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PHYTOCHEMICAL ANALYSIS, IN VITRO TESTING OF ANTIBACTERIAL PROPERTIES, ANTI- INFLAMMATORY ACTIVITY, AND CYTOTOXICITY OF AQUEOUS AND DICHLOROMETHANE LEAF EXTRACTS OF SOLANUM INCANUM AND TAMARINDUS INDICA

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Citations:

Ngurari S.W., Kiruki S & Gichumbi J. M. (2023). ***Phytochemical Analysis, In Vitro Testing Of Antibacterial Properties, Anti- Inflammatory Activity, And Cytotoxicity Of Aqueous And Dichloromethane Leaf Extracts Of Solanum Incanum And Tamarindus Indica*****. In: Isutsa, D. K. (Ed.).** ***Proceedings Of The Chuka University 9th Annual International Research Conference Held In Chuka University, Chuka, Kenya From 24th To 25th November*****, 2022. 370-390 Pp.**

ABSTRACT

Most communities use medicinal plants to treat and prevent various diseases. The use of the plants demonstrates the need to study the efficacy of selected medicinal plants. There is limited information on the antibacterial, anti- inflammatory activity, and cytotoxicity of *Solanum incanum* and *Tamarindus indica* leaves extracted using polar and less polar solvents. This study focused on analyzing phytochemicals present in aqueous and dichloromethane leaf extracts of the two plants and testing their antibacterial, anti-inflammatory, and cytotoxic properties. The phytochemical analysis was done using standard chemical tests followed by Gas Chromatography-Mass Spectroscopy of dichloromethane extracts. The antibacterial potency of the extracts was tested against *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* using disc diffusion, Minimum Inhibitory Concentration, and Minimum Bactericidal concentrations. The anti-inflammatory potency of the extracts was tested using an Erythrocyte stabilization assay, while the toxicity of the extract was tested using brine shrimp lethality tests. The chemical test revealed that all the plant extracts contained flavonoids, tannins, saponins, phenols, and alkaloids. The *T. indica* dichloromethane and *S. incanum* water extracts had no glycosides, while anthraquinones were absent in all the plant extracts. The chromatographic analysis demonstrated the presence of flavonoids, phenols, and alkaloids in the selected plant extracts. The antibacterial activity revealed no significant difference in antibacterial activities between the two plants at $\alpha = 0.05$. *E. coli* and *S. Typhi* were the most sensitive, while *S. aureus* was the least sensitive to the extracts. The Minimum

Inhibitory Concentration of *T. indica* ranged between 62.5 µg/mL and 125 µg/mL among the test organisms, while the Minimum Inhibitory Concentrations of *S. incanum* ranged between 62.5 µg/mL and 250 µg/mL. The Minimum Bactericidal Concentration of both plants ranged between 125 µg/mL and 500 µg/mL. The *T. indica* dichloromethane extracts had the highest stabilization, 57.64 ±13.90%, while *S. incanum* water extract had the lowest erythrocyte stabilization percentage, 19.06 ±14.43%. *T. indica* plant was more effective at 45.59 ±27.09% compared to *S. incunum*, 37.12 ±15.70%. The dichloromethane extracts of the two plants were more effective, 56.41 ±19.71% than water extracts at 26.30 ±12.53%. *T. indica* dichloromethane extract has the highest toxicity (LD_{50} of 113.57 µg/mL) while *S. incanum* was least toxic (LD_{50} of 2341 µg/mL). The assays demonstrated that the two plants are effective in treating bacterial infections and managing inflammation. In addition, the two plants were toxic at higher doses.
Keywords: Anti-inflammatory, Anti-bacterial, Toxicity, Phytochemicals, Lethal Dose, Bactericidal concentrations

INTRODUCTION

Background Information

Most communities in Kenya have been using various plants to prevent, manage and treat diseases (Gakuya *et al*., 2020). The successful use of medicinal plants is attributed to their phytochemical composition, which explains the importance of phytochemical analysis in medicinal plants research (Yoo *et al*., 2018). Phytochemical analysis entails all studies done to determine the phytochemical composition of various extracts and their concentrations. The preliminary testing is then followed by elucidation of chemical structures of phytochemicals of interest. Yoo *et al*. (2018) also revealed that understanding the molecular composition of a plant extract is essential in accurately predicting its health effects. There is limited information on the phytochemical composition of *S. incanum* and *T. indica* leaf extracts from Tharaka Nithi county. Therefore, it is important to conduct a preliminary phytochemical analysis of the two plant extracts and link the phytochemicals present with various health benefits.

Traditional practitioners have been using *S. incanum* and *T. indica* to cure bacterial infections and manage diseases caused by inflammation (Akanmu *et al*., 2018; Enoc *et al*., 2018). The herbalists justify their efficacy by the ability of the plant extracts to promote the healing of chronic wounds and manage gastrointestinal Tract illnesses (Taye *et al.*, 2011). Studies using plants from other regions have demonstrated that these plants could have antibacterial potency. Sbhatu and Abraha (2020) explained that both ethanolic and aqueous leaf extracts of *S. incanum* were effective against *S. aureus, S. typhi,* and *E. coli*. Similar results were reported when methanolic leaf extracts of the plant were used against *Streptococcus pyogenes* and *Pseudomonas aerugninosa* (Akanmu *et al.,* 2018). Other studies concluded that

ethanolic and methanolic leaf and fruit extracts of *T. indica* were active against several bacterial species, including *Bacillus subtilis, E. coli*, and *P. aeruginosa* (Abdallah and Ali, 2018; Hassan *et al*., 2019). Based on the solvents used, the studies focused more on polar phytochemicals, leaving a knowledge gap on the efficacy of less polar phytochemicals present in the extracts. Less polar solvents such as Dichloromethane (DCM) can be used to extract nonpolar phytochemicals. DCM extract can then be used to assess the effectiveness of the target phytochemicals.

S. incanum and *T. indica* from other world regions have been assessed for their anti-inflammatory potency. da Costa *et al*. (2015), using *S. incanum* from Brazil, reported that the plant effectively controlled inflammation at dosages above 75 mg/kg. Enoc *et al*. (2018) found that *S. incanum* extracts effectively managed acute inflammation at dosages above 6.5 mg/kg. Researchers confirmed that the ethanolic extracts of the *T. indica* roots and back have antioxidant and anti-inflammatory effects, with the roots being superior to the back (Borquaye *et al*., 2020). These studies on the antibacterial and anti-inflammatory potency

of the plant explain why traditional healers have successfully managed bacterial and inflammatory diseases over the years. However, no studies have been published on the anti-inflammatory potency of *S. incanum* and *T. indica* used in eastern Kenya despite their regular use in Meru traditional medicine.

Several studies have been done to assess the toxicity of various plant extracts. da Costa *et al*. (2015) reported that dichloromethane extracts of *Solanum lycocarpum* leaves had lower toxicity than ethyl acetate and hexane extracts. A study using *T. indica* seeds demonstrated that vincristine sulfate extracts of the seeds had lower toxicity compared to ethanolic and methanolic extracts (Hassan *et al*., 2019). These studies show variation in toxicity levels in plants extracted using different solvents. Due to this, it was also important to evaluate the toxicity levels of various concentrations of *S. incanum* and *T. indica* extracts.

Statement of the Problem

The emergence of multidrug-resistant bacteria strains such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) and toxicity caused by most anti-inflammatory medications pose a significant health problem globally. Traditional practitioners in Eastern Kenya use *S. incanum* and *T. indica* to manage bacterial-related diseases and inflamation. However, the plants in the region have not been scientifically evaluated for their antibacterial and anti-inflammatory properties. In addition, there is limited information on the toxicity profile of the two plants. Using plants with toxic metabolites can affect the users' health in the long run.

General Objective

To determine the phytochemical composition, antibacterial properties, anti-inflammatory activities, and cytotoxicity of aqueous and dichloromethane leaf extracts of *Solanum incanum* and *Tamarindus indica*.

Specific Objectives

I. To determine the phytochemical composition of aqueous and dichloromethane leaf extracts of *S. incanum* and *T. indica*.

II. To determine the antibacterial potency of aqueous and dichloromethane leaf extracts of *S. incanum* and *T. indica* using diffusion and dilution methods.

III. To determine the anti-inflammatory activity of aqueous and dichloromethane leaf extracts of *S. incanum* and *T. indica* in erythrocytes.

IV. To determine the cytotoxicity of aqueous and dichloromethane leaf extracts of *S. incanum* and *T. indica* using the brine shrimp lethality test.

MATERIALS AND METHODS

Study Site

The leaves of *S. incanum* and *T. indica* were obtained from their natural habitat in Karingani ward, Tharaka Nithi County (0° 17' 60.00" N Longitude: 38° 00' 0.00" E). The plants were identified based on their ethnobotanical information. The sampling of *S. incanum* was based on their age (plants that have not produced any flowers were the most preferred), while only fresh *T. indica* leaves were picked for the study.

Research Design

The antibacterial assay was performed using a 3×3×2×2 factorial design laid out in Complete Randomized Design (CRD). The four factors used were three bacterial stains, three concentrations two plant species, and two extraction modes. The anti-inflammatory assay was done using a 4×2×2 factorial design laid out in CRD. Three factors to used were concentrations at four levels, plant species at two levels and modes of extraction at two levels. Three replicates

were used in the two research layouts. The toxicity test was conducted using a 3×2×2 factorial design laid in CRD. The three factors used for the study were concentration at three levels, two modes of extractions and two plant species. Each concentration in the toxicity assay was replicated five times.

Data Collection

Sample Preparation and Extraction of Phytochemicals

The leaves were collected while green and washed with running water at room temperature to remove dust. They were dried at room temperature away from direct sunlight until they were completely dry. The duration taken to complete the drying process depended on the succulence of the leaves. Each dried sample was milled independently using a blender to obtain a fine powder. The powdered samples were stored in waterproof bags to avoid contamination and exposure to moisture. The extraction process was done according to Jimoh *et al*. (2019) with minor modifications. Two hundred grams of each powdered sample were soaked in four different reagent bottles containing 1000 mL of the respective solvents (Distilled water and DCM) for 48 hours. The mixtures were vigorously hand-shaken every 12 hours to facilitate extraction. The mixtures were filtered using a cotton cloth and Whatman No. 1 filter papers into four separate beakers. A rotary evaporator was used to concentrate the DCM crude extract at 40 ºC and 100 mbar. The water filtrates were chilled at -20 ºC to facilitate the freeze-drying process. To maximize the extraction process, each sample was divided into approximately 200 mL portions, which were poured into different plates of the freeze-drier. The freeze-drying process was done for 72 hours.

Determination of Phytochemical Composition Test for Phenols

The test was performed as described by Mumtaz *et al*. (2014). One milliliter of each extract was added to test tubes containing one milliliter of distilled water. Two drops of Iron III chloride ($FeCl_3$) were added, and the mixture was shaken several times. A bluish-green colour indicated the presence of phenols. The data was recorded in a table.

Test for Saponins

The test was performed according to the method described by Mumtaz *et al*. (2014). Two grams of powdered samples were added to 20 mL of distilled water and boiled in a water bath for five minutes. The mixture was filtered using filter paper. To 10 mL of the filtrate, 5 mL of distilled water was added, and the mixture was shaken vigorously. A stable foam indicated the presence of saponins. The data was then recorded.

Test for Flavonoids

The test was performed as described by Mumtaz *et a*l. (2014). One gram of the powdered extracts was heated in 10 mL ethyl acetate for five minutes. The mixture was filtered, and one milliliter of dilute ammonia was added to the samples. The yellow colour indicated the presence of flavonoids. The data was recorded in a table.

Test for Alkaloids

The test was conducted as described by Rao *et al*. (2016). Two milliliters of each extract were mixed with 0.2 mL of 2 N HCl. One milliliter of Meyer's reagent was added, and the mixture was shaken. The yellow colour indicated the presence of alkaloids. The data was recorded in a table.

Test for Terpenoids

Two milliliters of chloroform were mixed with 0.5 mL of each extract, and three milliliters of concentrated sulfuric acid were added. The mixture was gently shaken. Red or brown colour at the interphase between sulfuric acid and chloroform will indicate the presence of

terpenoids (Rao *et al*., 2016). The data obtained was then recorded.

Test for Glycosides
In one milliliter of distilled water, 0.5 g of each extract was added. Three drops of aqueous sodium hydroxide were added, and the mixture was shaken. The yellow colour indicates that glycosides are present (Rao *et al*., 2016). The obtained data were recorded in a table.

Test for Anthraquinones
Twenty milliliters of chloroform were added to a test tube containing one gram of powdered leaf. The mixture was heated in a water bath for five minutes. The extract was filtered and allowed to cool before 10% ammonia was added

(Auwal *et al*., 2014). The mixture was vigorously shaken, and the upper later of the mixture was observed for pink colouration. The results were recorded in a table.

The described tests were used to determine the presence or absence of the selected phytochemicals, which are important in understanding the basis of the bioactivity of the plant extracts.

Gas Chromatography-Mass Spectrometer (GC-MS)
The plant extracts were subjected to GC-MS analysis to obtain a spectrum that was used to estimate the concentrations of each compound. Solid extracts obtained during the extraction phase were dissolved in DCM. To 1 mL of DCM, 0.2 g of the extract was added. The mixtures were hand-shaken until the extracts are dissolved. The samples were filtered using filter paper to obtain an injectable sample (Komappa *et al*., 2020). The carrier gas for analysis was helium, which was set at a flow rate of 1.0 mL/min. Other conditions set on the GC-MS machine include 16.2 psi, 1 µL injector, a split ratio of 1:50, interphase temperature at 300 ºC and ion-source temperature at 250 ºC. The average percentage of each phytochemical class was determined by comparing the peak area of the phytochemicals to the total areas. The data from this analysis was used to estimate the concentration of the phytochemicals in each extract.

Determination of Antibacterial Activity
Three bacterial strains (*Staphylococcus aureus* ATCC 25923*, Escherichia coli* ATCC 25922, and *Salmonella typhi* ATCC 6539) were used to test the antibacterial potency of the plant extracts. The bacteria strains were obtained from the biological sciences laboratory at Chuka University.

Disc Diffusion Assay
The disc diffusion method was done using the modified Kirby-Bauer assay as described by Hudzicki (2019). Muller Hinton Agar (MHA) was prepared by dissolving 19 g of the agar powder in 500 mL distilled water. The mixture was autoclaved at 121 ºC for 15 minutes. Thirty-Six Petri dishes were labelled and subdivided into four quadrants using a marker pen. The label contained the name of the bacteria, the plant species, the extraction solvent used, and the concentration of the extract. Fifteen milliliters of the media were poured into each of the thirty-six sterile Petri dishes and allowed to solidify. The microorganisms were suspended in 10 mL of normal saline. Using a sterile wire loop, the microorganisms were applied to the Petri dishes with MHA while rotating the dishes for uniform inoculation. Discs (6 mm diameter) were made from a Whatman filter paper No.1 using a paper punch. The discs were covered with an aluminum foil and sterilized by autoclaving at 121ºC for 15 minutes. Three concentrations (3000 µg/mL, 1500 µg/mL, and 750 µg/mL) of each extract were prepared as described by Burman *et al*. (2018). Ten microliters of each sample were applied to the 6 mm discs so that each disc was soaked with the different concentrations of the extracts. The discs were dried at room temperature covered in sterile Petri dishes for thirty minutes. The dried discs were placed on MHA plates with the bacterial strains, one

at a time, using a pair of sterilized forceps. Each disk was placed at the center of a quadrant. A positive control disk (ciprofloxacin commercial disk) was placed on the fourth quadrant. Each Petri dish will contain three discs with the extract at the same concentration and one positive control placed in independent quadrants. The forceps were sterilized after each transfer stage. The plates were incubated for 24 hours at 37 ºC after which the zones of inhibition produced by the extracts and control were measured in millimeters using a ruler. The data was used to compare the efficacy of the plant extracts with the positive control and differences in antibacterial potency of the two plant extracts. In addition, the zone of inhibitions was used to compare the differences in antibacterial activities of aqueous and DCM extracts of the same plant.

Determination of Minimum Inhibition Concentration

Determination of Minimum Inhibition Concentration (MIC) was conducted according to Bussmann *et al*. (2011) with minor modifications. The process was done only for the extract that showed a strong antibacterial activity (zone of inhibition ≥ 10 mm) (Mariita *et al*., 2011). All the plant extracts were diluted to attain a 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, concentrations. Five hundred milliliters of nutrient broth were prepared by dissolving 6.5 g of nutrient broth powder into 500 mL distilled water in a conical flask. The mixture was gently mixed and heated to ensure all the powder had dissolved. The conical flask was tightly covered with aluminium foil and the mixture autoclaved at 121 ºC for 15 minutes. The broth was allowed to cool at room temperature for subsequent procedures. Fifteen milliliters of microorganism suspensions were prepared by picking a small sample of each strain from sample vials. The bacteria were transferred into two test tubes each containing 15 mL of distilled. The tubes were gently shaken to ensure that the microorganisms were evenly suspended in distilled water. For the first extract, A set up for MIC contained two sets of four test tubes, with each tube containing 1 mL of nutrient broth. To the first tubes in each

set, 500 µg/mL (1 mL) of the first extracts was added and mixed by pipetting the extract up and down. From the first tubes, one milliliter of the mixture was obtained and transferred to the second tubes. The same procedure was used for the third and fourth tubes. The next stage involved the addition of 100 µL of the individual bacterial strains into each tube containing plant extracts such that set one contained a gram-positive bacterium (*S. aureus*) while set two contained a gram-negative bacterium (*E. coli*). The same procedure was repeated for the other three extracts. For the positive control, amoxicillin was diluted to attain the same concentration as the extracts. The control setup was similar to that for the extracts, but the extracts were replaced with the standard drug. Two tubes, one inoculated with *S. aureus* and another inoculated with *E. coli*, were used as negative controls. All the tubes were incubated at 37 ºC for 24 hours, after which visible turbidity in the tubes was assessed. The minimum concentration without any visible turbidity was used for the MBC assay.

Determination of Minimum Bactericidal Concentration

The assay was conducted as described by Mostafa *et al*. (2018) with minor modifications. Twenty-four Petri dishes were labelled, and MHA was prepared as described in procedure 3.3.3.1. The sub-culturing process was done by taking 100 µL samples from the two lowest concentration tubes without any visible bacterial growth. The samples were inoculated in independent MHA plates. A sterile wire loop was used to spread the samples evenly on the media. All the Petri dishes were incubated for 24 hours at 37 ºC, after which the MBC values were obtained. The lowest concentration without visible growth on MHA after the specified duration was recorded as MBC. The subculturing was done in duplicates for each concentration. The data from this assay will help determine whether the extracts have bacteriostatic or bactericidal effects on the three strains.

Determination of anti-Inflammatory Activity

An anti-inflammatory assay was performed using the membrane stabilization method as described by Yesmin *et al*. (2020) with minor modifications. Twelve milliliters of blood samples were collected from healthy sheep using 20- gauge needles and 5 mm syringes.

The blood was gently transferred to three-4 mL EDTA tubes and gently shaken five times. This helped prevent hemolysis and ensure that the blood samples were well mixed with the anticoagulant. The three blood-containing tubes were transported in cool boxes. In preparation for the assay, the blood was removed from the cool boxes and kept at room temperature for 20 minutes. The samples were centrifuged at 2500 rpm for 5 minutes, and the packed cells were washed three times with isosaline (0.9% w/v NaCl, pH 7.2). A 10 % v/v suspension of the erythrocytes was prepared by adding 4 mL of the cells to 36 mL of Isosaline. A phosphate buffer (0.15 M, pH 7.4) was prepared by mixing 121.5 mL of 0.15 M $NaH_2PO_4.2H_2O$ and 28.5 mL of 0.15 M Na_2HPO_4 solutions (Kezia *et al*., 2020). Positive control was prepared by dissolving 0.1g of diclofenac sodium in 100 mL isosaline to form the mother liquor. The solution was diluted using isosaline to attain a 100 µg/mL concentration.

A 4.5 mL solution containing 0.25% w/v hyposaline (2 mL), 0.15 M sodium phosphate buffer (1 mL), 10% erythrocytes (0.5 mL) and plant extracts (1 mL) at different concentrations (500 µg/mL, 1000 µg/mL, 2000 µg/mL, and 4000 µg/mL were prepared (Kezia *et al*., 2020). In the negative control, the extracts were replaced with 1 mL isosaline, while in the positive control, the extracts were replaced with 1 mL diclofenac sodium solution. The tubes were incubated in a water bath at 50 ^{o}C for 20 minutes and then centrifuged at 3000 rpm for five minutes. Two milliliters of the supernatant were gently pipetted into cuvettes. The absorbance of the supernatant was read at 560 nm. The experiment was performed in three replicates for all the test samples. The percentage of hemolysis was estimated by assuming the hemolysis produced by the negative control was 100%. The percentage of erythrocyte membrane stabilization or protection was calculated using the following formula described by Yesmin *et al*. (2020).

Percentage protection =100-(OD sample/ OD control) ×100

The data obtained from this assay were analyzed to compare the effectiveness of the extracts and the positive control (diclofenac sodium). The data also demonstrated the difference in efficacy between the plant extracts.

Determination of Cytotoxicity of Plant Extracts Hatching the Brine Shrimp

Three liters of distilled water were measured and poured into a rectangular jar. Twenty-seven grams of table salt were added to the water and stirred to ensure that the salt had completely dissolved. An airline was placed on one side of the jar to enhance air circulation. Fifteen grams of brine shrimp eggs were added to the top of the water jar, followed by mixing (Sarah *et al*., 2017). A 75-watt bulb was placed a few inches from the jar and was switched on. The nauplii hatched after 24 hours, after which they were obtained for toxicity testing.

Toxicity Testing

All the extracts prepared in procedure 3.3.1 were diluted to attain three different concentrations (10 µg/mL, 100 µg/mL and 1000 µg/mL) in 10 mL seawater containing 1% dimethyl sulfoxide (DMSO). Five replicates were prepared for each concentration. Nauplii were picked by sterilized forceps and exposed to the three concentrations such that each tube with the extracts contained ten live nauplii (Sarah *et al*., 2017). The positive control setup was prepared using vincristine sulphate, while the hatching solution containing 1% DMSO was used as the negative control. The number of live nauplii was counted after 24 hours. A nauplius was considered dead after 30 seconds of no forward motion. The percentage of death of nauplii was calculated as follows.

Number of dead nauplii

$$\text{Percentage Death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii + Number of live nauplii}} \times 100$$

The LD_{50} was calculated using the probit method. An extract was considered toxic if 50% of the nauplii died at lower concentrations. The data is important in deciding the safest concentrations of the extract with maximum health benefits.

Statistical Analysis

The values of average zones of inhibition produced by the four plant extracts and RBC stabilization assay results were analyzed using one-way Analysis of Variance (ANOVA). The significance level for the differences will be set at a 5% probability level, after which Least Significant Difference mean separation procedures were applied. The percentage death data obtained from the brine shrimp lethality test was analyzed using probit analysis, and LD_{50} will be obtained using regression at a 5% probability level. All the analysis was done using STATA software.

RESULTS

Yields of the Extracts

The yield of the extract was calculated using the formula below.Percentage Yield=

$$\frac{\text{Weight of the extracts after concentration}}{\text{Weight of the powdered leaves used}} \times 100$$

Each plant had a different yield, based on their phytochemical compositions. Also, the yield varied based on the mode of extraction used. After calculating the yields using the formula stated above, *S. incunum* water extract had the highest yield (4.7% w/w) while *T. indica* had the lowest yield (1.07% w/w). For both plants, water extracts had a higher yield than DCM extracts. The percentage yields of the extracts are summarized in Table 1 below.

Table 1: A summary of the percentage yield of the extracts obtained after the final concentration of the extracts

Extract	**Percentage Yield (w/w)**
S. incanum (DCM)	2.54
T. indica (DCM)	1.07
S. incanum (Water)	4.7
T. indica (Water)	2.53

Qualitative Phytochemical Analysis

Chemical Identification of Phytochemicals

Phytochemical analysis was done using standard chemical tests as described by various authors. The analysis is based on the chemical reaction between phytochemicals and selected solvents. From the analysis, all the extracts contained phenols, saponins, flavonoids, alkaloids, and terpenoids. However, glycosides were absent in *T. indica* (Water) and *S. incunum* (DCM). None of the extracts had anthraquinones. Table 2 below summarizes the qualitative phytochemical analysis results.

Table 2: A representation of qualitative phytochemical analysis results. A Positive (+) indicates presence, while a negative (-) indicates an absence of selected phytochemicals.

Extract / **Phytochemicals**	***S. incanum* (Water)**	***T. indica* (Water)**	***S. incanum* (DCM)**	***T. indica* (DCM)**

Phenols	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Terpenoids	+	+	+	+
Glycosides	+	-	-	+
Anthraquinones	-	-	-	-

Estimation of phytochemical composition using GC-MS

The GC-MS analysis revealed compounds that are unique to selected extracts. The phytochemicals are of different classes and are summarized in table 3. The limited number of phytochemicals listed is based on the library IDs present in the GC-MS system. Although the GC-MS could not identify all compounds as expected, the listed compounds are similar to those identified by other researchers.

Table 3: A Summary of GC-MS Results of Two Plant Extracts

Plant Extract	Phytochemical	Molecular Formulae	Peak Area	Phytochemical Class
T. indica	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{22}O_4$	488940	Flavonoid
	Diisooctyl phthalate	$C_{24}H_{38}O_4$	448929	Flavonoid
	Phthalic acid, butyl tetradecyl ester	$C_{26}H_{42}O_4$	327849	Flavonoid
	1,2-Benzisothiazol-3-amine	$C_7H_6N_2S$	299972	Alkaloid
	Phthalic acid (2-cyclohexylethyl isobutyl ester)	$C_{16}H_{22}O_5$	272036	Flavonoid
	Phthalic acid, butyl isohexyl ester	$C_{18}H_{26}O_4$	212980	Flavonoid
	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O$	175642	Phenols
S. incanum	2-chloro-2-methyl-Butane	$C_5H_{11}Cl$	1142168	Alkane
	1,2-Benzisothiazol-3-amine	$C_7H_6N_2S$	17797	Alkaloid
	N-(4-methoxyphenyl)-2,2-dimethyl	$C_{12}H_{17}NO_2$	173554	Phenols

Determination of antibacterial Activity Disc Diffusion Assay

There were significant differences in the four extracts applied in different bacterial strains at $\alpha = 0.05$. All the controls had the highest inhibition zones, with the largest highest zone being that of ciprofloxacin against *E. coli* (29.27 ±0.40 mm). *S. incanum* water extract was the least effective, with a zone of inhibition of 7.79 ±1.76 mm. On the other hand, the *S. incanum* DCM extract had the largest zone of inhibition (18.90 ±2.38 mm). The mean inhibition of the four extracts is summarized in table 4 below.

Table 4: Means Zone of inhibition produced by the four plant extracts as tested against different bacteria strains.

Plant/ Control	Extraction	Bacteria	Zones of Inhibition (mm ± SEM)
Control	Water	*E. coli*	29.27 ± 0.40^{a}
Control	Water	*S. typhi*	27.23 ± 0.40^{ab}
Control	Water	*S. aureus*	24.83 ± 0.42^{b}
S. incanum	DCM	*E. coli*	18.90 ± 2.38^{c}
S. incanum	Water	*S. typhi*	18.12 ± 2.69^{c}
T. indica	DCM	*S. typhi*	17.99 ± 1.61^{c}
T. indica	Water	*E. coli*	17.60 ± 3.77^{c}
T. indica	DCM	*E. coli*	17.39 ± 0.54^{c}
T. indica	Water	*S. typhi*	17.38 ± 1.76^{c}
S. incanum	Water	*E. coli*	17.31 ± 2.49^{c}

S. incanum	DCM	*S. typhi*	16.38 ±1.35[c]
T. indica	DCM	*S. aureus*	11.68 ±2.13[d]
S. incanum	DCM	*S. aureus*	11.66 ±3.94[d]
T. indica	Water	*S. aureus*	9.73 ±2.61[de]
S. incanum	Water	*S. aureus*	7.79 ±1.24[e]
LSD			2.6133

[a] Means represented by the same letter are not significantly different at α= 0.05. Water/ DCM represents extraction modes. LSD= Least Significant Difference.

Significant differences were also noted between the zones of inhibition produced by the control and the two plant species. However, there were no significant differences in zones of inhibition produced by *S. incanum* and *T. indica* at α= 0.05. The control had larger zones (28.25 ±2.46 mm) compared to the *T. indica* (15.29 ±4.68 mm) and *S. incanum* (15.03 ±3.98 mm), as demonstrated in Table 5.

Table 5: A summary of th e zone of inhibitions produced by the two plants and the control

Sample	Zones of Inhibition (mm ± SEM)
Control	28.25 ±2.46[a]
T. indica	15.29 ±4.68[b]
S. incanum	15.03 ±3.98[b]
LSD	1.0279

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD= Least Significant Difference.

The effectiveness of the two species also varied between the bacteria strains used. *S. incanum* had highest inhibition zones against *E. coli*, while it was least effective against *S. aureus*. Similarly, *T. indica* was more effective against *S. typhi* and least effective against *S. aureus*. The data shows that the two plant species were more effective against gram-negative bacteria. However, despite the differences, the statistical analysis reported no significant differences between
S. incanum zones of inhibition against *E. coli* and inhibition diameters produced by *T. indica* against the same bacteria at α= 0.05. Also, there were no significant differences in the effectiveness of the two plant species against *S. aureus*. More details of the efficacy of the plant species are summarized in Table 6 below.

Table 6: A summary of zones of inhibition of *S. incanum* and *T. indica* tested against three bacterial strains

Plant Species	Bacterial	Zones of Inhibition (mm ±SEM)
Control	*E. coli*	29.27 ±0.40[a]
Control	*S. typhi*	27.33 ±0.40[ab]
Control	*S. aureus*	24.83 ±0.42[b]
S. incanum	*E. coli*	18.12±2.50[c]
T. indica	*S. typhi*	17.68±1.67[c]
T. indica	*E. coli*	17.49±2.62[c]
S. incanum	*S. typhi*	17.25±2.25[c]
T. indica	*S. aureus*	10.70±2.52[d]
S. incanum	*S. aureus*	9.72±3.46[d]
LSD		2.71

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD= Least Significant Difference

There were no significant differences in the zone of inhibition between the two extraction modes at α= 0.05. The DCM extracts of the two plants produced the highest zones of inhibition (15.66 ±4.13 mm) than the water extracts (14.65 ±4.89 mm). The extract from the two extraction modes was less effective than the control, which produced a mean of

28.25 ±2.46 mm. The information on the differences in zones of inhibitions produced by the two extracts is summarized in table 7 below.

Table 7: Mean Zones of Inhibition produced by Control, DCM and water extracts

Samples	Zones of Inhibition (mm ±SEM)
Control	28.25 ±2.46[a]
DCM	15.66 ±4.13[b]
Water	14.65 ±4.89[b]
LSD	1.0279

[a] Means represented by the same letter are not significantly different at α= 0.05.

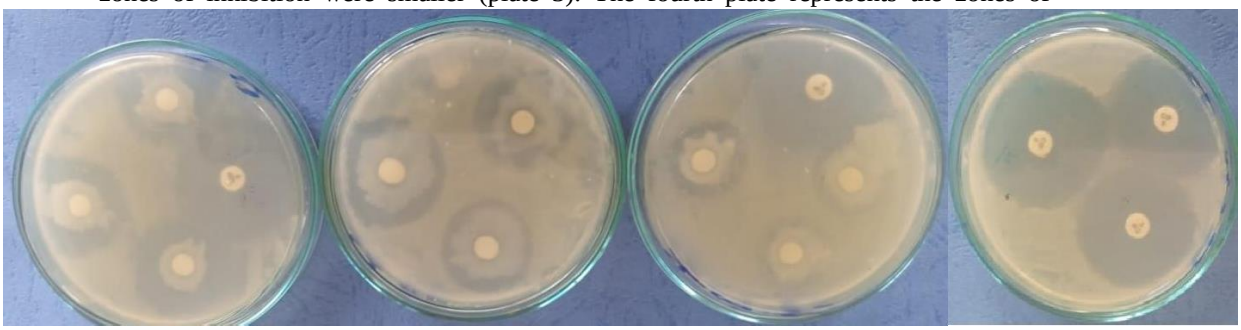
The assay also demonstrated significant differences in zones of inhibition between the three concentrations used for the extracts and the two control at α= 0.05. The positive control against *E. coli* had the largest zone of inhibition (28.25 ±2.46 mm), while 750 µg/mL dilutions had the value of the zones (12.67 ±4.17 mm), as demonstrated in the LSD Table 8 below.

Table 8: Zones of inhibition produced by the extracts at different concentrations

Concentration (µg/mL)	Zones of Inhibition (mm ±SEM)
Control	28.25 ±2.46[a]
3000	17.46 ±4.08[b]
1500	15.34 ±3.38[c]
750	6.37 ±0.39[d]

[a] Means represented by the same letter are not significantly different at α= 0.05.

Below are samples of plates with various zones of inhibition. At higher concentration, the zones were clear (as demo started by plates one and two) while at low concentration, the zones of inhibition were smaller (plate 3). The fourth plate represents the zones of inhibition of the positive control.

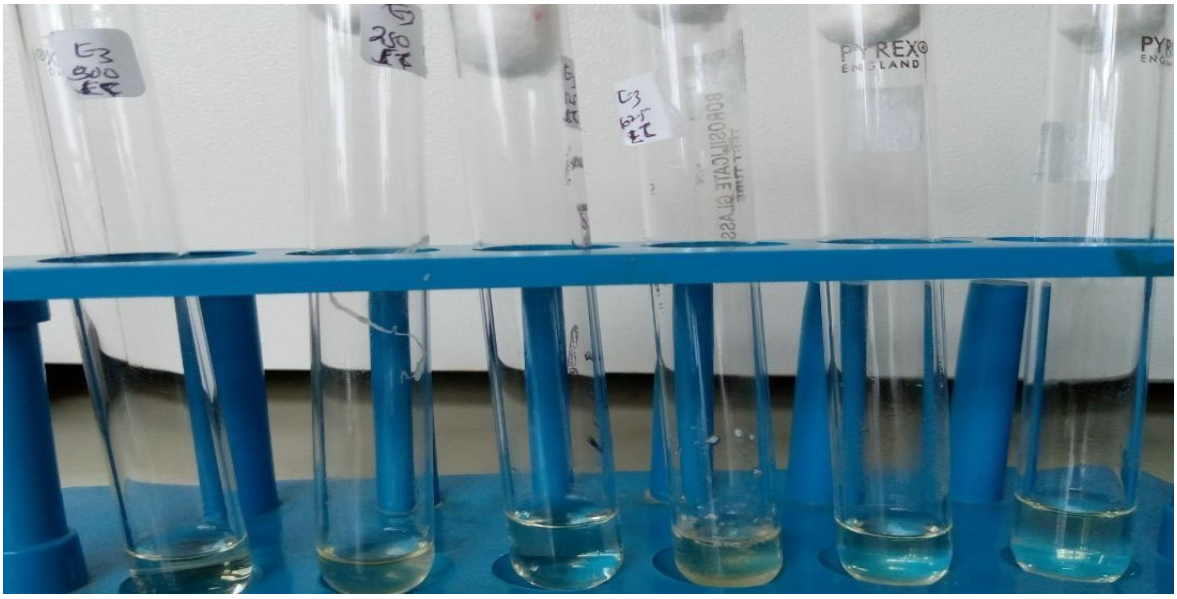


1 2 3 4

Figure 1: Images of Petri dishes with *E. coli* cultures exposed to varying concentration of *T. indica* DCM extract. The concentrations used were 3000 µg/Ml (Plate 1), 1500 µg/mL (Plate 2) and 750 µg/mL (Plate 3) respectively. Plate 4 was the control plate.

Minimum Inhibitory concentration

E. coli and *S. aureus* were subjected to the dilution method to determine MIC. The first extract (*T. indica* aqueous extract) had a 125 µg/mL MIC against *E. coli* and 125 µg/mL *S. aureus*. For extract two (*S. incanum* water extract), the MIC was recorded as 62.5 µg/mL against *E. coli* and 250 µg/mL and *S. aureus*. The *T. indica* DCM extract demonstrated an MIC of 125 µg/mL against *E. coli* and 125 µg/mL against *S. aureus. S. incanum* DCM extract has an MIC of 62.5 µg/mL against *E. coli* and 125 µg/mL against *S. aureus*. Figure 2 represents MIC dilution tubes for *T. indica* DCM extract against *E. coli*. From the setup, there was bacterial growth in tube 4, while tube 1 to 3 were clear. No bacterial growth on the positive control test tubes.

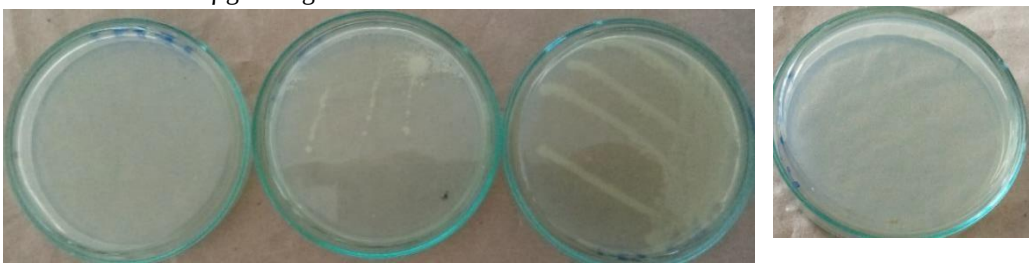


1 2 3 4 5 6

Figure 2: MIC dilution tubes for *T. indica* DCM extract against *E. coli.* The concentrations of the extracts were 500 µg/Ml (tube 1), 250 µg/Ml (Tube 2), 125 µg/Ml (Tube 3) and 62.5 µg/Ml (tube 4). The last two tubes (5 and 6) were the positive controls.

Minimum Bactericidal Concentration

Samples from tubes without visible turbidity were plated on nutrient agar, and MBC was recorded. The *T. indica* aqueous extract had an MBC value of 250 µg/mL against *E. coli* and 250 µg/mL against *S. aureus*. *S. incanum* water extract had an MBC of 250 µg/mL against *E. coli* and 500 µg/mL against *S. aureus* as demonstrated in figure 10 below. The images of the MBC plates for extract two are demonstrated in figure 3 on the next page. The *T. indica* DCM extract demonstrated an MBC of 125 µg/mL against *E. coli* and an MBC of 500 µg/mL against *S. aureus*. *S. incanum* DCM extract has an MBC of 125 µg/mL against *E. coli* and 250 µg/mL against *S. aureus*.



1 2 3 4

Figure 3: Images of MBC plates of *S. incanum* water extract against *S. aureus*. The dilutions were, 500 µg/Ml (Plate 1), 250 µg/mL (Plate 2) and 125 µg/Ml (Plate 3). Plate 4 was the positive control.

Determination of Anti-Inflammatory Activity of Plant Extracts

This assay evaluated the ability of the two plants (*S. incanum* and *T. indica*) extracted using two modes of extraction (aqueous and DCM extractions) to stabilize RBCs at different concentrations. The four extracts were tested at four concentrations (500 µg/mL, 1000

µg/mL, 2000 µg/mL and 4000 µg/mL). The essay demonstrates no significant difference in stabilization between *T. indica* DCM extract, *S. incanum* DCM extract, and the control at α= 0.05. In addition, there was no significant difference in stabilization between *T. indica* water extracts and *S. incanum* water extract. However, there were significant differences in stabilization between the DCM and water extracts of the two

plants at α= 0.05. The mean stabilization of *T. indica* DCM extract was the highest, while *S. incanum* water extract had the lowest stabilization. In addition, the *T. indica* DCM had higher percentage stabilization than the control. The mean separation data for percentage stabilization of the four extracts and the control are represented in Table 9 below.

Table 9: A Representation of the Mean Stabilization of Four Extracts and the Control

Sample/ Control	Extraction Method	Percentage Stabilization ± SEM
T. indica	DCM	57.64 ±13.90[a]
Control	Water	55.33 ±0.08[a]
S. incanum	DCM	55.18 ±24.81[a]
T. indica	Water	33.54 ±2.39[b]
S. incanum	Water	19.06 ±14.43[b]
LSD		16.641

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD= Least Significant Difference.

There were significant differences in stabilization between *S. incanum* and *T. indica*. From the data representations in Table 10 below, *T. indica* had a higher stabilization mean compared to *S. incanum*. However, the percentage mean stabilization for both plants was lower than the control.

Table 10: A Summary of Percentage Mean Stabilization of the Two Plant Samples and the Control

Sample	Percentage Mean Stabilization ±SEM
Control	55.33 ±0.08[a]
T. indica	45.59 ±27.09[b]
S. incanum	37.12 ±15.70[c]
LSD	0.547

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD= Least Significant Difference.

The LSD mean separation demonstrated significant differences in percentage mean stabilization of the four extracts at different concentrations. All the four concentrations were significantly different from each other at α= 0.05. The mean stabilization of the control was also significantly different from that of the four extracts. A concentration of 1000 µg/ml had highest stabilization (48.05 ±25.99 %), while 500 µg/mL had the lowest percentage stabilization (27.33
±11.00 %) as demonstrated in Table 11.

Table 11: A representation of Percentage Mean Stabilization of the Four Extracts at Different concentrations

Concentration (µg/mL)	Percentage Stabilization Mean ± SEM
Control	55.33 ±0.08[a]
1000	48.05 ±25.99[b]
4000	47.32 ±11.85[c]
2000	42.71 ±29.81[d]
500	27.33 ±11.00[e]
LSD	0.5359

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD= Least Significant Difference.

The assay also showed significant differences in percentage stabilization between the two extraction modes at α= 0.05. The DCM extract had a higher stabilization mean (56.41 ±19.71%) compared to the control (55.33 ±0.08%) and water extract (26.30 ±12.53%). The summary of the differences is represented using the LSD Table 12 below.

Table 28: A Representation of the Mean stabilization of Two Modes of Extraction Used for the Two Extracts.

Sample	Percentage Stabilization ± SEM
DCM	56.41 ±19.71[a]
Control	55.33 ±0.08[b]
Water	26.30 ±12.53[c]
LSD	0.547

[a] Means represented by the same letter are not significantly different at α= 0.05. Water/ DCM represents modes of extraction. LSD= Least Significant Difference.

Determination of Cytotoxicity of the Plant Extracts

All the plant extracts had high toxicity thresholds. All the extracts had a higher toxicity threshold than the positive control (Vincristine Sulfate). *S. incanum* DCM extract had a LD_{50} of 2341 µg/mL. The probit analysis of the extract is represented in Table 13.

Table 13: A representation of the LSD Values of the Model Used in the RBC Stabilization Assay. The LD_{50} was determined at a 95% probability level.

Probability	Concentration (µg/mL)	95% Fiducial Limits	
0.10	2.056	0.000523	14.437
0.20	27.620	0.637	93.637
0.30	155.193	32.695	712.077
0.40	638.825	196.348	15769.00
0.50	2341.00	560.532	491144.00
0.60	8575.00	1379.00	17754360.00
0.70	35298.00	3470.00	938025872.00
0.80	198331.00	10347.00	1.22645×10^{11}
0.90	2662237.00	51949.00	1.93134×10^{14}

The *S. incanum* water extract had a lower toxicity threshold compared to *S. incanum* DCM extract. The regression analysis demonstrates that the extract had an LD_{50} of 1000 µg/mL. The summary of the regression analysis is represented in Table 14.

Table 14: A Summary of the regression model for S. incanum water extract. The LD_{50} was determined at a 95% probability level.

Probability	Concentration (µg/mL)	95% Fiducial Limits	
0.10	833.690	796.296	872.841
0.20	891.578	851.587	933.448
0.30	932.261	890.445	976.041
0.40	966.991	923.617	1012.402
0.50	1000.000	955.145	1046.960
0.60	1034.135	987.749	1082.699
0.70	1072.660	1024.546	1123.033
0.80	1121.605	1071.296	1174.277
0.90	1199.485	1145.682	1255.814

T. indica water extract had an LD_{50} of 1104.53 µg/mL. The toxicity threshold of the *T. indica* water extract was higher than *S. incanum* water extract. Therefore, the *T. indica* water

extract is less toxic than *S. incanum* water extract. The results of the *T. indica* water analysis are represented in Table 15.

Table 15: A Summary of the regression model for *T. indica* water extract. The LD_{50} was determined at a 95% probability level.

Probability	Concentration (µg/mL)	95% Fiducial Limits	
0.10	913.844	864.093	966.459
0.20	980.051	926.696	1036.478
0.30	1026.690	970.796	1085.802
0.40	1066.571	1008.506	1127.980
0.50	1104.532	1044.400	1168.126
0.60	1143.844	1081.572	1209.701
0.70	1188.276	1123.585	1256.692
0.80	1244.823	1177.054	1316.495
0.90	1335.010	1262.331	1411.874

T. indica DCM extracts had the lowest LD_{50} values of 113.58 µg/mL. The results show that the *T. indica* DCM extract was more toxic than the *T. indica* water extract and *S. incanum* extracts. The summary of LD_{50} values of the extracts is summarized in Table 16 below.

Table 16: A Summary of the regression model for the *T. indica* DCM extract. The LD_{50} was determined at 95% probability level.

Probability	Concentration (µg/mL)	95% Fiducial Limits	
0.10	95.948	90.541	101.679
0.20	102.108	96.353	108.207
0.30	106.419	100.421	112.775
0.40	110.089	103.884	116.664
0.50	113.568	107.167	120.351
0.60	117.156	110.553	124.154
0.70	121.196	114.365	128.435
0.80	126.313	119.194	133.858
0.90	134.422	126.846	142.451

The positive Control had the lowest toxicity threshold. The positive control had an LD_{50} of 11.92 µg/mL. Therefore, the positive control is more toxic than the four extracts. The results of the analysis of the positive control are summarized in Table 17.

Table 17: A Summary of the regression model for the positive control (Vincristine sulphate). The LD_{50} was determined at a 95% probability level.

Probability	Concentration	95% Fiducial Limits	
0.10	3.620	1.138	5.711
0.20	5.620	2.549	7.971
0.30	7.528	4.253	10.185
0.40	9.567	6.266	12.858
0.50	11.920	8.548	16.661
0.60	14.853	11.056	22.767
0.70	18.875	13.940	33.586
0.80	25.285	17.798	56.086
0.90	39.254	24.828	125.593

DISCUSSION

Percentage Yield of the Extracts

The extraction process aims at obtaining and characterization of active compounds in plants. In this case, the extraction generated a crude extract of active compounds. A comprehensive extraction process is required because the concentration of various phytochemicals is low (Zhang *et al*., 2018). Water and DCM were selected as extraction solvents. Water is highly polar with a polarity index of 10.2, while DCM is less polar with a polarity index of 3.1 (Altemimi *et al*., 2017). The two solvents facilitated the extraction of phytochemicals with different polarities.

Several factors determine the yield of phytochemicals produced. The aqueous extraction protocol had a higher extract yield than the DCM methods. The higher yield through water extraction demonstrates that most phytochemicals in the leaves were polar. Wakeel *et al.* (2019) explained that the extraction efficiency is dependent on the polarity index of the phytochemicals and the miscibility of solvents used. The results on the yield of the extracts revealed a difference in concentration of selected phytochemicals in the two plants.

Qualitative Phytochemical Analysis

The initial phytochemical tests of the extracts demonstrated that the four extracts had phenols, saponins, flavonoids, alkaloids, and terpenoids. In addition, both *S. incanum* DCM extracts and T. indica water extracts did not have glycosides. Anthraquinones were absent in all four extracts. Belayneh *et al*. (2021) also reported that *S. incanum* leaf extracts did not contain anthraquinones. The chemical test results from the *T. indica* water extracts were similar to results published by Abdalla and Muhammad (2018). However, the researcher noted that the *T.indica* leaf extracts had significant amounts of anthraquinones, but this phytochemical class was absent in the extracts used in this study.

The findings from qualitative phytochemical analysis of *S. incanum* extracts were similar to those reported by Sbhatu and Abraha (2020). In addition to this, the researchers also reported that *S. incanum* ethanolic leaf extracts had significant amounts of glycosides (Sbhatu and Abraha, 2020). The GC-MS results of the current studies were different from those reported by other authors. However, the analysis revealed several compounds that had similar chemical structures (with minor variations) to those identified by other researchers. For example, the *S. incanum* extract used in this study had 2-chloro-2-methyl-butane, while the study by Yetayih and Ravichandran (2020) found significant amounts of 2,3-butanediol.

A similar trend was noted in *T. indica* GC-MS results. The extracts had unique phytochemicals such as 1,2- Benzisothiazol-3-amine. However, there were other phytochemicals with similar chemical structures. For example, the *T. indica* extract had Phthalic acid, which is also present in other extracts (Sharma *et al.,* 2021). In addition, the plant had several esters with chemical structures similar to those identified by other researchers. Butyl tetradecyl ester from *T. indica* used in this study had a similar chemical structure to Butyl Octyl Ester, as identified by Sharma *et al*. (2021). The differences in phytochemicals present in the plants of the same species are influenced by climatic variation, interspecies interactions, soil properties, and solvent differences (Defossez *et al*., 2021; Wakeel *et al*., 2019; Silva *et al.,* 2018; Kumar *et al*., 2017). The solvents used have different polarities, with water being more polar than DCM (Abubakar and Haque, 2020). Due to this, phytochemicals present in the water extracts are more polar than those in the DCM extracts. The difference in solvents could have also contributed to the variation in phytochemicals identified using GC-MS. In most studies, the researcher used more polar solvents such as methanol and ethanol, which in this case, only the DCM extracts were subjected to the GC-MS analysis.

Determination of Antibacterial Activities of the Plant Extracts

The disc diffusion assay revealed significant differences in inhibition zones between the plant extracts used for the assay. However, no significant differences were noted when the extracts were tested against gram-negative bacteria alone. From the study, DCM and water

extracts of *S. incanum* and *T. indica* had no significant differences in zones of inhibition when tested against *E. coli* and *S. typhi*. When tested against a gram-positive bacterium (*S. aureus*), the zones of inhibition of *S. incanum* DCM extract and *S. incanum* water extract were significantly different. There was no significant variation between *T. indica* DCM and water extract against *S. aureus*. The plant extracts had varying antibacterial activities, and thus the null hypothesis was rejected.

The minor differences in the antibacterial activities of the two plant species can be attributed to the similarities in the phytochemical composition of the extracts. From the preliminary chemical tests for the phytochemicals, all the four extracts have similar metabolites, apart from *S. incanum* DCM and *T. indica* water extract, which tested negative for glycosides. Several phytochemicals tested in the preliminary chemical analysis are known to possess antibacterial activities, and some are effective against MRSA (Okwu *et al*., 2019). All four extracts tested positive for phenols. Several studies have shown that phenolics are active against gram-positive and gram-negative bacteria. The compound inhibits bacterial growth by interfering with biofilm formation and inhibiting β lactamases (Mandal *et al*., 2017). The findings could be why other studies have identified that phenolics can be used to increase drug sensitivity among drug- resistant bacterial strains such as *S. aureus* (Miklasińska-Majdanik *et al*., 2018).

Saponins also have antibacterial activities against several bacterial strains. Sanfack *et al*. (2019) revealed that saponins extracted from *Albizia adianthifolia* were effective against gram-negative bacteria, with MIC values ranging from as low as 16 µg/mL to 128 µg/mL. Studies on specific flavonoid classes have also shown that this phytochemical class significantly affects bacterial growth (Farhadi *et al*., 2019). Farhadi *et al*. (2019) also explained that some flavonoids are more effective than standard drugs. For instance, Adamczak *et al*. (2019) reported that the MIC values of flavone against *S. aureus* were 1000 µg/mL, while its MIC against *E. coli* and *P. aeruginosa* was 500 µg/mL. Similar trends were noted with the four extracts used in this study. All the extracts were less effective against *S. aureus* and more effective against gram-negatives.

All the four plant extracts had a high alkaloid composition. Most alkaloids have high antibacterial activity against both gram-negative and gram-positive bacteria. Manosalva *et al*. (2016) reported that alkaloid extracts of *Berberis microphylla* had MIC values of 83-333 µg/mL and MBC values of 167- 717 µg/mL. However, some alkaloids are less effective, with MIC values as high as 1.67 mg/mL (Mabhiza *et al*., 2016). Plant phytochemicals have varying modes of action. The most common conclude DNA damage, inhibition of synthesis of bacterial proteins, losing ATP, disruption of cell walls and cell membranes which causes leakage in cell constitutes and direct interference with

synthesis of cell walls and cell membrane (Khameneh *et al*., 2021). Also, some studies have shown that the phytochemicals often affect bacterial communication, biofilm formation, and the efficiency of efflux pumps. The pumps reduce the intracellular concentration of toxic compounds and metabolites (Khare *et al*., 2021). In addition, some phytochemicals, such as phenols, inhibit β- lactamases produced by various bacterial trains (Mandal *et al*., 2017). The ability to compromise the efficiency of efflux pumps and inhibit β- lactamases justify the efficacy of the extracts when used alongside conventional medications (Manosalva *et al*., 2016).

Several unique phytochemicals in the extracts also have antibacterial activities. Phthalic Acid Esters (PAE) produced by *T. indica* have been found to have antibacterial effects against *S. aureus, E. faecalis, P. aeruginosa* and *E. coli* (Huang *et al*., 2021). The *T. indica* extracts had significant amounts of diisooctyl phthalate has antifungal and antibacterial activities (Habib and Karim, 2009). The compound is effective against several bacterial species, including
S. aureus, E. coli, K. pneumoniae, P. aeruginosa and *Streptococcus pyogenes* (Zellagui *et al*., 2012). Although 1,2- Benzenedicarboxylic acid concentration was the lowest among

all compounds, the phytochemical has high antibacterial activity (Shoge *et al*., 2016). 1,2-Benzisothiazol-3-amine identified in *S. incanum*, and *T. indica* has significant antibacterial effects. Priyanka *et al*. (2015) revealed that the compound and other alkaloids were the reason for the high antibacterial activity of *Acacia karoo* against several bacterial strains. These unique compounds were the reason for the high antibacterial activity of the selected plant extracts.

Determination of Anti-inflammatory Activity of Plant Extracts

There were significant differences in percentage RBC stabilization between the four plant extracts. However, there were no significant differences in stabilization between the *T. indica* DCM extract, *S. incanum* DCM extract and the control. In addition, there were significant differences in stabilization between the water and DCM extracts of the two plants. The study demonstrated that *T. indica* DCM extract was more effective than the four extracts, while *S. incanum* water extract had the least stabilization. The stabilization effects were associated with the differences in phytochemicals present in the extracts and their concentrations. The presence of the phytochemicals in each extract was affected by their polarity. Water has a higher polarity, attracting highly polar phytochemicals, while DCM is less polar and extracted less polar phytochemicals.

The findings from this assay support other studies conducted on the anti-inflammatory activities of plants of the same genus as *S. incanum* and *T. indica*. Anosike *et al*. (2012) reported that *Solanum aethiopicum,* the plant extract inhibited hemolysis of RBCs at all doses between 800 µg/mL to 100 µg/mL. These researchers reported percentage membrane stabilization of between 86.67 ± 3.06% and 46.53 ± 2.52%, with the highest stabilization noted at 800 µg/mL while the lowest stabilization was reported at 400 µg/mL. Stabilization at 100 µg/mL was higher than at 400 µg/mL. Benvenutti *et al*. (2021) demonstrated that *Solanum diploconos* hydroalcoholic extracts had anti-inflammatory activities at low concentrations. da costa *et al*. (2015) revealed that *Solanum lycocarpum* hydroethanolic extract had *in vivo* anti-inflammatory properties at dosages between 75 mg/kg and 150 mg/kg. *Solanum xanthocarpum* ethanolic extracts were also proven effective at 150 mg/kg (More *et al*., 2013).

While assessing the analgesic and anti-inflammatory efficacy of *T. indica* roots, bark, seeds stem and leaves, Komakech *et al*. (2019) noted that the extracts from the parts effectively managed inflammation at a low concentration. The researchers recommended that more research should be done on other plant parts. In addition, Borquaye *et al*. (2020) reported that ethanolic stem extracts of *T. indica* is effective at 154.5 ± 2.6 mg/kg, while the root extracts of the plant effectively managed oedema at 118.1 ± 1.9 mg/kg concertation. Sundaram *et al*. (2015) linked the efficacy of the *T. indica* seeds to the ability of the plant to manage arthritis.

Several phytochemicals present in the extracts have been shown to pose anti-inflammatory activities. The chemical tests reveal that all the extracts had significant amounts of terpenoids. Liu *et al*. (2021) reported that terpenes control inflammation by activating AMP-Activated Protein Kinase (AMPK). AMPK is a threonine/serine kinase which stimulates various energy metabolic pathways, such as lipid oxidative pathways (Miki *et al*., 2019). Terpenes also control inflammation by inhibiting pathways such as the NF-Kβ that lead to the production of inflammatory mediators such as IL-1 and TNF-α (Prado-Audelo *et al*., 2021). Also, Liu *et al*. (2021) reported that the terpenoids inhibit the production of LPS-induced inflammatory mediators. Studies on the mode of action of alkaloids have shown that phytochemicals of this class often inhibit the COX-2 enzyme (Hashmi *et al*., 2018). The enzyme is involved in the synthesis of PGE, which stimulates inflammatory pathways. In addition to the inhibition of COX-2, alkaloids also inhibit LOX-5, an enzyme involved in thromboxane production (Wang *et al*., 2021). Other studies have also shown

that alkaloids from various plant extracts can be used to manage IBD due to their inhibitory

activity on MAPK and NF-κB signalling pathways (Yang *et al*., 2021; de Almeida *et al*., 2017). The major alkaloid identified in the *T. indica* DCM extract is 1,2-Benzisothiazol-3-amine. Alam *et al*. (2021) reported that the alkaloid and other isothiazole derivatives have anti-inflammatory properties. *S. incanum* also had a significant amount of isothiazole derivatives. In addition to 1,2-Benzisothiazol-3-amine, *S. incanum* also had a high concentration of propenamide derivatives, which are proven to have anti-inflammatory and anti-oxidant activity (Gökçe *et al*., 2009). The two compounds could be the reason for the high anti-inflammatory activity of the extract. Flavonoids and saponins also inhibit similar enzymes and signaling pathways. However, Lim *et al*. (2019) reported that, besides the activity of flavonoids against COX-2 and LOX-5, they also inhibit nitric oxide synthase, autophagy and inflammasome activation. Also, flavonoids affect other pathways such as Nrf2, PPAR, and AP-1 (Chen *et al*., 2018).

Determination of the Cytotoxicity of the Plant Extracts

Assessing the toxicity of plant extracts is essential in determining the safety of the plants. In traditional medicine, most traditional practitioners use plant extracts to treat various ailments without knowing the medically important concentration and concentration that can cause harm to the human body. The brine shrimp lethality test demonstrated different toxicity levels for the four extracts. In addition, the DCM extracts had lower toxicity compared to the water extracts. The difference in toxicity is dependent on the differences in phytochemicals present in extracts. Several phytochemicals have significant toxicity levels. For example, Seremet *et al*. (2018) demonstrated that pyrrolizidine alkaloids have high cytotoxicity levels in animal models. The chemical tests of plant extracts revealed that the four extracts contained alkaloids, which was confirmed using the GC-MS analysis. Flavonoids and sesquiterpenes also determine the toxicity levels of plant extracts (Butala *et al*., 2021). The chemical tests demonstrated that all the plant extracts had a significant amount of flavonoids and terpenes. Besides this, the GC-MS revealed that the plant extracts had several classes of flavonoids, most of them being phthalic acids. Other studies have also proven that crude saponin extracts are highly toxic to animal cells and can be used in cancer treatment (Sobolewska *et al*., 2020; Alam *et al*., 2017). The chemical tests demonstrated high amounts of saponins. Although the GC-MS analysis could not reveal the actual saponins present, this class of phytochemicals could have contributed to the toxicity of the plant extracts. Vu *et al*. (2019) reported that several glycosides have cytotoxic activities. *T. indica* DCM extracts and *S. incanum* water extracts contained glycosides that could have also contributed to the toxicity of the two plant extracts.

Studies on different parts of *S. incanum* revealed that the plant contains cytotoxic phytochemicals. For example, an *in-vitro* toxicity analysis of *S. incanum* hydromethanol root and leaf extracts demonstrated that the extracts were toxic at 2 g/kg (Belayneh *et al*., 2021). Feyera *et al*. (2017) demonstrated that *S. incanum* fruit extracts induced sub-acute toxicity at 400 mg/kg dosages. Thaiyah *et al.* (2011) reported that *S. incanum* fruits were toxic to sheep. Most sheep treated with the extracts died due to brain congestion, damage to hepatocytes and kidney cells and lung emphysema. In addition, the researchers noted increased necrosis of neurons and Purkinje cells. Although no *in vivo* tests on *S. incanum* were identified, other studies have shown that plants of the genus *Solanum* have varying toxicity levels (Niño *et al*., 2006). *Solanum ovalifolium* had LD_{50} values of 1.00 mg/mL, *Solanum leucocarpun* (LD_{50} 0.44 mg/mL), *Solanum deflexiflorum* (0.68 mg/mL) and *Solanum lepidotum* (1.00 mg/mL) (Niño *et al*., 2006). The study linked the high toxicity of the extracts against brine shrimps to the high steroids, triterpenes and saponins present in the extracts. In this study, *S. incanum* DCM extract had LD_{50} of 1000 µg/mL, close to the value reported in other plants within the *Solanum* genus.

Other studies have shown varying toxicity of *T. indica* extracts. Nwondo *et al*. (2011) demonstrated that *T. indica* ethanol extracts had LD_{50} levels of between 832 µg/mL and 5 019 µg/mL. The LD_{50} values reported in this study are within the range reported by

Nwondo *et al*. (2011). Hasan *et al*. (2019) reported LD_{50} values of between 30 µg/mL and 100 µg/mL from *T. indica* seeds extracted using ethanol and methanol. The higher toxicity values could be due to the difference in the phytochemical present in the fruits and leaves of the extract. Shirisha and Varalakshmi (2017) showed that the toxicity of *T. indica* DCM bark extracts could help manage cancer. The researchers noted that the extracts induced apoptosis in PA-1 cells and HeLa cells. Besides this, the toxicity of various extracts of *T. indica* justifies its application in root canal treatment in endodontic procedures (Wulandari, 2008).

Researchers have identified several modes of actions linked with plant toxicity. For example, researchers reported that presence of ribosome inactivating metabolites interfere with rRNA N-glycosidase activity (Tumer, 2015). Inactivation of the enzyme affects translation process, which in turn causes cell death. Other studies have shown that some toxic metabolites interact with nucleic acid and biomembranes affecting their stability (Wink, 2015). Wink (2015) also

reported that most terpenoids and phenolics interact with receptors of various neurotransmitters, which affects their function. The metabolites interact with various cellular elements through ionic, hydrophobic and hydrogen bond. Besides, the toxicity of some plants is linked with presence of elements such as lead, arsenic, Cadmium and Aluminum (Brima, 2017). Brima (2017) explained that, due to the presence of toxic elements in medicinal plants, the WHO decided to formulate guidelines to ensure that the population is not exposed to higher concentration of the toxic elements per dose.

The toxicity tests of the plants reveal that all four extracts were toxic at different concentrations. Although cytotoxicity of plant extracts is associated with negative effects in the human body, such as damage to neurons, kidney and liver cells, the toxic compound can also be effective in managing cancer. Yu *et al*. (2017) reported that cytotoxic phytochemicals in *S. incanum* induced apoptosis of cancer cells and can be used to treat different types of cancer. The study also reported that the extracts could minimize cancer metastasis. On the other hand, Hussein *et al*. (2017) reported that *T. indica* seeds induced cell death in several cell lines at concentrations between 0.1-1000 µg/mL. Due to this, the researchers reported that the extracts could effectively manage different types of cancer.

CONCLUSION AND RECOMMENDATIONS

S. incanum and *T. indica* produced significant amounts of phytochemicals after water and DCM extraction. *S. incanum* had a higher yield compared to *T. indica*. The chemical tests for the phytochemicals revealed that the four extracts contained most phytochemicals tested. The extracts were more effective against gram-negative bacteria (*E. coli* and *S. typhi*) and least effective against gram-positive bacteria (*S. aureus*). The MIC and MBC values varied between various extracts. The MIC and MBC values against *S. aureus* were higher than the gram-negative bacteria. *T. indica* DCM extract was the most effective in stabilizing RBCs, while *S. incanum* water extract was the least effective. Regardless of the extraction mode, *T. indica* was more effective than *S. incanum*. In addition, DCM extracts were more effective than water extracts. All four extracts had varying toxicity levels. *S. incanum* water extract was more toxic than DCM extract, while *T. indica* DCM extract was more toxic than *T. indica* water extract.

Further studies should focus on fully identifying the phytochemicals present in the two plants. The studies can achieve this by using a specific standard for each phytochemical. Also, fractionation of various phytochemical classes to identify the actual class of phytochemicals with antibacterial, anti-inflammatory and cytotoxic effects followed by testing them against multi-drug resistant bacteria such as MRSA and resistant *E. coli* will improve scientific data on the two plants. Lastly, more studies should focus on using animal models for *in-vitro* testing of anti-inflammatory and cytotoxicity of the two plants.

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