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Phytochemical analysis and antimicrobial activity of *Tamarindus indica* extracts against *Fusarium oxysporum* and *Xanthomonas campestris*

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Abstract. *Gitari FM, Githae EW, Kuria EK. 2023. Phytochemical analysis and antimicrobial activity of* Tamarindus indica *extracts against* Fusarium oxysporum *and* Xanthomonas campestris. *Intl J Trop Drylands 7: 73-82.* Plant-pathogenic bacteria and fungi are a major threat to biodiversity and food security worldwide. The pathogens are difficult to control using cultural methods and have sometimes acquired resistance to conventional pesticides. This has necessitated the search for more efficient active compounds against them. One promising source of such compounds is tropical-medicinal plants such as *Tamarindus indica* L. This study first determined the phytochemical composition of *T. indica* extracts from different parts (leaves, bark, roots, and pods). Then it evaluated in-vitro the antimicrobial activity against plant pathogenic bacteria (*Xanthomonas campestris*) and fungi *(Fusarium oxysporum)*. Crude extracts were obtained using different solvents (dichloromethane, methanol, and acetone). The analysis revealed the presence of nine pharmaco-active phytochemicals; methanol extracts had the highest concentrations of these phytochemicals. All extracts demonstrated inhibitory effects against *F. oxysporum*. However, the extracts did not show any antimicrobial effect against *X. campestris*. There was a significant difference ($p<0.05$) in the percentage of inhibition of *F. oxysporum* growth by different extracts. Generally, high growth inhibition was observed in media containing different plant extracts at 250 and 500 ppm concentrations. For acetone extracts, the highest inhibition (71.042%) was induced by root extract at a 250 ppm concentration, whereas for dichloromethane extracts, the highest inhibition (68.811%) was induced by 500 ppm of leaf extract. Methanol extracts from stem recorded the highest inhibition of 86.953% at concentrations of 125 ppm. This was followed by root extracts (75.169% inhibition) at 500 ppm. The *T. indica*, therefore, has great potential as a source of bio-pesticide for use in integrated pest management of *F. oxysporum*.

Keywords: Antimicrobial, *Fusarium oxysporum*, phytochemicals, *Tamarindus indica, Xanthomonas campestris*

INTRODUCTION

Crops pathogens are of great economic importance and have warranted widespread and frequent use of pesticides (Mahmood et al. 2016). Bacterial and fungal pathogens negatively impact agriculture (Singh et al. 2015; Murithi et al. 2016). Such pathogens include the bacterium *Xanthomonas campestris* and the fungus *Fusarium oxysporum,* whose spread is helped by climate, soil, and human activities (Gitonga and Githae 2022). The *X. campestris* is a gram-negative bacterium that infects plants, causing diseases such as black rot in cruciferous vegetables and bacterial spot disease in tomatoes and pepper (Destefano et al. 2003; Mkandawire et al. 2004; Thieme et al. 2005; Mbaka et al. 2009). After infecting plants through wounds and openings such as stomata, the bacterium migrates to the xylem, where it accumulates and causes blockage, hence wilting of the host (Kidist 2003; Tripathi et al. 2009). The various disease control methods have failed (Nuñez et al. 2018). For instance, the bacterium has acquired resistance to some chemical pesticides (Shenge et al. 2014).

The *F. oxysporum* is an important group of fungi, with more than 150 strains that infect plants (Fourie et al. 2011). These strains cause *Fusarium* Wilt (FW) and *Fusarium* Crown Root Rot (FCRR) in different plants (Mustaffa and Thangavelu 2011; Bharat and Sharma 2014; Dita et al. 2018; Srinivas et al. 2019; Kalman et al. 2020; Awere et al. 2021). The *F. oxysporum* impairs plant water transport, leading to wilting and ultimate death (Ouyang et al. 2014). This fungus remains in the soil for a long time, even without a host, and only attacks plants when conditions are favorable (Velarde-Félix et al. 2018).

Cultural disease control methods effectively avoid infections (Agrios 2009). These methods include proper sanitation, crop rotation, and early or delayed planting (Bajwa and Kogan 2004). However, they become less effective once the plants have been attacked (Katan 2004). Therefore, it is paramount to integrate these cultural methods with other control strategies (Shenge et al. 2014). Such strategies include Chemical control which weakens or kills the pathogens (Vidaver and Lambrecht 2004; Agrios 2005). Managing *F. oxysporum* and *X. campestris* is mainly through chemical soil fumigation. However, this has adverse effects on the environment (Velásquez et al. 2018). For instance, killing non-target organisms upsets biodiversity (Ramaiah and Garampalli 2015). Additionally, Pesticide toxins remain in the produce after harvest, and their ingestion is suspected to cause human diseases (Dorri et al. 2018; Nuñez et al. 2018). Further, continuous use of these chemicals can cause resistance to pathogens (Pal and Gardener 2006). Therefore, replacing pesticides with environmentally safe methods will increase the quantity and quality of crop produce and reduce pollution (Pal and

Gardener 2006). For instance, plant-based pesticides provide an environmentally friendly option for combating plant pathogens (Yuan et al. 2012; Ramaiah and Garampalli 2015). Further, their modes of action are similar to those of chemical pesticides. Therefore, they can be applied in agriculture, medicine, and industry (Oyelana et al. 2011; Awere et al. 2021).

Tamarind *(Tamarindus indica* L.), a plant of the family Fabaceae, is a multipurpose leguminous tree that is drought-tolerant and thrives well in tropical climates (Rao et al. 2015; Kidaha et al. 2017). It is widely distributed in Kenya's arid and semi-arid areas and is common on farmlands (Muok et al. 2000; Maundu and Tengnas 2005). Tamarind has medicinal phytochemicals with antimicrobial, anti-inflammatory, antifungal, anti-nematode, antidiabetic, molluscicidal, and cytotoxic activities (El-Siddig et al. 2006; Escalona-Arranz et al. 2010; De Caluwé et al. 2010; Bhadoriya et al. 2011; Gumgumjee et al. 2012; Simon 2019). These compounds are contained in the leaves (Escalona-Arranz et al. 2010; Bhadoriya et al. 2011), the pods (Bhadoriya et al. 2011), the bark and fruit pulp (Nwodo et al. 2011). However, little information is documented about their activity against plant pathogens.

Plants have numerous phytochemicals of different solubilities in different solvents (Doughari 2006; Vaghasiya and Chanda 2009; Gumgumjee et al. 2012; Majekodunmi 2015). This study extracted different parts of tamarind using acetone, dichloromethane, and methanol. Their phytochemical composition and antimicrobial effect against *X. campestris* and *F. oxysporum* were evaluated, and promising prospects for controlling plant pathogens using tamarind were demonstrated.

MATERIALS AND METHODS

Study area

This study was carried out in Tharaka-Nithi County in Kenya. The county borders Embu County in the south, Meru County in the north, Kitui County in the east, and Mount Kenya in the west. This region lies between longitudes 38.0631460 east and 0.29650 south. The county is divided into six sub-counties: Tharaka North, Tharaka South, Chuka, Igambang'ombe, Muthambi, and Maara. Samples of *T. indica* were collected in the lower Igambang'ombe, classified as a semi-arid area (Tharaka-Nithi County Government 2016). It has an annual temperature range of 20-30°C and an altitude of around 800 m above sea level. The area receives an average annual rainfall of 125 mm/year (Tharaka-Nithi County Government 2016).

Collection of plant samples

Plant samples were collected according to Upadhayay (2016). Identification of *T. indica* was done with the assistance of a botanist from the National Museums of Kenya. Plant samples were collected randomly from 10 trees located within a radius of 50 m. Young and fresh leaves, green pods, bark, and roots were collected from each tree and bulked to form a composite sample. Each composite sample was placed in a separate bag, sealed, labeled, and placed in cooler boxes. The samples were taken to the Biochemistry Laboratory at the National Museums of Kenya for analysis.

Analysis of phytochemicals

According to Wadood et al. (2013) and Altemimi et al. (2017), crude extracts were prepared. Each plant sample was cleaned and rinsed in distilled water before drying for seven days at room temperature. One hundred grams of each sample were weighed and ground into a fine powder using a high-speed multi-function grinder (RRH-2000A). The crude extraction used different solvents (acetone, methanol, and dichloromethane). The mixture was put in a sealed container and left to stand for three days to ensure maximum extraction of soluble components. The samples were then filtered using Whatman filter paper number 1. The filtrate was concentrated using a rotary evaporator to get a crude extract. The residue was then re-soaked for 48 hours, and the extraction process was repeated to ensure it was exhaustive (Azwanida 2015). The filtrate was purified using fractional distillation to remove the solvent and leave only the pure crude extract. The extracts were then analyzed for the presence of phytochemicals, according to Gomathi et al. (2017). They were tested for the presence of alkaloids, steroids, saponins, flavonoids, terpenoids, phenols, tannins, glycosides, and resins. Dimethyl Sulfoxide (DMSO) was used to make the dilutions that were used for the antimicrobial assay at concentrations of 62.5 ppm, 125 ppm, 250 ppm, 500 ppm, and 1000 ppm (Do et al. 2014; Umaru and Umaru 2018).

Isolation of the plant pathogens

The *F. oxysporum* was isolated from the stems of tomato plants that showed symptoms of *Fusarium* wilt (Fourie et al. 2011). The stem was divided into one-millimeter portions using a sterile scalpel. After sterilizing the sections for one minute with a 1% sodium hypochlorite solution, the sections were washed in distilled water to eliminate any remaining disinfectant. The extra water was then blotted off using sterile blotting paper. The portions were then put on culture plates with Potato Dextrose Agar (PDA) media. The plates were then closed and incubated for three days at 25°C in an inverted position to allow growth. Further sub-culturing was done to obtain pure cultures using a single-spore isolation approach (Zhang et al. 2013). In preparation for the sensitivity test, the acquired pure culture was inoculated in fresh PDA media, allowed to grow at 25°C, then kept in the refrigerator at 5°C, awaiting the antifungal assay.

The *X. campestris* was isolated from the leaves of tomato plants that showed symptoms of *Xanthomonas* wilt (Wasukira et al. 2012). The leaves were cut using a sterile scalpel and packed in aluminum foil. The symptomatic leaves were surface-sterilized using 1% sodium hypochlorite for two minutes, then rinsed with distilled water and dried using sterilized blotting paper. Next, using a sterile blade, the contaminated area of the leaves was aseptically cut, then put into a sterilized mortar. Next, 1 mL

of normal saline solution was added before the leaf pieces were crushed with a pestle. The sap from the crushed leaf was then injected into nutrient agar plates, where it was incubated for 24 hours at 28°C. The *X. campestris* colonies were morphologically identified and subcultured into Muller-Hinton Agar for 24 hours at 28°C to obtain pure cultures. Gram staining and oxidase tests were conducted to identify the pathogen. The pure cultures were then inoculated into freshly prepared media, grown overnight, and then stored at 4°C in the refrigerator, awaiting the antibacterial assay.

Antimicrobial assay

Antimicrobial tests were done according to Balouiri et al. (2016). One hundred and eighty plates for culture media were prepared for the antimicrobial assay of each pathogen. Next, 1 mL of the crude extracts, diluted at the different concentrations, was added to each plate and replicated thrice. Pure cultures of the pathogens were picked aseptically with a 3 mm diameter sterile cork borer and placed in their respective treated plates for the antimicrobial assay. The positive controls were Ridomil© for *F. oxysporum* and Liquicop© for *X. campestris*, while the negative control was pure distilled water. Mycelial growth diameter was measured using a vernier caliper on days 3, 6, and 9. Percentage inhibition was calculated using the formula:

$$inhibition = \frac{mycelial\ diameter\ of\ the\ negative\ control - mycelial\ diameter\ of\ treated}{mycelial\ diameter\ of\ the\ negative\ control}\ x\ 100$$

Statistical analysis

All variables were subjected to a two-way Analysis of Variance (ANOVA) in the Statistical Analysis System (SAS) version 9.4. Significant means were separated using the Least Significant Difference (LSD) at P = 0.05.

RESULTS AND DISCUSSION

Phytochemical analysis of *Tamarindus indica* extracts

A total of nine phytochemicals were identified from the stem, root, pods, and leaves of *T. indica*, with different concentrations. These were saponins, flavonoids, alkaloids, terpenoids, steroids, phenols, tannins, glycosides, and resins. In the acetone extract, seven phytochemicals were identified in all the *T. indica* extracts (Table 1). Phenols, steroids, and tannins were present in all the extracts. Alkaloids were highest in the stem, while steroids were highest in both leaves and pods. Glycosides were only present in the root extract, whereas terpenoids and resins were not detected in any of the extracts.

Only four phytochemicals were detected in all the dichloromethane extracts, some at very low concentrations (Table 2). A moderate concentration of flavonoids was found in the stem extract. Glycosides and tannins were only found in low concentrations, while steroids were found in high concentrations in the leaves and pods. No phytochemicals were detected in the root extract.

Furthermore, like the acetone extract, seven phytochemicals were identified in all the methanol extracts (Table 3). However, terpenoids and resins were not detected in all the extracts. On the contrary, Saponins were detected in all parts of the plant, with very low concentrations in some parts. Alkaloids were also detected in very low concentrations. Glycosides were only present in leaves, whereas phenols were only present in leaves and pods.

Table 1. Phytochemicals present in different parts of *T. indica* extracted using acetone

Phytochemical	Stem	Root	Leaves	Pods
Saponins	-	+	++	-
Flavonoids	+	+	+	-
Alkaloids	+++	++	+	-
Terpenoids	-	-	-	-
Steroids	+	+	+++	+++
Phenols	++	++	+	++
Tannins	+	++	+	++
Glycosides	-	+	-	-
Resins	-	-	-	-

Note: Key: Absent (-), Low Concentration (+), Moderate Concentration (++), High Concentration (+++)

Table 2. Phytochemicals are present in different parts of T. indica when the extract is obtained using dichloromethane

Phytochemical	Stem	Root	Leaves	Pods
Saponins	-	-	-	-
Flavonoids	++	-	-	+
Alkaloids	-	-	-	-
Terpenoids	-	-	-	-
Steroids	+	-	+++	+++
Phenols	-	-	-	-
Tannins	+	-	+	-
Glycosides	-	-	+	+
Resins	-	-	-	-

Note: Key: Absent (-), Low Concentration (+), Moderate Concentration (++), High Concentration (+++)

Table 3. Phytochemicals are present in different parts of *T. indica* when the extract is obtained using methanol

Phytochemical	Stem	Root	Leaves	Pods
Saponins	+	+++	+	+++
Flavonoids	+++	+++	-	+
Alkaloids	-	+++	+	-
Terpenoids	-	-	-	-
Steroids	-	-	+++	+++
Phenols	-	-	+	+
Tannins	+	+	+++	+
Glycosides	-	-	+	-
Resins	-	-	-	-

Note: Key: Absent (-), Low Concentration (+), Moderate Concentration (++), High Concentration (+++)

Antimicrobial assay of *Tamarindus indica* extracts against *Xanthomonas campestris*

In Mullier-Hinton agar, *X. campestris* appeared pale yellow (Figure 1). The antimicrobial tests of *T. indica* extracts obtained using different solvents against *X. campestris* were all negative and did not show any inhibition. However, the positive control showed a one-centimeter ring of growth inhibition from the point of inoculation of the pathogen after 48 hours of incubation. At this point, there was full growth in the negative control plate.

Antimicrobial assay of *Tamarindus indica* extracts against *Fusarium oxysporum*

Acetone plant extracts

The *F. oxysporum* produced white powdery mycelium in the PDA medium and microconidia that were single pink-red and hyaline (Figure 2). As observed under the microscope, the macroconidia were oval, thick-walled, hyaline, in short chains, and with hooked apices. The results showed a significant difference ($p < 0.05$) in the percent inhibition between acetone extracts at different concentrations. The maximum percentage inhibition (71.042%) was obtained in the stem extract at 250 ppm, followed by the root extract (57.556%) at the same concentration (Table 4). The lowest percentage of inhibition was in the leaf (13.551%), and pod (13.926%) extracts at a concentration of 1,000 ppm. The inhibition by the positive control (Ridomil) was significantly higher ($p < 0.05$) than all concentrations of the extracts except the stem extract at a concentration of 250 ppm, which was not significantly different. Generally, the highest inhibition mean was obtained at 250 ppm and the lowest at 1,000 ppm concentrations.

Inhibition of F. oxysporum *by dichloromethane plant extracts*

There was a significant difference ($p < 0.05$) in the percent of inhibition displayed by dichloromethane extracts against *F. oxysporum*. The highest inhibition (68.81%) was exhibited by leaf extract at 500 ppm, followed closely by stem extract (68.664%) at 125 ppm and leaf extract (65.004%) at a concentration of 62.5 ppm (Table 5). Extracts from pods exhibited the lowest inhibition compared to extracts from other parts. The lowest inhibition was exhibited at 62.5 ppm (23.224%) and 1,000 ppm (25.047%). There was a significant difference ($p <0.05$) in the inhibition between all extracts and the positive control, except for the leaf extract at a concentration of 500 ppm. Like acetone extracts, the highest inhibition mean was obtained at 125 ppm, while the lowest was at 1,000 ppm concentrations. The positive control also exhibited a significantly higher inhibition percentage than the dichloromethane plant extracts.

Inhibition of F. oxysporum *by methanol plant extracts*

There was a significant difference ($p<0.05$) in the percent of inhibition exhibited by methanol extracts against *F. oxysporum* (Figure 3). The highest inhibition was recorded in the stem extract at 125 ppm (86.953%) and in the root extract at 500 ppm (75.169%) (Table 6). These were followed by stem extract at 250 ppm (74.742%) and leaf extract at a concentration of 500 ppm (74.041%). The lowest percent of inhibition was recorded in the stem extract at 62.5 ppm (34.705%) and in pod extracts at 1000 ppm (42.889%). There was no significant difference ($p<0.05$) in inhibition between the positive control and the stem extract (at 125 ppm and 250 ppm), the root extract (at 250 ppm and 500 ppm), and the leaf extract (at 500 ppm).

Table 4. Percentage inhibition of *Fusarium oxysporum* by acetone extracts from different parts of *Tamarindus indica* at different concentrations

Concentrations (ppm)	Treatment	Inhibition (%)	Means	LSD	CV
62.5	AC_LF	18.893 d	44.429	14.257	39.223
	AC_PD	25.055 cd			
	AC_RB	52.827 b			
	AC_SB	37.739 c			
	Ridomil	87.632 a			
125	AC_LF	19.400 d	52.358	21.456	11.953
	AC_PD	28.374 cd			
	AC_RB	48.569 b			
	AC_SB	32.762 c			
	Ridomil	87.686 a			
250	AC_LF	35.321 c	59.310	19.918	9.665
	AC_PD	53.208 b			
	AC_RB	57.556 b			
	AC_SB	71.042 a			
	Ridomil	79.423 a			
500	AC_LF	22.312 c	44.393	31.616	11.483
	AC_PD	28.379 c			
	AC_RB	50.452 b			
	AC_SB	40.176 b			
	Ridomil	80.645 a			
1000	AC_LF	13.551 b	36.600	29.201	9.700
	AC_PD	13.926 b			
	AC_RB	55.196 a			
	AC_SB	21.805 b			
	Ridomil	78.523 a			
Means		46.418			
LSD		4.873			
CV		29.212			

Note: AC_LF: Acetone leaf extract, AC_PD: Acetone pod extract, AC_RB: Acetone root extract, AC_SB: Acetone stem extract. The means denoted by the same letter show no significant difference in the percent inhibition.

Figure 1. Pure cultures of *Xanthomonas campestris* in Mullier Hinton Agar Media

Table 5. Percentage inhibition of *Fusarium oxysporum* by dichloromethane extracts from different parts of *Tamarindus indica* at different concentrations

Concentrations (ppm)	Treatment	Inhibition (%)	Means	LSD	CV
62.5	DC_LF	65.004 b	53.905	31.881	11.688
	DC_PD	33.224 d			
	DC_RB	51.004 c			
	DC_SB	41.164 c			
	Ridomil	87.632 a			
125	DC_LF	56.204 c	58.013	23.604	11.203
	DC_PD	29.082 d			
	DC_RB	48.430 c			
	DC_SB	68.664 b			
	Ridomil	87.686 a			
250	DC_LF	48.797 c	55.062	23.078	10.396
	DC_PD	37.324 d			
	DC_RB	49.434 c			
	DC_SB	60.333 b			
	Ridomil	79.423 a			
500	DC_LF	68.811 ab	55.672	37.909	17.267
	DC_PD	30.772 c			
	DC_RB	59.799 b			
	DC_SB	38.332 c			
	Ridomil	80.645 a			
1000	DC_LF	35.940 b	33.300	1.739	19.303
	DC_PD	25.047 b			
	DC_RB	35.940 b			
	DC_SB	39.758 b			
	Ridomil	78.523 a			
Means		32.499			
LSD		6.187			
CV		32.499			

Note: DC_LF: DCM leaf extract, DC_PD: DCM pod extract, DC_RB: DCM root bark extract, DC_SB: DCM stem bark extract. The means denoted by the same letter show no significant difference in inhibition

Table 6. Percentage inhibition of *Fusarium oxysporum* by methanol extracts different parts of *Tamarindus indica* at different concentrations

Concentrations (ppm)	Treatment	Inhibition (%)	Means	LSD	CV
62.5	ME_LF	50.663 bc	57.692	34.414	16.243
	ME_PD	54.249 b			
	ME_RB	61.210 b			
	ME_SB	34.705 c			
	Ridomil	87.632 a			
125	ME_LF	50.082 b	68.092	21.456	11.953
	ME_PD	60.594 b			
	ME_RB	55.143 b			
	ME_SB	86.953 a			
	Ridomil	87.686 a			
250	ME_LF	53.468 c	69.140	17.585	9.947
	ME_PD	68.262 b			
	ME_RB	69.807 ab			
	ME_SB	74.742 ab			
	Ridomil	79.423 a			
500	ME_LF	74.041 ab	70.014	17.043	9.762
	ME_PD	68.364 b			
	ME_RB	75.169 ab			
	ME_SB	51.851 c			
	Ridomil	80.645 a			
1000	ME_LF	56.601 b	57.608	35.355	16.664
	ME_PD	42.889 b			
	ME_RB	52.358 b			
	ME_SB	57.670 b			
	Ridomil	78.523 a			
Means		64.509			
LSD		5.823			
CV		25.117			

Note: ME_LF: Methanol leaf extract, ME_PD: Methanol pod extract, ME_RB: Methanol root bark extract, ME_SB: methanol stem bark extract. The means denoted by the same letter show no significant difference in inhibition

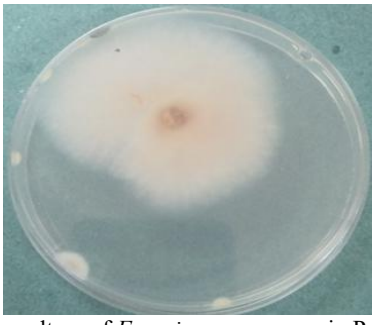

Figure 2. Pure culture of *Fusarium oxysporum* in Potato Dextrose Media (PDA)

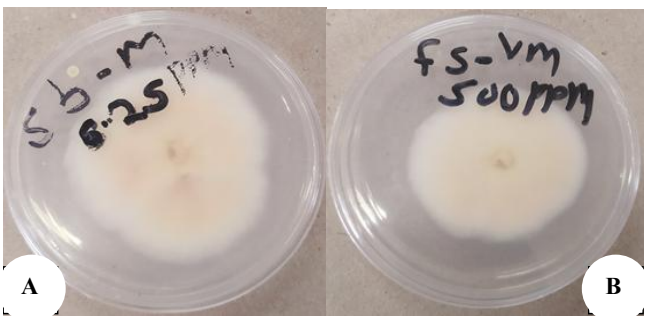

Figure 3. Inhibition of growth of *Fusarium oxysporum* by *T. indica* pods methanol extracts at a concentration of A. 62.5 ppm and B. 500 ppm

Discussion

Extraction of phytochemical compounds from plants

In this study, different extraction solvents resulted in differences in the phytochemicals extracted from different samples. Different solvents extract different phytochemical compounds based on the polarity of the solvent. Yadav and Agarwala (2011) researched seven medicinal plants using four solvents (water, methanol, ethanol, and acetone) and detected different phytochemical compounds. Extraction of the phytochemical compounds from plants depends on various factors, such as solvents, polarity, and temperature. These factors influence the types of metabolites in the crude extracts (Stéphane et al. 2021). Nwodo et al. (2011) found a significant variation in the phytochemicals present in a stem sample taken from *T. indica* when extracted using hot water, cold water, and methanol. Methanol and acetone extracts of *T. indica* provided higher phenol, flavonoids, and saponins yields than other solvent extracts (Moteriya et al. 2015; Estevinho et al. 2018). This demonstrates that crude aqueous and organic solvent extracts yield medicinally significant bioactive substances. When several extraction solvents (water, petroleum ether, and water) were used in the extraction process, the extracts obtained from *T. indica* displayed potential antibacterial action (Warda et al. 2007). Another study by Padalia et al. (2015) combined hexane and water to extract phenols, while

Abdallah and Muhammad (2018) combined water and methanol to detect different phytochemicals. However, the best extraction method should be realized quickly with minimal solvent consumption (Ameer et al. 2017; Rahimi et al. 2022).

In this study, methanol had a relatively high number of phytochemicals detected. The extracts obtained using methanol also showed the highest inhibition of growth of *F. oxysporum* as compared to extracts obtained using acetone and dichloromethane. These findings could be explained by the fact that various phytochemicals are more soluble in methanol than other solvents (Young 2004). In a study conducted by Abdallah and Muhammad (2018), methanol extracts of *T. indica* exhibited higher antimicrobial activity than aqueous extracts. According to Kalpana and Prakash (2016), methanol extracts exhibited greater antimicrobial sensitivity than extracts obtained using acetone. In a similar study, methanol extracts were more effective than other extracts in a comparable trial to test 12 medicinal plants for possible antibacterial activity against five medically important bacteria (Parekh et al. 2005). Similarly, Nauman and Arshad (2011) assessed the antibacterial activity of aqueous methanol extracts of 10 plants against two gram-negative and three gram-positive bacteria and found positive results. In addition, according to Biscaia and Ferreira (2009), the extraction yields of methanol extracts were higher than those of other solvent extracts, with a decrease in polarity.

Acetone also exhibited considerable extraction potential for seven phytochemicals, though at a lower concentration; this solvent has been used for extraction in many related studies because it is more affordable and reproducible to assess plant extracts' efficacy than to conduct animal experiments (Adamu et al. 2013). However, various studies use acetone only for precipitation and concentration of total proteins while simultaneously removing interfering substances (Niu et al. 2018). Dichloromethane was the least applicable solvent compared to acetone and methanol. Of the nine phytochemicals under study, only four were found at much lower concentrations. According to Mohammadi et al. (2016), *Urtica dioica* dichloro methanolic extract can efficiently induce apoptosis in PC3 cells; as a result, it may be employed as a novel therapeutic candidate for prostate tumor treatments. In a related investigation, the dichloromethane extract of the aerial portions of *Satureja khuzistanica* was used to characterize four substances with promising outcomes (Moghaddam et al. 2007). The differences observed in the phytochemicals present could be due to various solvent factors such as concentration, temperature, pressure, and polarity (Kar et al. 2019).

Phytochemical compounds of Tamarindus indica *extracts*

A total of nine phytochemicals were identified from the different parts of *T. indica*. This diversity of phytochemical compounds was reported in similar research (Nwodo et al. 2011; Gomathi et al. 2017). The presence of different enzymes in different plant parts, the different roles of the parts, and the level of exposure of each plant part could be caused this variation (Doughari et al. 2006; Saxena et al. 2014; Nemzer et al. 2020). In this study, steroids were the most abundant phytochemicals in *T. indica* leaves and pods, probably due to the high presence of cyclization enzymes that form steroids in various biological processes. Steroids are synthetic organic compounds crucial during hormone formation and activation (Wood and Gower 2010). They possess numerous intriguing medical, pharmacological, and agrochemical activities (Ericson-Neilsen and Kaye 2014). Numerous steroid compounds are produced by plants and are employed as hormones and allelochemicals (Dinan et al. 2001).

Tannins were present in every plant part of *T. indica*. Due to their antimicrobial and insecticidal effects, their presence probably protects the plant from pests and diseases. These important water-soluble plant phenolics fight pathogens integrally (Hussain et al. 2019) and have exhibited antimicrobial activity against some phytopathogenic fungi (Gade et al. 2020). Besides, Tannins and alkaloids also have a very high antimicrobial and insecticidal effect (Qiu et al. 2014; Hussain et al. 2019). Additionally, Terpenoids are non-polar phytochemicals that are insoluble in most polar solvents; hence, non-polar solvents are the best options for their extraction (Martins et al. 2017). Natural resins are known for their antiseptic and antibacterial benefits (Shuaib et al. 2013) and are also incredibly beneficial for helping plants repair their wounds (Timmermann et al. 2013). In this study, the resins were not found in any parts of the plant. These phytochemicals were most likely present in their precursor forms as diterpenoids and triterpenoids and would only be activated when the plant was injured. However, different locations and the plant part's general health and stress level may determine the phytochemical composition present (Gil et al. 2002; Özcan and Chalchat 2005; Timmermann et al. 2013; Papazian and Blande 2020).

Antifungal assay of Tamarindus indica *extracts against* Fusarium oxysporum

Extracts from *T. indica* showed significant antimicrobial activity against *F. oxysporum*. The inhibition was similar to that Rongai et al. (2012), and Awere et al. (2021) reported. Similarly, Dissanayake (2013) demonstrated radial mycelial growth inhibition when plant extracts were applied to their growth media. A different study studied the antifungal activity of *T. indica* (leaves, stem bark, and pulp) against *Aspergillus niger, A. flavus, and F. oxysporum* (Abubakar et al. 2010). Of the three plant parts, the stem bark inhibited the growth of *A. flavus* and *F. oxysporum*. In a similar study, the crude extracts of leaves, stems, fruit pulp, seeds, and bark of *T. indica* were toxic against *A. flavus* and *F. oxysporum* in vitro (Ramaiah and Garampalli 2015). In a phytochemical analysis done on *T. indica* by Hussain et al. (2019), the root and stem samples exhibited the highest yield of phytochemicals compared to all other parts analyzed. They showed high concentrations of tannins and flavonoids, which are very important in fighting plant pathogens. According to Barros et al. (2011), roots and leaves show great antifungal potential. Bautista-Baños et al. (2002) also found that the stem, fruit, and leaves of *T. indica* manifest significant inhibition of the growth of *Colletotrichum gloeosporioides*.

In this study, it was observed that an increase in the plant extract concentration reduced the percentage of inhibition. This can be attributed to an increase in the amount of bioactive compounds. This is similar to a study by Al-Hetar et al. (2011) and Ramaiah and Garampalli (2015). The reduced free reactive radicals can explain a gradual decrease in the percentage inhibition observed at 1,000 ppm. Thus the phytochemicals form a stable structure with tight bonds that are not easy to break, resulting in a reduction in kinetics (Ochoa-Gómez et al. 2009). The increase in concentration also meant that there was an increase in the viscosity of the extract. A study conducted by Meyer et al. (2014) showed an inverse relationship between viscosity and diffusion. That suggests as the viscosity increased, the distribution of the antimicrobial in the culture medium may have been reduced, lowering the overall inhibition of *F. oxysporum* growth. The positive control showed the highest percentage of inhibition compared to the plant extracts. The *T. indica* extracts inhibited growth in crude form, indicating that further purification and isolation of the bioactive compounds is possible.

Antibacterial assay of Tamarindus indica *extracts against* Xanthomonas campestris

All *T. indica* extracts showed no antibacterial activities against *X. campestris,* probably due to the pathogen's tolerance degree towards the bioactive compounds in the extracts. Compared to a study by Satish et al. (2009), where 30 plant extracts were assayed for their antibacterial effect against *X. campestris*, only eight showed significant inhibition against the *F. oxysporum* pathogen. Interestingly, the dryland plants showed less inhibition than the wetland plants. In a different study, the efficiency of *Bacillus subtilis* was evaluated against three strains of *X. campestris* pv. *campestris* in four *Brassica* crops (cabbage, cauliflower, rape, and broccoli) (Wulff et al. 2002). Biological control was effective in broccoli but not in cabbage. According to Bobis et al. (2015), gram-negative bacteria exhibit higher antibiotic tolerance than gram-positive bacteria. That was also reported by Paterson (2006), who demonstrated a wide range of resistance in gram-negative bacteria. The Gram-negative bacteria possess a periplasmic space between the outer lipid membrane and the inner peptidoglycan layer that contains enzymes that may lead to the breakdown of secondary compounds, thus offering great resistance (Costerton et al. 1974; Bondarczuk and Piotrowska-Seget 2013). The findings contradict those of Patel et al. (2013) and Kalpana and Prakash (2016), who described experiments in which plant extracts tested positive for pathogen sensitivity. While there are so many *X campestris* strains, maybe *T. indica* extracts may be effective in some but not others.

In conclusion, different phytochemicals can be found in different parts of *T. indica*; these phytochemicals are obtained with different solvents used for extraction. Compared to acetone and dichloromethane extracts, methanol-derived extracts have the highest percentage of *F. oxysporum* inhibition. The percentage of inhibition also varies among the various plant parts, with the roots and stem bark displaying the highest percentage of inhibition. *T. indica* extracts have a significant ability to combat *F. oxysporum* and can, therefore, play a crucial role in integrated pest and disease management. However, the extracts showed no inhibition against *X. campestris,* probably due to the pathogen's tolerance. More research is needed to determine the structures of the phytochemical compounds present in *T. indica* extracts.

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