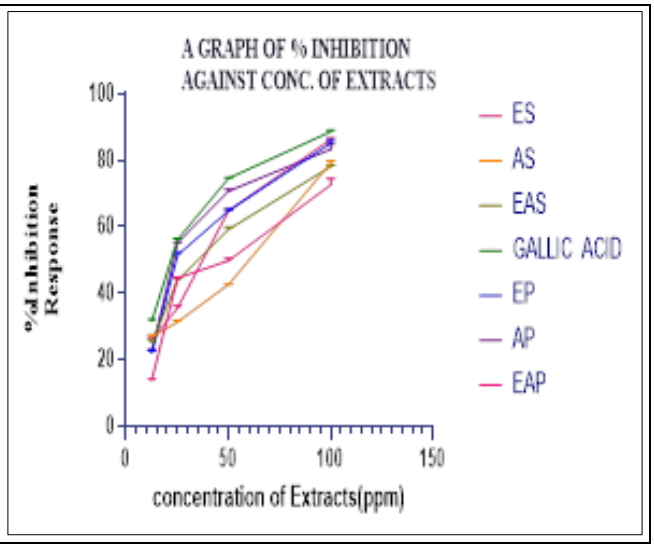

Figure 5 Hydrogen peroxide scavenging assay

3.2. Broth microdilution assay

The antimicrobial activity of the ethanolic, aqueous and ethyl acetate extract was tested against six (6) microorganisms of which, two (2) were Gram-positive, two (2) Gram-negative, and two (2) fungi, by the use of broth microdilution method. The MIC was taken as the least concentration that inhibited any visible growth of organisms after a 24-hour incubation period.

3.2.1. Minimum Inhibition Concentration (MIC)

Ethanol pulp, Ethyl acetate pulp, Aqueous pulp and Ethanol seed were prepared in concentrations of 50 mg/ml since they gave zones of inhibition less than 12.5 mg/ml.

Ethyl acetate seed and Aqueous seed extract concentrations were prepared at 100 mg/ml since no zones of inhibition were noticed for the agar well.

250 ml nutrient broth was prepared.

ORGANISM KEY: Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Candida albicans, Tinea corporis.

4. Conclusion

Tamarindus Indica L. has a reasonable amount of crude fat, crude fibre, crude protein, ash, and carbohydrates, according to the discussion of the current results. The outcomes demonstrate the need for additional research into fruit pulp and seed. The presence of saponins, general glycosides, flavonoids, terpenoids, steroids, alkaloids, tannins, phenols, anthraquinones, glycosides, and phenols may account for the medicinal properties of the seed and fruit extract. Because it provides essential nutrients like iron, zinc, magnesium, calcium, potassium, phosphorus, sodium, and potassium, *Tamarindus indica* Linn seed is considered a nutritional supplement for many families. This work has also demonstrated that *Tamarindus indica* Linn seed has good antioxidant activity, which can be attributed to some of the phytochemicals that were detected. It has also demonstrated intriguing antimicrobial properties. Future projects should take into account toxicology studies. Because of all of these properties, tamarind seed can be added as a food supplement to baked goods and foods that contain moisture, like corn dough, to enhance the nutrients in those foods and protect them from microbes.

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