PHYTOCHEMICAL DETECTION AND ANTI-BACTERIAL ACTIVITY OF *Tamarindus indica* EXTRACT AGAINST ISOLATED BACTERIA FROM BANANA PEEL

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Abstract- Tamarindus indica is a tree usually recognized as Tamarind, which belongs to *Leguminosae* family. Tamarind pulp comprise a diversity of biologically active phytochemical components like phenolic compounds, vitamin C, flavonoids, anthocyanin and carotenoids. These components show antibacterial, anti-inflammatory, anti-diabetic, antioxidant and also anti-cancer activities. The aim of this study was to find out the phytochemical components exist in ethanolic, distilled water and n-hexane extract of the fruit pulp of *Tamarindus indica*. And then check the antibacterial activity of Tamarind fruit pulp extract against *Escherichia coli* and *Staphylococcus aureus*, which were isolated from Banana peel. By phytochemical analysis, the occurrence of flavonoids, tannins, terpenoids, saponins and reducing sugar were determined. High Performance Liquid Chromatography (HPLC) was also conducted for the analysis or separation of phytochemical constituents in tamarind fruit pulp extract. Bacteria from Banana peel was isolated through serial dilution method and then bacteria was morphologically identified. Antibacterial activity was determined by agar well diffusion method. The antibacterial activity was determined against the strains of two bacteria (*Escherichia coli* and *Staphylococcus aureus*). The result of the study is that fruit pulp of *Tamarindus indica* exhibited a significant antibacterial activity against bacteria.

Key words-Tamarindus indica, phytochemical analysis, exhibited, HPLC, anti-bacterial activity.

1. INTRODUCTION

Plants basically have a key part in the existence of humans and they are the base of traditional herbal medication. Plant treatment has a better treatment of disease than the synthetic drug treatment. Plant medication is basically safe, more helpful and have lesser side effects. These types of plants produce numerous types of secondary bioactive metabolites which give main goal that why they are more effective therapeutic purpose even since pre historical time (Plants not only provides decorative, flavors, cosmetics, clothing, fumigants, fragrance and insect prevention but also helped the humankind in the treatment of illness. The herbal plants are used for medicinal purpose (Abukakar et al., 2008). *Tamarindus indica*, usually recognized as Tamarind, having *Leguminosae* family, this family is the third largest flowering plants family. Tamarind seeds and pericarp comprise antioxidant components like phenolic compounds. The ethanolic fruit pulp extract exhibited important hypolipidemic and antioxidant activity of hypercholesterolemic in hamsters (Zohrameena et al., 2017). Due to the occurrence of highly amount of tartaric acid and reducing sugar, the pulp of Tamarind fruit has acidic and sweet taste. The pulp of tamarind plant is also used as a foodstuff constituent to taste confectionaries, sauces or curries and also contribute in certain beverages and juice flavors. The pulp of Tamarind is freshly eaten and also used in sweets and jam (Ayat et al., 2019).

Tamarind pulp extract showed antibacterial activity against those bacteria which harm the fruits. Fruits play a chief role in diet of human beings by providing vitamins and minerals. However, fruits production become insufficient during the handling procedure of the crop from its grower stage to finally consumers (Hasan & Zulkahar, 2018).

Tamarind arrangement are most commonly documented for the treatment of fever and as purgatives and carminatives. By mixing the fruit pulp with lime juice, milk, honey, spices and dates then this pulp is considered for the treatment of many digestive disorders and as an anti-scorbutic (Dorcas et al., 2020).

Tamarind pulp comprise a diversity of naturally active phytochemical compounds, such as phenolic components, vitamin C, flavonoids, anthocyanin and carotenoids. These phytochemicals completely impact the health of human beings and specify high antioxidant action. Henceforth, it is measured vital to rise the antioxidant consumption in the diet of human beings and one way of attaining this is can be over enriching foodstuffs, containing high amount of phytochemicals. Tamarind is favored as a beneficial maintenance for the patients of malnourished (Natukunda et al., 2016).

Phytochemicals are chemicals present in plant that have defensive or having properties of disease prevention. They useful in preserving optimum body immune response, as in lacking or unnecessary consumptions can have harmful effect on the health of humans. Nutritional consumption of phytochemicals may encourage benefits of health, defensive against chronic degenerative illnesses cardiovascular, cancer and neurodegenerative syndromes. Defense against many disorders like, these are chemicals which present in plant that have defensive or having properties of disease prevention. Those plants having these bioactive compounds providing human beings various advantages, tamarind plant is also include in this category of plants which provide various advantages to human beings. (Batta, 2016). The study aim is to the identification of phytochemical components of the fruit pulp extracts of Tamarindus indica plant. Then analyze the Tamarindus indica fruit pulp ethanolic extract by using High performance liquid chromatography (HPLC) and evaluate the antibacterial potential against two bacteria which were isolated from banana peel.

2. MATERIALS AND METHODS

2.1 Materials Required

Conical flask, pipettes, beakers, sterile petri plates, measuring cylinders, test tubes, droppers, inoculation loops, weight machine, orbital shaker, rotary evaporator, whatman's filter paper 1, biochemical reagents, distilled water, ethanol, nhexane, zonal scale for measuring zone of inhibition.

2.2 Plant Material

Fresh Tamarind fruit pulp was collected from the market of Faisalabad, Punjab. Collected fruit pulp was firstly taxonomically identified from University of Agriculture Faisalabad, Department of Botany.

2.3 Sample Preparation

For sample preparation, seeds were removed from the collected tamarind fruit pulp. After the removal of seeds, cleaned the fruit pulp from distilled water and then dried this pulp under shade for a week. By the process of maceration, the dried fruit pulp was firstly ground into fine powder for further extract preparation (Dorcas et al., 2020).

2.4 Extract Preparation

Three different types of extracts were prepared with the help of maceration procedure. About 50g of powdered sample was dissolved separately in 500ml of distilled water, ethanol and n-hexane in 1:10 ratio separately. These extracts have to left and shake in orbital shaker for 72 hours. After 72 hours, whatman's filter paper 1 was used to filter these extracts separately and removed the extra solid particles. By rotary evaporator, the remaining filtrate was evaporated and converted into its dry from (Ayub et al., 2017).

2.5 Phytochemical screening

Qualitative analysis was carried out to investigate the presence of natural bioactive components. The presence or absence of following phytochemicals were determined; alkaloid, saponins, terpenoids, tannins, steroids and flavonoids. This was accomplished by performing different types of biochemical test (Bandiola, 2018). Quantitative analysis was carried out to investigate the total flavonoid components and total phenolic components in plant extract. Total flavonoid compounds of *Tamarindus indica* was determined by aluminium chloride colorimetric assay with some modifications. Total phenolic compound (TPC) of *Tamarindus indica* was determined by FC method with some modifications. (Ashraf et al., 2016).

2.6 HPLC analysis

High performance liquid chromatography was carried out to analyze the compound mixture. HPLC analysis of ethanolic extract was carried out with the help of chromatographic system which contains auto-sampler with fixed loop of $100 \,\mu$ l and an Ultraviolet-Visible detector. With the help of UV-Vis detector, the detection was completed at 280 nm (Tripathi et al., 2012).

2.7 Isolation of bacteria from Banana peel

Bacteria was isolated from serial dilution method from spoiled Banana peel. 0.5g of banana peel was crushed with distilled water in pre-sterile mortar and pestle and form suspension. The suspension was consecutively diluted from 101 to 106 dilutions. Over nutrient agar medium (NAM) plate, 100µL of each suspension of diluted fruit was spread. For the growth of bacteria, the inoculated petri plates was incubated for 24 hours at 37°C. Different types of bacterial colonies were appeared on the petri dish. Then the specific colonies were grow on selective media. For E. coli the selective media was EMB (Eosin-Methylene blue) for the identification. For S. aureus the selective media was MSA (Mannitol Salt Agar). After the identification on selective media then morphological analysis was determined on the base of selective bacterial colonies. Bacteria was isolated and then subcultured on NAM slants at 40°C for further antibacterial analysis (Hasan & Zulkahar, 2018).

2.8 Identification of the bacterial isolates

On the basis of morphological and biochemical characterization the bacterial isolates were identified corresponding to Bergey's Manual of Systematic Bacteriology (Abdallah & Muhammad, 2018). For cellular morphology of isolated bacteria gram staining was conducted. Then isolates were further identified on the base of biochemical characterization (IMViC) (Hasan & Zulkahar, 2018).

2.9 IMViC test

IMViC is extensively used biochemical test series for the identification of bacteria. It is useful for the identification and characterization of several types of bacteria. It is used for primary screening determination. It included four different types of test; Indole test, Methyl-red test, Voges-Proskauer reaction test, Citrate Utilization test. All the test in the series were easy to perform and it give consequences within 24-48 hours (Dikhit & Sohani, 2022).

2.10 Anti-bacterial activity

The antibacterial activity was performed by agar well diffusion method (Ayub et al, 2018). Escherichia coli and Staphylococcus aureus were isolated from banana peel extract. Then these bacterial isolates were morphologically identified and biochemically characterized by the laboratory of Microbiology, University of Agriculture Faisalabad. Then E. coli and S. aureus were further used to analyze each samples separately. For agar well diffusion method, firstly prepared the agar nutrient medium. For its preparation 25g of nutrient was added in 100mL of distilled water and then it was maintained at 7 pH and for 15 minutes autoclaved it and then cooled down. 7mL of nutrient agar was added in petri plates for the preparation of petri plates and then solidify it. In the wells 100 µL of test compound and Ciprofloxacin as a positive control was introduced and then firstly placed it at ambient temperature for 24 hours and then at 37°C the plates were incubated overnight. By calculating the zone of inhibition diameter the microbial growth was determined (Shahid et al., 2021).

2.11 Statistical analysis

All the collected data was analyzed by using analysis of variance (ANOVA) and mean and standard deviation. For graphing statistical software SPSS was employed.

3. RESULTS AND DISCUSSIONS

3.1 Phytochemical analysis

Qualitative analysis was used to investigate the various classes of natural bioactive components (alkaloids, saponin, tannin, terpenoid, steroid, flavonoids) which also recognized as secondary metabolites, present in the extract.

Table 1:	<i>Phytochemical</i>	Screening	of	Tamarindus
indica frui	it pulp			

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Phytochemical	Distilled	Ethanolic	n-
groups	water	extract	hexane
	extract		extract
Alkaloids	+	+	+
Saponins	+	+	_
Tannins	+	+	_
Steroids	+	-	+
Flavonoids	+	+	_
Terpenoids	-	+	+

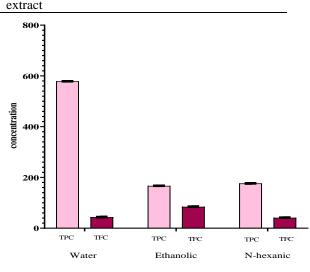
Oualitative phytochemical screening revealed that alkaloids were exist in distilled water. nhexane and ethanolic tamarind fruit pulp extract. In distilled water extract and ethanolic extract alkaloids were existed in higher amount. But on the other hand in n-hexane extract it were existed in lower amount than other two extracts. Alkaloids are plant derived component. It has been reported that it is responsible for antibacterial activity. Alkaloid is a powerful pain reliever component and have an antipyretic action (Rana et al., 2018). Saponins were present in ethanolic and distilled water extract of tamarind fruit pulp but it were not n-hexane extract. Tannins were present in distilled water extract and ethanolic extract but it were not detected in n-hexane extract of tamarind fruit pulp. Another secondary metabolite, steroids were not detected in ethanolic extract of tamarind fruit pulp but it were detected in other two n-hexane and distilled water extract. Flavonoids were detected in distilled water and ethanolic extract but it were not detected in n-hexane extract. Terpenoids were not detected in distilled water extract of fruit pulp but it were detected in both n-hexane and ethanolic extract. A distilled water extract of tamarind pulp was found to have a larger amount of bioactive secondary metabolites than any of the other extracts. All these bioactive components are reported as antibacterial effect (Farooq et al., 2022). As reported in Nwodo et al (2011), alkaloids were detected in ethanolic extracts along with distilled water extract of the fruit pulp of tamarind. Steroids were not detected in any extract and terpenoids were found only in the ethanolic extract of the tamarind fruit pulp. Phytochemical analysis of the Tamarind fruit pulp demonstrated that the occurrence of alkaloid, saponin, reducing sugar, terpenoids and flavonoid. The occurence of these phytochemicals in tamarind parts was answerable for its antibacterial activity reported by Abdallah et al (2018).

3.2 Quantitative analysis

Quantitative phytochemical analysis were used for the detection of the amount of bioactive constituents in different extracts of plant. The quantitative phytochemical screening for the estimation of total flavonoid and phenolic compounds in extract was determined by standard laboratory methods with some modifications. Total phenolic component concentration was determined by FC method and the total flavonoid components concentration was determined by aluminium chloride colorimetric assay (Rana et al., 2018).

Table	2:	Quantitative	phytochemical	screening	of
Tamar	ind	pulp extracts			

	Gallic acid Equivalent	Catechin equivalent	
Sample	Final TPC (mg GAE/mL)	Final TFC (ug CE/mL)	
	(Mean±S.D)	(Mean±S.D)	P value
Tamarind D. water extract	578.71±0.556	43.78±0.961	0.000
Tamarind ethanolic	167.1±0.850	84.69±0.995	0.000
extract Tamarind n-hexane	176.7±0.821	41.52±0.96	0.000



Extracts

Figure 1: *Graphical representation of the TPC and TFC concentrations in Tamarind pulp extracts*

Graphical representation of the TPC and TFC concentrations in Tamarind pulp extracts illustrated that the concentration of TPC was higher in distilled water extract and lower in ethanolic extract of fruit pulp. On the other hand the concentration of TFC was

higher in ethanolic extract and lower in n-hexane extract of fruit pulp.

Phytochemical constituents in tamarind pulp of distilled water extract included 578.71±0.45 phenolic components and 43.78±0.78 flavonoid components. In the ethanolic extract of tamarind pulp, 167.1±0.69 phenolic components and 84.69±0.81 flavonoid components were present. And in the n-hexane extract of tamarind fruit pulp, 176.7±0.72 phenolic components and 41.52±0.79 flavonoid components were present. The ethanolic extract of tamarind fruit pulp contained flavonoids, 19.06 - 25.66% and phenol, 9.78 - 25.41% has been reported in Oluwole-Banjo (2019). One-way ANOVA test was applied to check the significant difference between the mean values of TPC and TFC. The result was compared with p values. The values of p were 0.000 of all the samples which was lower than 0.05, its means that the result was significant.

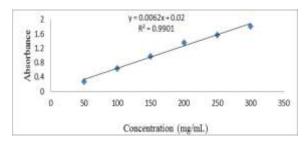


Figure 2: Gallic acid standard curve for determination of total phenolic components in Tamarind pulp extracts

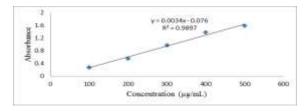


Figure 3: Catechin standard curve for determination of total flavonoids components in Tamarind pulp extracts

3.3 HPLC analysis of Tamarind fruit pulp ethanolic extract

HPLC analysis is a significant tool for the analysis of phytochemicals in herbal plants. This technique is useful for numerous secondary metabolites identification found in the herbal plants which further used for the medication of numerous diseases. HPLC technique consist of mobile phase and stationary phase. Pigments were separated based on the interaction with the stationary phase. HPLC analysis was used for ethanolic extract of tamarind pulp analysis. The instrument column was Shim-Pack. The gradient A of mobile phase was H_2O and CH_3COOH in 94:6 and the pH was 2.27 and the gradient B was Acetonitrite (100%). The flow rate was 1ml/min. The HPLC was done at the optimum temperature. The analysis was detected on UV-Vis Detector, the detection was done at 280 nm. The peak width was detected 0.200 per minute. The threshold 0.050 mV was detected. Figure 4 shows the graphical demonstration of HPLC result of tamarind pulp ethanolic extract.

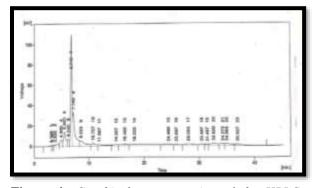


Figure 4: Graphical representation of the HPLC results of Tamarind pulp ethanolic extract

Graphical representation of ethanolic extract exhibited that many bioactive components were detected in the extract in different concentrations and retention time. The retention time for the detection of Quercetin was 3.107. The retention time for Gallic acid, Benzoic acid, Syringic acid, Sinapic acid was 4.900, 14.907, 16.480, 25.887 respectively.

Table 3: Table result of HPLC- Calculation method

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Compoun	Reten	Area	Are	Amoun
d	Time	mV.S	a (94)	t in Ppm
name	3.107	20.685	<u>(%)</u> 0.3	1
Quercetin	5.107	20.085	0.5	1.09pp m
Gallic acid	4.900	177.67	2.9	6.39pp
		7		m
Benzoic	14.90	80.050	1.3	8.48pp
acid	7			m
Syringic	16.48	150.78	2.5	3.76pp
acid	0	4		m
Sinapic	25.88	91.714	1.5	1.19pp
acid	7			m

As shown in Table 3, major five types of compounds were distinguished in the ethanolic pulp extract. Quercetin is a pigmented compound which belongs to the flavonoid family was detected in the extract with the concentration of 1.09ppm. Another compound Gallic acid which is classified as a phenolic acid was detected with the concentration of 6.39ppm. Benzoic acid was identified with the concentration of 8.48ppm. Syringic acid is a phenolic component was detected with the concentration of 3.76 ppm. Sinapic acid is a natural polyphenol component was detected Sinapic acid of 1.19 ppm. Comparison between these compounds showed that in the ethanolic pulp extract of tamarind highest concentrated component was Benzoic acid detected with 8.48ppm concentration. The lowest concentrated compound was Quercetin with 1.09 ppm concentration was detected.

3.4 Isolation of bacteria from Banana peel

Fruits are natural source of minerals and vitamins for human beings. Because of the high amount of water fruits are spoiled from microorganisms. Bacteria were isolated from serial dilution method for the antibacterial activity. Two types of bacteria; *E. coli* and *S. aureus* were isolated from banana peel. These two bacteria were firstly identified on selective media, *E. coli* on EMB strain and *S. aureus* on MSA strain. Then further identification was performed on the base of simple Gram staining technique. Bacterial isolates were further identified by biochemical characterization. Morphological characteristics of isolated bacteria is explained in table 4.

Table 4: Morphological characterization of isolated bacteria

Bacteria	Escherichia coli	Staphylococcus aureus
Morphological characteristics on nutrient agar	Colorless, white, yellow circular, smooth colonies on	Circular, opaque, smooth colonies with golden yellow pigment on nutrient agar
Gram staining	nutrient agar with entire edge Gram negative bacteria which turned into pink color	Gram positive bacteria turned into purple color

As shown in table 4, *E. coli* was produced a colorless, circular, smooth colonies and yellowish white in color on nutrient agar. While on the other hand *S. aureus* was produced smooth, opaque and golden yellow colonies on nutrient agar.

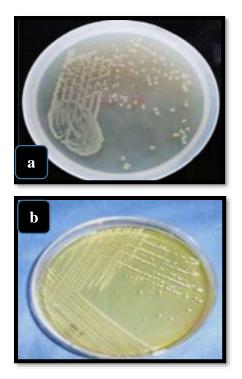


Figure 5: (a) Development of Escherichia coli on nutrient agar plate (b) Development of Staphylococcus aureus on nutrient agar plate

3.5 Biochemical characterization

Biochemical characterization of bacterial isolates were performed through IMViC test. Table 5 shows the biochemical characterization of isolates through IMViC test.

 Table 5: Biochemical characterization of isolates

 through IMViC test

agn im vie iesi	
est	Results
ndole test	Red layer at the top of tube
lethyl-Red test	Red color produce in tube
oges-Proskauer eaction test fitrate	Red color produce
tilization test	Blue color produce
	est ndole test fethyl-Red test 'oges-Proskauer eaction test itrate

According to table 5, IMViC tests were used for the biochemical characterization of isolates. IMViC tests involved four types of tests. For bacterial characterization, in Indole test red layer was produced at the top of the test tube which detected the occurrence of bacteria. In Methyl-Red test, red color was produced in a test tube which detected the existence of isolate. In Voges-Proskauer reaction test, also red color was produced which detected the occurrence of isolate. In Citrate Utilization test, blue color was produced which showed positive result for the biochemical characterization of bacteria. Table 6

shows the IMViC test results for both *Escherichia coli* and *Staphylococcus aureus*.

Bacteria	Escherichia coli	Staphylococcus aureus		
Indole test	+	_		
Methyl-Red	+	+		
test				
Voges-				
Proskauer	_	+		
reaction test				
Citrate				
Utilization	_	+		
test				

Table 6: IMViC test results

IMViC test are used for the recognition of the member of the family of *Enterobacteriaceae*. For the identification of *Escherichia coli* and *Staphylococcus aureus* in a Banana peel sample IMViC tests were used. By using Indole test, *E. coli* was detected but on the other hand *S. aureus* was not detected. Methyl-red test exhibited +ive result for both *E. coli* and *S. aureus*. In Voges-Proskauer reaction test, *E. coli* was not detected but *S. aureus* was detected in this reaction test. Citrate Utilization test showed negative result for *E. coli* but it exhibited positive result for *S. aureus*.

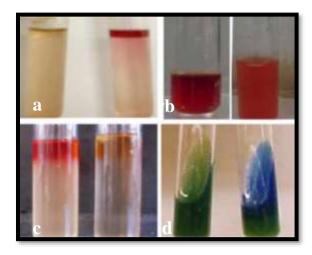


Figure 6: *IMViC test results (a) Indole test (b) Methyl-red test (c) Voges-Proskauer reaction test (d) Citrate Utilization test*

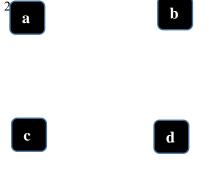
According to figure 6, (a) represents the colored result of Indole test which was used for the detection of bacterial isolates. *E. coli* was detected by indole test by producing a layer of red in color at the topmost of the tube but on the other hand *S. aureus* was not detected and showed no colored ring, (b) represents the colored result of Methyl-red test. Methyl-red test exhibited positive result for both *E*.

coli and *S. aureus* by producing red color, (c) represents the colored result of Voges-Proskauer reaction test. *E. coli* was not detected but *S. aureus* was detected in this reaction test by producing a layer of red in color at the topmost of the tube, (d) represents the colored result of Citrate Utilization test. Citrate Utilization test showed negative result for *E. coli* but it exhibited positive result for *S. aureus* by producing blue color.

As reported in IMViC test are used for the identification of the members of the family of *Enterobacteriaceae*. Isolated *E. coli* exhibited positive result and *S. aureus* exhibited negative result for indole test. Methyl-Red test was used to confirm an organism's capacity to produce strong acid from glucose and to conserve a low pH. Bacteria produce red color exhibited positive result for both *E. coli* and *S. aureus*. For Voges-Proskauer test, *E. coli* exhibited negative result. For citrate Utilization test, *E. coli* exhibited negative result and *S. aureus* exhibited positive result.

3.6 Antibacterial analysis

The antibacterial activity was performed by agar well diffusion method (Ayub et al, 2018). *Escherichia coli* and *Staphylococcus aureus* were isolated from banana peel extract with the help of serial dilution method. Then these bacterial isolates morphologically identified and biochemically characterized. Then *E. coli* and *S. aureus* were further used to analyze each samples <u>separately</u> (Shahid et al.,



Treatment	Escherichia coli	Staphylococcus
	(Zone in mm)	aureus
		(Zone in mm)
Tamarind pulp	13mm	15mm
ethanolic extract		
Positive control	29mm	30mm
Tamarind pulp	5mm	4mm
n-hexane extract		
Positive control	30mm	29mm
Tamarind pulp	10mm	12mm
distilled water		
extract		
Positive control	31mm	30mm

Table 7: Zone of inhibition of tamarind fruit pulp

 extracts against E, coli and S. aureus

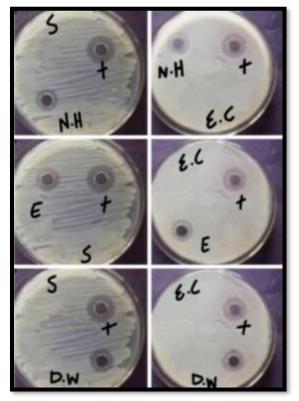
As shown in table 7 three different tamarind pulp extracts exhibited antibacterial activity contrary to Escherichia coli and staphylococcus aureus. Three different extracts included ethanolic extract, n-hexane extract and distilled water extract showed positive response to the Escherichia coli and Staphylococcus aureus. Positive control of ethanolic, n-hexane and distilled water extracts of Tamarindus indica for Escherichia coli was 29mm, 30mm, 31mm respectively. Positive control of ethanolic, n-hexane and distilled water extracts of Tamarindus indica for Staphylococcus aureus was 30mm, 29mm, 30mm respectively. Comparison between the inhibition zones of plant extracts and Ciprofloxacin which was used as a positive control showed that n-hexane tamarind plant extract showed minimum amount of antibacterial activity. Ethanolic extract of tamarind pulp exhibited maximum amount of antibacterial activity but lower than the Ciprofloxacin

.It has been reported that the antibacterial activity varies with the concentration of the extracts. When the amount of the extract increases the antibacterial activity also increases and when concentration of extract decreases the antibacterial activity also decreases. Several authors reveal that fruit pulp of tamarind plant effective against *S. aureus* (125mg/ml) and *E. coli* (100mg/ml). Methanolic extracts having higher antibacterial activity than the n-hexane extracts (Adeola et al., 2010).

As reported in Abukakar et al (2008), antimicrobial potential of *Tamarindus indica* showed antibacterial activity higher in *Staphylococcus aureus* than the *pseudomonas aeruginosa* and *Escherichia coli*. In Nigeria reported by Abdallah & Muhammad (2018), when the bioactive compounds in methanol extract compared with the aqueous extract it was demonstrated that methanol extract display higher antibacterial activity than the aqueous extract. The *E. coli* with zone of inhibition of 14.62mm is higher

complex than the *Shigella* sp. with inhibition zone of 11.47mm.

Figure 7: Anti-bacterial activity of three different	Figure	7:	Anti-bacterial	activity	of	three	different
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extracts of Tamarind pulp against Escherichia coli and Staphylococcus aureus

CONCLUSION

According to the qualitative phytochemical analysis, alkaloids, Saponins, flavonoids, Steroids and Terpenoids were detected in three different extracts; n-hexane, distilled water and ethanolic extract. It has been concluded that in tamarind fruit pulp distilled water extract and ethanolic extract have higher concentration of bioactive components. According to the quantitative phytochemical analysis distilled water extract having higher concentration of flavonoids components. It has been concluded that the bioactive components. It has been concluded that the bioactive components in ethanolic extract of tamarind fruit pulp having strong antibacterial potential against *E. coli* and *S. aureus*.

Conflict of Interest: The Authors have no conflict of interest.

REFERENCES

Abukakar, M. G., Ukwuani, A. N., & Shehu, R. A. (2008). Phytochemical screening and antibacterial

activity of *Tamarindus indica* pulp extract. *Asian Journal of Biochemistry*, 3(2), 134-138.

Adeola, A. A., Adeola, O. O., & Dosumu, O. O. (2010). Comparative analyses of phytochemicals and antimicrobial properties of extracts of wild *Tamarindus indica* pulps. *African Journal of Microbiology Research*, 4(24), 2769-2779.

Ashraf, A., Sarfraz, R. A., Rashid, M. A., Mahmood, A., Shahid, M., & Noor, N. (2016). Chemical composition, antioxidant, antitumor, anticancer and cytotoxic effects of Psidium guajava leaf extracts. *Journal of Pharmaceutical biology*, *54*(10), 1971-1981.

Ayat, A. A., Shimaa, A. A., Sahar, H. E., Mawa, I. A., Marvit, O. W., Layla, F. Y., & Saad, M. H. A. (2019). The effect of blending of extracts of Sudanese Adansonia digitata and *Tamarindus indica* on their antioxidant, anti-inflammatory and antimicrobial activities. *Journal of Pharmacognosy and Phytotherapy*, *11*(2), 28-34.

Ayub, M. A., Hussain, A. I., Hanif, M. A., Chatha, S. A. S., Kamal, G. M., Shahid, M., & Janneh, O. (2017). Variation in phenolic profile, β -Carotene and flavonoid contents, biological activities of two Tagetes species from Pakistani flora. *International Journal of Chemistry & biodiversity*, 14(6), 1600463-1600481.

Bandiola, T. M. B. (2018). Extraction and qualitative phytochemical screening of medicinal plants: a brief summary. *International Journal of Pharmacy*, 8(1), 137-143.

Batta A. A. (2016). Review on Phytochemicals and their activities. *International Journal of Current Research in Medical Sciences* 2(7), 1-3.

Dikhit, M. S., & Sohani, S. (2022). Microbial investigation through Isolation and identification of water bodies from selected hotels of Indore city. *Mukt Shabd Journal*, 6(8), 2347-3150.

Dorcas, K., Palakodeti, P., Yadav, H., Yerra, V., & Majjiga, V. (2020). Antibacterial Properties of Tamarind. *International Journal of recent Scientific Research*, 11(05), 38618-38621.

Fagbemi, K. O., Aina, D. A., Adeoye-Isijola, M. O. Naidoo, K. K., Coopoosamy, R. M., & Olajuyigbe, O. O. (2022). Bioactive compounds, antibacterial and antioxidant activities of methanol extract of *Tamarindus indica* Linn. *Scientific Reports*, *12*(1), 112.Farooq, S., Munir, R., Imtiaz, K., Sehar, S., Khurshid, A., Yunus, N., & Nian, F. A. N. G. (2022). Phytochemical investigation and antioxidant activities of tamarind (*Tamarindus indica* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *50*(4), 12892-12892.

Hasan, N. A and I. M. Zulkahar. (2018), October. Isolation and identification of bacteria from spoiled fruits. *In AIP Conference Proceedings*, *12*(1), 20-73.

Hasan, N. A., & Zanuddin, N. A. M. (2018, October). Molecular identification of isolated fungi from banana, mango and pineapple spoiled fruits. In *AIP Conference Proceedings*, (1), 020074.

Natukunda S, Muyonga JH, Mukisa IM. (2016). Effect of tamarind (*Tamarindus indica* L.) seed on antioxidant activity, phytocompounds, physicochemical characteristics, and sensory acceptability of enriched cookies and mango juice. *Journal of Food Science Nutrition*, 4(4):494-507.

Nwodo, U. U., Obiiyeke, G. E., Chigor, V. N., & Okoh, A. I. (2011). Assessment of Tamarindus indica extracts for antibacterial activity. *International Journal of Molecular Sciences*, *12*(10), 6385-6396.

Oluwole-Banjo, A. K. (2019). Phytochemical and antioxidant properties of methanolic extracts of pulp, seed, leaf and stem bark of velvet tamarind (Dialium guineense) plant. *Journal of Underutilized Legumes*, *1*(1), 159-168.

Rana, M., Sharma, P., Mahima, R. C., & Sharma, P. (2018). Proximate and phytochemical screening of the seed and pulp of *Tamarindus indica*. *Journal of Medicinal Plants*, 6(2), 111-115.

Shahid, M., Naureen, I., Riaz, M., Anjum, F., Fatima, H., & Rafiq, M. A. (2021). Biofilm inhibition and antibacterial potential of different varieties of garlic (Allium sativum) against sinusitis isolates. *An International Journal of Dose-Response*, 19(4). 1-7.

Tripathi, I. P., Mishra, M. K., Pardhi, Y., Dwivedi, A., Dwivedi, N., Kamal, A., & Gupta, P. (2012). HPLC analysis of methanolic extract of some medicinal plant leaves of Myrtaceae family. *International Pharmacy Science*, 2, 49-53.

Zohrameena, S., M. Mujahid, P. Bagga, M. Khalid, H. Noorul, A. Nesar and P. Saba. (2017). Medicinal uses & pharmacological activity of *Tamarindus indica*. *Journal of Pharmaceutical Sciences*, *35*: 121-133.