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ORIGINAL RESEARCH ARTICLE

Phytochemical Profiling and Comparative Analysis of the Bioactive Compounds in *Adansonia digitata, Morinda citrifolia* and *Tamarindus indica* from Igabi Local Government Area, Kaduna State

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ABSTRACT

For decades, man has utilized plants as traditional medicine to cure various illnesses. The majority of modern medications now available have been generated from plants. This study subjected the ethanolic crude extract of *Adansonia digitata* stem bark, *Morinda citrifolia* leaves, and *Tamarindus indica* leaves to phytochemical analysis. Ultraviolet/visible spectrophotometric techniques were used to quantify the bioactive compounds detected in the plants. Qualitative analysis indicated the presence of alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids, terpenoids, reducing sugars, and quinones in all three plant extracts. Anthraquinones were detected only in *A. digitata*, while coumarins, though present in *M. citrifolia* and *T. indica,* were absent in *A. digitata*. *Adansonia digitata* recorded a higher content of anthraquinones (59.3 mg/g), phenols (79.3 mg/g), and tannins (286.1 mg/g) than *M. citrifolia* and *T. indica*. Alkaloids (45.5 mg/g), flavonoids (124.0 mg/g), saponins (38.7 mg/g), and triterpenes (23.8 mg/g) were found to be higher in *T. indica* than in *A. digitata* and *M. citrifolia*. Statistical analysis shows significant variation in the concentration of these compounds between the sampled plants ($p < 0.05$). The high bioactive content of *A. digitata* and *T. indica* indicates their potential as outstanding bioactive compound repositories suitable for drug development, purification, and functional food uses.

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INTRODUCTION

In developing nations, medicinal plants are deeply entwined with everyday existence. They provide not only alternatives to modern medicine but also vital contributions to the overall well-being of native communities. These plants are crucial and intricately linked to social and cultural practices that mark the inevitability of aging and mortality. Indigenous people have relied on medicinal plants for generations, using them traditionally to manage and cure a variety of ailments as well as infections (Agidew, 2022). Phytochemicals, also known as secondary metabolites, are naturally occurring compounds within plants that can have both beneficial and detrimental effects on health. Medicinal plants, used to combat various diseases and ailments, are rich sources of diverse phytochemicals. Its unique blend of phytochemicals determines the medicinal properties of any plant. These compounds are broadly categorized into two categories: the primary metabolites, which include proteins, carbohydrates, amino acids, and chlorophyll, in addition to secondary metabolites, which comprise phenolic chemicals, tannins, terpenoids, alkaloids, essential oils, and flavonoids (Thomaz *et al.*, 2018). These phytochemicals are strategically distributed throughout different parts of the plant (Shaikh & Patil, 2020). While phytochemicals may not directly influence the plant's growth, development, or reproduction, they play a crucial role in its survival. They mediate ecological interactions with competitors and protect the plant from external threats, including diseases, parasites, insects, herbivores, pollution, and UV radiation. Humans have long harnessed these phytochemicals for their medicinal properties, as flavoring agents, and even as recreational drugs (Sobuj *et al.*, 2024).

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Adansonia digitata (common name: baobab), a towering deciduous tree reaching heights of 20-30 meters and diameters of 2-10 meters, belongs to the family Malvaceae and is a familiar sight across many African nations. Various parts of this remarkable tree, including its leaves, fruit pulp, seeds, and bark fibers, have long been utilized for their medicinal and nutritional properties (Adeoye *et al.,* 2017). Traditionally, baobab has been utilized in the management of many illnesses such as measles, smallpox, fever, diarrhea, dysentery, hemoptysis, and as a pain reliever (Abubakar *et al*., 2015). Baobab leaves are also employed in sitz baths for the treatment of parasitic infestations. *A. digitata* seed pulp has a versatile range of uses. It can be applied topically or consumed in a drink to address issues related to the stomach, kidneys, and joints (Silva *et al*., 2023).

Morinda citrifolia (common name: noni), a tiny evergreen tree that is well-adapted to the unique conditions of seaside regions and volcanic landscapes, boasts a wide array of nutraceutical and therapeutic benefits. It belongs to the family Rubiaceae. Traditionally, it is used to cure a range of illnesses (Haritha *et al*., 2021). Noni offers a wealth of health advantages, including anti-bacterial, analgesic, anti-inflammatory, anti-cancer, and antioxidant activities, and it may strengthen the immune system. Furthermore, *M. citrifolia* leaf extracts have long been utilized to prevent and improve numerous chronic illnesses, such as those related to malaria, diabetes, hypertension, and damage from reactive oxygen species (ROS) (Zhu *et al*., 2020).

Tamarindus indica (common name: tamarind), belonging to the family Fabaceae, is an evergreen, long-lived tree reaching heights of 20-30 meters with a thick trunk. It is a vital multi-purpose sub-tropical fruit tree found in the subcontinent of India. It holds a significant place in folk medicine in many tropical nations, particularly Nigeria, Bangladesh, Pakistan, and India (Naeem *et al*., 2017). Traditionally, tamarind has been utilized to treat diarrhea, dysentery, stomach pain, helminthiasis, wounds, gonorrhea, malaria, fever, constipation, inflammation, and eye conditions. The tamarind tree is a rich source of phytochemicals and boasts numerous medicinal properties. From the roots to the tips of the leaves, every component of the tamarind plant is useful for human requirements (Bhadoriya *et al*., 2011).

Although the medicinal properties and phytochemical constituents of these plants have been studied individually in various locations across the globe with established data (Gaffo *et* al.,2022; Sam-ang *et al.,* 2020; Mohamadou *et al*., 2024), the phytochemical content of plants varies based on climatic and ecological conditions in different ecosystems. A comprehensive comparative analysis of the phytochemical content of these plants in Igabi, where most inhabitants rely on natural products as a primary source of medicine, remains understudied. Hence, the need to validate and compare the scientific data on the plants.

MATERIALS AND METHODS

Reagents used

The following analytical-grade chemicals and reagents were used: ethanol, chloroform, Benedict's reagent, Mayer's reagent, Dragendoff's reagent, picric acid, concentrated sulphuric acid, hydrochloric acid, acetic acid, ammonia (MOLYCHEM, Mumbai, India), magnesium ribbon, benzene, bromocresol green (SPECTRUM, New Jersey, USA), olive oil (HEMANI, Karachi, Pakistan), phosphate buffer solution, atropine powder, quercetin, sodium nitrite, aluminum chloride (EMSURE, Merck Darmstadt, Germany), vanillin, diosgenin, ursolic acid, anthracene, gallic acid, and anthracene (SIGMA-ALDRICH, Missouri, USA).

Plant Material Collection and Identification

Three (3) different types of plant were gathered and used in this study, namely, the stem bark of *Adansonia digitata,* leaves of *Morinda citrifolia,* and *Tamarindus indica* obtained from Igabi Local Government Area, within Kaduna State, Nigeria. The plants were adequately authenticated, and voucher specimens were placed in the Department of Biological Sciences herbarium at the Nigeria Defence Academy in Kaduna, Nigeria.

Preparation of Plant Samples

The different plant specimens obtained were properly cleaned with tap water to detach any adhering materials. To prevent potential deterioration or denaturation of their putative compounds, the various plant materials were allowed to air dry at ambient temperature (25-30^{0}C) away from direct sunlight. Using a mortar and pestle, the plant components that had been air-dried were pulverized into a coarse powder, which was subsequently aseptically ground using an electric grinder (Labaran *et al*., 2021). Afterward, the powdered plant materials were kept in airtight containers until further use.

Extraction of Plant Material

Extraction was done via the maceration method using 70% ethanol. Ethanol results in a higher yield of extracts and can extract a broad range of secondary metabolites, including both polar and non-polar compounds (Roka Aji *et al.,* 2020). The maceration method was selected for its simplicity, versatility, and efficiency in preserving the integrity of various compounds, including thermolabile compounds (Abubakar & Haque, 2020). The plant materials were put into a container and coated entirely with the solvent. To guarantee full extraction, the container (containing the plant material and solvent) was then sealed and left for 72 hours with periodic shaking and stirring. Following the extraction process, a filter paper-lined Büchner funnel was employed to filter the macerates. The debris after filtration was concentrated at 40^{0}C in an evaporator. To produce a solvent-free extract, the

concentrates were heated over a water bath, dried in a desiccator, weighed, labeled, and kept in the fridge until needed (Dagne *et al.,* 2021). The formula below was utilized to ascertain each extract's yield in percentage (%);

$$\textit{Percentage Yield (\%)} = \frac{(\text{Weight of Extract + Container} - \text{Weight of Container})}{\text{Weight of Dried Plant Material}} \times 100$$

Qualitative Phytochemical Screening

Secondary metabolites present in the plant materials were identified by qualitative phytochemical screening of the crude extracts for alkaloids, saponins, flavonoids, carbohydrates, tannins, terpenoids, phenolic compounds, quinones, anthraquinones, coumarins, reducing sugar, anthocyanins, steroids, and cardiac glycosides. All experiments were done in triplicates.

Test for Alkaloids

- *Dragendoff's Test*

Dragendoff's reagent (2 mL) was added to 2 mL of every plant extract. Observation of a reddish-brown precipitate demonstrated the existence of alkaloids (Shaikh & Patil, 2020).

-*Mayer's Test*

In a test tube containing 2 mL of each plant extract, 2 drops of Mayer's reagent were added along the sides of the test tube. The appearance of a creamy whitish precipitate indicated the presence of alkaloids (Shaikh & Patil, 2020).

-Picric Acid Test

Three drops of 2% picric acid solution were incorporated into 2 mL of every extract. An orange coloration indicates the existence of alkaloids (Shaikh & Patil, 2020).

Test for Cardiac Glycosides

Approximately 50 mg of every extract was hydrolyzed in a water bath using concentrated hydrochloric acid (HCl) for two hours. The hydrolysate was filtered and utilized in the subsequent tests;

-Keller-Killani Test

To 1 mL of each extract, glacial acetic acid (1.5 mL) was added, then a drop of 5% ferric chloride and a drop of concentrated H_2SO_4 was gently introduced by the side of the test tube. The development of a brown circle at the interface indicates the existence of deoxy sugars, a characteristic feature of cardenolides. It's also possible for a magenta-colored circle to be seen under the brown circle within the layer of sulfuric acid. Additionally, a green-colored circle can form directly above the brown circle in the layer of the acetic acid, gradually dispersing throughout the entire layer. This observation suggests the existence of cardiac glycosides (Sobuj *et al*., 2024).

-Bontrager's test

A 2 mL portion of the hydrolysate of each extract that has been filtered was combined with chloroform (3 mL) and vigorously shaken. Then, a 10% ammonia solution was added to the separated chloroform layer. The emergence of a pinkish color signifies the existence of glycosides (Banu & Cathrine, 2015).

Test for Saponins

- Frothing Test

Approximately 50 mg of every extract was combined with distilled water (3 mL) and vigorously shaken for 1 minute to produce a steady, long-lasting foam. The foam was then mixed with 3 drops of olive oil to observe the development of an emulsion. This emulsion formation shows the existence of saponins (Sobuj *et al*., 2024).

Test for Phenolic Compounds and Tannins

-Ferric Chloride Test

To approximately 50 mg of each extract, distilled water (5 mL) was added to dissolve it. Then, some drops of a solution of 5% ferric chloride were added. The development of a dark greenish coloration shows that phenolic compounds are present (Banu & Cathrine, 2015).

-Lead Acetate Test

Distilled water was added to around 50 mg of each extract to dissolve it. Next, 3 mL of lead acetate solution (10%) was added. The presence of phenolic compounds was revealed by the production of a creamy white precipitate (Banu & Cathrine, 2015).

Test for Steroids

- Salkowski's Test

Each extract (50 mg) was treated with 5 mL of chloroform, and the mixture was then filtered. The filtrate was treated with a small amount of concentrated sulfuric acid (H_2SO_4) and left undisturbed for several minutes. A few drops of concentrated H_2SO_4 were added to the filtrate, and the solution was allowed to stand for several minutes. The presence of steroids was confirmed by the development of a red color in the bottom layer (Shaikh & Patil, 2020).

-Libermann-Burchard's Test

Each extract (50 mg) was dissolved in 2 mL of acetic anhydride. Then, two drops of concentrated H_2SO_4 were

cautiously added to the test tube's side. The presence of steroids was indicated by a color change in the solution, transitioning from violet to either blue or green (Shaikh & Patil, 2020).

Test for Carbohydrates

Each extract, weighing approximately 50 mg, was dissolved in distilled water, filtered, and then used to conduct the following tests.

-Molisch's Test

To test for carbohydrates, approximately 2 mL of each extract's filtrate was combined with 2 drops of an alcoholic solution containing α-naphthol. After giving the mixture a good shake, a small amount of concentrated H_2SO_4 was gently added to the test tube's sidewalls. The presence of carbohydrates was verified by the formation of a violet ring at the liquid-liquid interface (Banu & Cathrine, 2015).

-Test for Starch

Approximately 5 mL of a 5% potassium hydroxide (KOH) solution and around 2 mL of the aqueous extract were combined. Starch can be detected by looking for a cinary coloration (Shaikh & Patil, 2020).

-Test for Pentoses

Approximately 2 mL of concentrated hydrochloric acid (HCl) in a test tube, a small quantity of phloroglucinol, and heat were introduced to 2 mL of each aqueous extract. The emergence of red color indicates pentoses (Shaikh & Patil, 2020).

Test for Flavonoids

-Shinoda's Test

Approximately 5 mL of alcohol was used to dissolve about 50 mg of each plant extract. Next, a small amount of concentrated HCl was added to the mixture after magnesium ribbon pieces were added. A pink-to-red solution will develop when flavonoids, also called flavonal glycosides, are present (Shaikh & Patil, 2020).

-Alkaline Reagent Test

A two percent sodium hydroxide (NaOH) solution (2 mL) was mixed with 1 mL of each extract and a few droplets of diluted hydrochloric acid. Flavonoids are characterized by a bright yellow color that becomes colorless upon the addition of diluted HCl (Shaikh & Patil, 2020).

Test for Terpenoids

-Salkowski's Test

About 3 mL of concentrated H_2SO_4 and 2 mL of chloroform were combined with each extract (5 mL). A reddish-brown coloration developing at the interface of the two liquids indicates that terpenoids are present in the sample extracts (Sobuj *et al.*, 2024).

Test for Quinones

-Concentrated Hydrochloric (HCl) Acid Test

In a test tube containing 2 mL of each test extract, a few droplets of concentrated HCl were applied along the side. Quinones are present when the resultant color turns green (Shaikh & Patil, 2020).

Test for Anthraquinones

-Bontrager's Test

Each plant extract was mixed with benzene (10 mL), left for 20 minutes to soak, then filtered. Next, 10% ammonia (10 mL) was added to the filtrate and shaken well for 30 seconds. A pink, violet, or reddish-colored solution appears when anthraquinones are present (Shaikh & Patil, 2020).

Test for Reducing Sugars

-Benedict's Test

Distilled water (5 mL) was used to dilute each extract (50 mg), which was then filtered. A 0.5 mL portion of the filtrate was combined with 0.5 mL of Benedict's reagent, and the mixture was heated for 2 minutes to a boil. According to Shaikh and Patil (2020), reducing sugars are present if a green, yellow, or reddish color is observed.

-Fehling's Test

The aqueous filtrate of each of the extracts was mixed with approximately 1 mL of Fehling's solutions A and B and then heated to a boil in a water bath. Reducing sugars are present when a red precipitate is observed (Shaikh & Patil, 2020).

Test for Coumarins

- Sodium Hydroxide (NaOH) Test

Some droplets of 10% NaOH were mixed with approximately 2 mL of the test extracts and chloroform. According to Shaikh and Patil (2020), the appearance of a yellow color denotes the presence of coumarins.

Test for Anthocyanins

-Hydrochloric (HCl) Acid Test

Approximately 2 mL of each test extract was combined with 2 mL 2N HCl, followed by a few droplets of ammonia. Anthocyanins are present when a pink-red solution is observed that turns blue-violet when ammonia is added (Shaikh & Patil, 2020).

Quantitative Phytochemical Analysis

Based on the preliminary qualitative screening of phytochemicals of the test extracts, the quantitative analysis of the phytochemicals detected was done to quantify the amount of some of the secondary metabolites using UV/VIS spectrophotometry.

Total Alkaloid Content (TAC) Estimation

The spectrophotometric approach was utilized to quantify the overall content of alkaloids. This technique relies on the interaction between bromocresol green (BCG) and alkaloids. To make the solution of BCG, approximately 69.8 mg of BCG was heated with 2N sodium hydroxide (3 mL) and distilled water (5 mL) until it dissolved fully. Then, 2M sodium phosphate (Na3HPO4) (71.6g Na3HPO4 in 1000ml of distilled or deionized water) was adjusted to a pH of 4.7 using 0.1N NaOH to create the phosphate buffer solution. To make a standard atropine solution stock of 1 mg/mL (1000µg/mL), pure atropine (10 mg) was dissolved in distilled water (10 mL). A series of precisely measured volumes (0.2, 0.4, 0.6, 0.8, and 1.0 mL) of the standard atropine solution were dispensed into separate funnels, corresponding to concentrations of 20, 40, 60, 80, and 100 µg/ml, respectively. Afterward, BCG (5 mL), as well as phosphate buffer solutions (5 mL), were incorporated into the individual separating funnels and agitated sequentially with different amounts of chloroform (1, 2, 3, and 4 mL). The extracts were collected in 10 mL volumetric flasks and diluted with chloroform to modify the volume to 10 mL. About 1 mg of each test sample was then dissolved with 1 mL of 2N HCl, which was then filtered. After moving this solution to a separating funnel, additions were made of 5 mL of BCG solution and 5 mL of phosphate buffer. Using 1, 2, 3, and 4 mL of chloroform, the formed complex was extracted after the mixture was thoroughly shaken. The extract was collected in a 10mL volumetric flask and diluted using chloroform. Using an ultraviolet-visible light (UV-VIS) spectrophotometer, the optical density of the samples, as well as the standard atropine solution, was determined at 470 nm. The atropine standard curve was created using the optical density generated by the atropine standard solutions. With the aid of the atropine standard curve, milligrams of atropine equivalent per gram (mg AE/g) of plant extract were used to calculate the total alkaloid content of each plant extract. All experiments were carried out in triplicates (Otitolaiye *et al.*, 2023).

Total Flavonoid Content (TFC) Estimation

A colorimetric assay method using aluminum chloride was utilized to establish the total flavonoid content in the test extracts. Quercetin standard stock solutions (1 mg/mL) for calibration were made by adding 10 mL of methanol to 10 mg of quercetin. A series of dilutions were performed on the standard solutions, resulting in a range of concentrations: 20, 40, 60, 80, and 100 µg/mL. Then, 1 mL of each quercetin concentration was added to a test tube holding distilled water (4 mL). Simultaneously, additions of 0.3 mL each of 10% sodium nitrite ($NaNO_2$) and aluminum chloride ($AlCl_3$) were made to the test tube, followed by NaOH (1 mL) after 6 minutes. Then, distilled water (4.4 mL) was added immediately, bringing the mixture's volume to 10 mL. The product was then well-mixed and left to stand for an additional 15 minutes. The test samples were made similarly, with each sample (1 mg) added to methanol (1 mL). Using an ultraviolet-visible light (UV-VIS) spectrophotometer, measurements of the optical density generated by the standard quercetin solutions and each sample were done. All experiments were performed three times. The optical density of the quercetin standard solution was used to create the standard quercetin curve for calibration. Based on the standard curve of quercetin, the milligram quercetin equivalent per gram (mg QE/g) was used to represent the total flavonoid concentration of each plant extract (Phuyal *et al.*, 2020).

Total Saponin Content (TSC) Estimation

To determine the total saponin content, the vanillin-sulfuric acid method was employed. Individual test tubes containing 0.25mL each of the extract or standard (1 mg/mL Diosgenin) at varying concentrations were prepared by adding 0.25 mL of 8% vanillin solution followed by 2.5 mL of 72% H_2SO_4. After fully combining the mixtures, they were incubated at 60^0C for 15 minutes. Following incubation, the combination (standard and test extract) was allowed to cool. The optical density of each sample, as well as the diosgenin standard solutions, were measured with a UV-VIS spectrophotometer at 560 nanometers. Every determination was made three times. The diosgenin absorbance values were used to create the standard calibration curve. Each extract's total saponin concentration was reported as mgDE/g or milligrams of diosgenin equivalent per gram of extract (Otitolaiye *et al.*, 2023).

Total Triterpenoid Content (TTC) Estimation

Approximately 1 mL of a 5% vanillin-acetic acid solution, 1.8 mL of sulphuric acid, and 200µL of the extract solution (1 mg/mL in glacial acetic acid) were mixed to evaluate the total triterpenoid concentration. After 30 minutes of heating to 70^0C in an ice-water bath, the reaction mixture was cooled to room temperature. Glacial acetic acid (2 mL) was then added. As standards, ursolic acid (in methanol) at 20, 40, 60, 80, and 100 µg/mL were

used. The optical densities of the extract/standard reaction mixtures were measured at 573 nm with a UV-VIS spectrophotometer. The standard curve for calibration was created using the optical density of the ursolic acid (UA) standard solution. Based on the ursolic acid calibration curve, each extract's total triterpenoid content was estimated as milligram ursolic acid equivalents per gram (mgUA/g) (Kamonlakorn *et al.*, 2020).

Total Anthraquinone Glycoside Content (TAGC) Estimation

The Bornträger-reaction test was utilized to determine the anthraquinone glycoside concentration in the test samples. First, 1.0 mL of a 10% sulphuric acid solution was combined with 1.0 mL of each test sample/standard (anthracene, 1 mg/mL). After giving the mixture a quick stir, the mixture was submerged for five minutes in hot water. Next, insoluble components were then filtered out, after which the mixture was cooled to normal temperature. Following filtration, 0.5 mL of 10% ammonia (NH_3) was added to the filtrate and swirled. Anthraquinone compound detection was characterized by the emergence of a pinkish-red color (Sakulpanich & Gritsanapan, 2009). To determine the anthraquinone glycoside content, 25 mg of each extract was diluted in 3 mL of 0.5N potassium hydroxide (KOH), and then 7 mL of 3% hydrogen peroxide (H_20_2) was added. In a water bath, the solution was heated to a boil, left to cool, and filtered. After adding ten drops of acetic acid to the mixture, the pH was measured using filter paper. Approximately 15 mL of chloroform was then utilized to filter and extract the sample solution. Next, the solution of the sample was split into two separate tubes, one as a control sample and the other as a test sample. Following the addition of ammonia (NH3) to the test sample, the tube was gently shaken. When anthraquinone glycoside was present, the color turned pinkish-red. The dilution factor of each extract was compared to the standard anthraquinone solution (anthracene) with a UV-VIS spectrophotometer at 515 nm to estimate the anthraquinone glycoside concentration (Khoomsab & Khoomsab, 2019).

Total Phenolic Content (TPC) Estimation

The Folin-Ciocalteu method was utilized to determine the total phenol content of each plant extract, with gallic acid serving as a standard. Initially, each extract (1 mL), distilled water (9 mL), and Folin-Ciocalteu phenol reagent (1 mL) were combined and thoroughly mixed. Then, 5 minutes later, 7% sodium carbonate solution (10 mL) was added to the mixture. Gallic acid standard solutions were made at varying concentrations (20, 40, 60, 80, and 100 µg/mL). Following a 90-minute room temperature incubation period, the optical densities of the test and standard solutions were measured at 550 nm using a UV-VIS spectrophotometer. The experiment was done in triplicates. The standard curve for calibration was created by making use of the optical density generated by the gallic acid solution. Based on the gallic acid standard curve, each extract's total phenol content was expressed as milligrams of gallic acid equivalent per gram of plant extract (mgGAE/g) (Lahare *et al.*, 2021).

Total Tannin Content (TTC) Estimation

Each plant extract's total tannin concentration was determined using the Folin-Ciocalteu method, with gallic acid acting as a standard. Approximately 0.5 mL of Folin reagent was added after about 1 mL of each plant extract had been dissolved in 7.5 mL of distilled water. Next, 1.0 mL of 35% sodium carbonate (Na_2CO_3) was added, and 10 mL of distilled water was used to dilute the mixture. After giving the reaction mixture a good shake, it was left to stand at room temperature for 30 minutes. A series of reference solutions of gallic acid standards with concentrations of 20, 40, 60, 80, and 100 µg/mL were prepared. By utilizing a UV-VIS spectrophotometer, the optical densities of the test and standard solutions were estimated at a wavelength of 725 nm. The experiment was done in triplicates. The optical density of the standard gallic acid solution was used to produce the standard curve for calibration. Each extract's total tannin content was expressed as milligrams of gallic acid equivalent per gram of plant extract (mgGAE/g) based on the standard gallic acid curve for calibration (Lahare *et al.*, 2021).

Data Analysis

Statistical analysis of the collected data was performed using the Statistical Package for the Social Sciences (SPSS version 25.0). Analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare the mean results. Data are presented as the mean ± standard error of the mean (SEM). Differences were considered statistically significant when the p-value was less than 0.05.

RESULTS

Plant Material Extraction

The plant materials were successfully extracted using 70% ethanol as the solvent via the maceration method. The percentage yield and organoleptic properties of each of the ethanolic crude extracts of *A. digitata, M. citrifolia*, and *T. indica* are shown in Table 1. From the results, *M. citrifolia* crude extract exhibited the highest percentage yield at 23.6%, followed by *T. indica* with 15.8%, while *A. digitata* had the lowest yield at 5.6% (Figure 1).

Phytochemical Screening

The presence of a variety of secondary metabolites, such as alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids, terpenoids, reducing sugars, and quinones, was discovered through qualitative phytochemical screening of the crude extracts of *A. digitata, M. citrifolia*, and *T. indica*. However, anthraquinones were detected in *A. digitata* only, while

coumarins were absent in *A. digitata* but present in both *M. citrifolia* and *T. indica* (Table 2).

Quantitative Phytochemical Analysis

The crude ethanol extracts of *A. digitata, M. citrifolia,* and *T. indica* were subjected to quantitative phytochemical analysis using UV/VIS spectrophotometry, which revealed the estimated quantities of various secondary metabolites extracted from these plants.

Total Alkaloid Content (TAC) Estimation

The mean total alkaloid contents varied significantly ($p < 0.05$) between the three sampled plants, with *T.indica* recording the highest content of 45.5 ± 0.06 mg/g (4.6%), followed by *A. digitata* with 37.9 ± 0.02 mg/g (3.8%), and *M. citrifolia* with 45.5 ± 0.06 mg/g (4.6%) (Figure 2). The atropine calibration curve used as a standard for estimating the total alkaloid concentration is shown in Figure 3.

Total Flavonoid Content (TFC) Estimation

There was a significant variation ($p < 0.05$) in the mean total flavonoid concentration of each of the plant extracts, with *A. digitata, M. citrifolia,* and *T. indica* having a mean content of 100.4 ± 0.77 mg/g (10.0%), 70.5 ± 1.07 mg/g (7.1%), and 124.0 ± 1.09 mg/g (12.4%), respectively as shown in Figure 4. The quercetin calibration curve used as a standard for evaluating the total flavonoid concentration is shown in Figure 5.

Total Saponin Content (TSC) Estimation

The total saponin content of all the examined extracts is shown in Figure 6. With a concentration of 38.7 ± 0.66 mg/g (3.9%), *T. indica* exhibited the highest saponin content, followed by *A. digitata* 31.4 ± 0.89 mg/g (3.1%) and *M. citrifolia* 23.1 ± 0.46 mg/g (2.3%). The diosgenin calibration curve used as a standard for estimating the total saponin concentration is shown in Figure 7.

Total Triterpene Content (TTC) Estimation

The analysis of the total triterpene content of the plants examined revealed a comparable mean content with no significant difference ($p < 0.05$) (Figure 8). *Adansonia digitata, Morinda citrifolia*, and *Tamarindus indica* exhibited values of 23.0 ± 0.15 mg/g (2.3%), 22.5 ± 0.12 mg/g (2.3%), and 23.8 ± 0.07 mg/g (2.4%), respectively. The ursolic acid calibration curve used as a standard for estimating the total triterpene concentration is shown in Figure 9.

Total Anthraquinone Content (TAC) Estimation

Qualitative phytochemical analysis revealed the presence of anthraquinones exclusively in the extract of *A. digitata.* The subsequent quantitative analysis determined the total anthraquinone content of *A. digitata* to be 59.3 ± 0.03 mg/g (5.9%), as shown in Figure 10. The anthracene calibration curve used as a standard for estimating the total anthraquinone concentration is shown in Figure 11.

Total Phenolic Content (TPC) Estimation

As indicated in Figure 12, the mean total phenolic content varied significantly ($p < 0.05$) between the three sampled plants with *A. digitata* recording the highest phenolic content of 79.3 ± 1.21 mg/g (7.9%), followed by *M. citrifolia* and *T. indica* with 14.5 ± 0.53 mg/g (1.5%), and 21.9 ± 0.04 mg/g (2.2%), respectively. The gallic acid calibration curve used as a standard for estimating the total phenol and tannin concentration is shown in Figure 13.

Tannin Content (TTC) Estimation

The total tannin content, as determined by our analysis, was found to be 286.1 ± 2.06 mg/g (2.9%) for *A. digitata,* 37.7 ± 1.08 mg/g (3.8%) for *M. citrifolia*, and 64.4 ± 0.95 mg/g (6.4%) for *T. indica.* These results indicate that *A. digitata* exhibited a significantly higher ($p < 0.05$) tannin content among the three plants, followed by *T. indica*, while *M. citrifolia* had the lowest tannin content (Figure 14).

Table 1: Organoleptic properties and percentage yield of the 70% ethanolic extracts of *A. digitata, M. citrifolia*, and *T. indica*

Plant Extract	Part used	Color/ Appearance	Odour	Initial weight of plant material (g)	Weight of Extract (g)	Yield (%)	Voucher number
A. digitata	Stem bark	Reddish brown/ powdery	Earthy slightly sweet	508.3	28.3	5.6	NDA/BIOH/2024/04
M.citrifolia	Leaves	Dark green/ viscous	Pungent slightly unpleasant	108.7	25.6	23.6	NDA/BIOH/2024/05
T. indica	Leaves	Dark brown/ viscous	Earthy slightly astringent	304	47.9	15.8	NDA/BIOH/2024/06

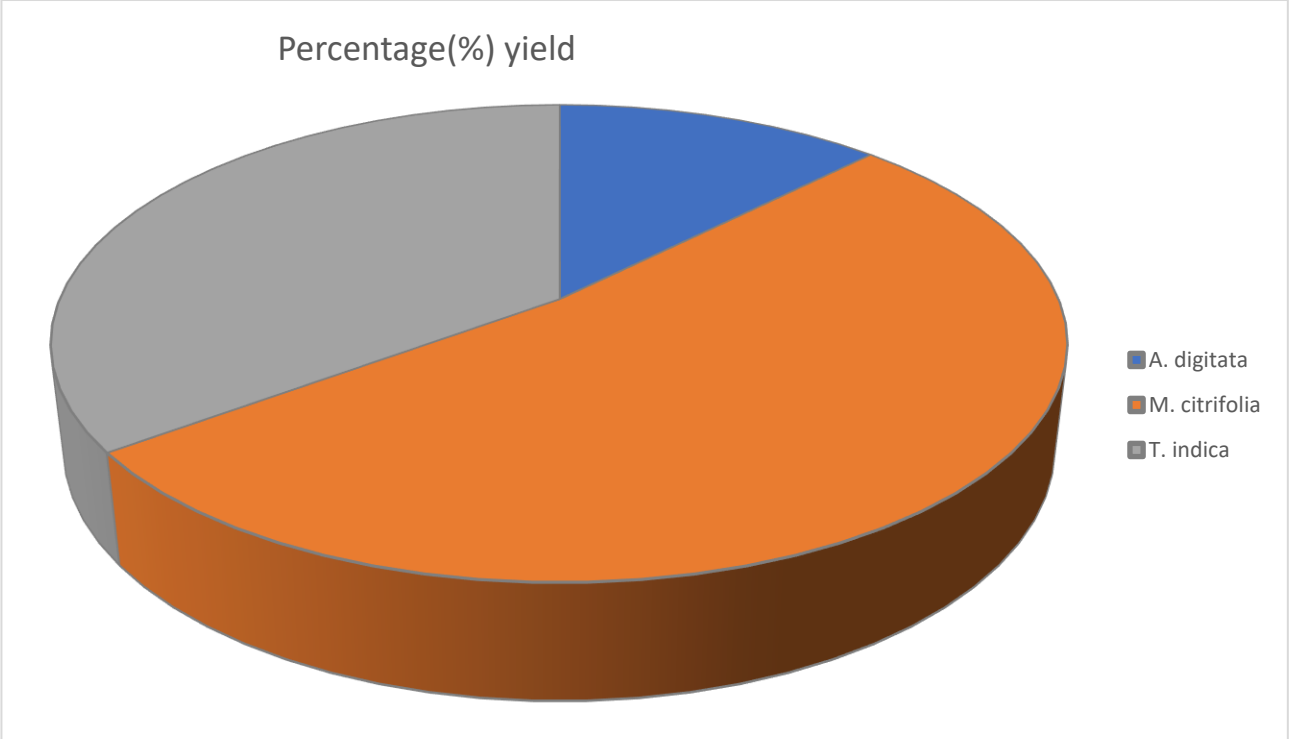

Figure 1: Percentage yield of 70% ethanolic crude extracts of *A.digitata, M. citrifolia* and *T. indica*

Table 2: Qualitative phytochemical components of 70% ethanolic crude extracts of *A. digitata, M. citrifolia,* and *T. indica*

Phytoconstituents	Test	ADE	MCE	TIE
Alkaloids	-Dragendorff test	+	+	+
	-Picric acid test	+	+	+
	-Meyer's test	+	+	+
Cardiac Glycosides	-Keller-Kiliani test	+	+	+
	-Bontrager's test	+	-	-
Saponins	-Frothing test	+	+	+
Phenolic compounds and tannins	-Lead acetate test	+	+	+
	-Ferric chloride test	+	+	+
Steroids	-Salkowiski test	+	+	+
	-Liebermann-burchard's test	+	-	-
Carbohydrates	-Molisch's test	+	+	+
	-Test for starch	-	+	+
	-Test for pentoses	+	+	+
Flavonoids	-Shinoda's test	+	+	+
	-Alkaline reagent test	+	+	+
Terpenoids	Salkowski's test	+	+	+
Anthraquinones	Bontrager's test	+	-	-
Reducing sugar	-Benedict's test	+	-	-
	-Fehling's test	+	-	-
Coumarins	NaOH test	-	+	+
Quinones	Conc. HCl test	+	+	+
Anthocyanins	HCl test	+	+	+

Key: + = Present; - = Absent; ADE = *Adansonia digitata* ethanolic extract; MCE = *Morinda citrifolia* ethanolic extract; TIE – *Tamarindus indica* ethanolic extract

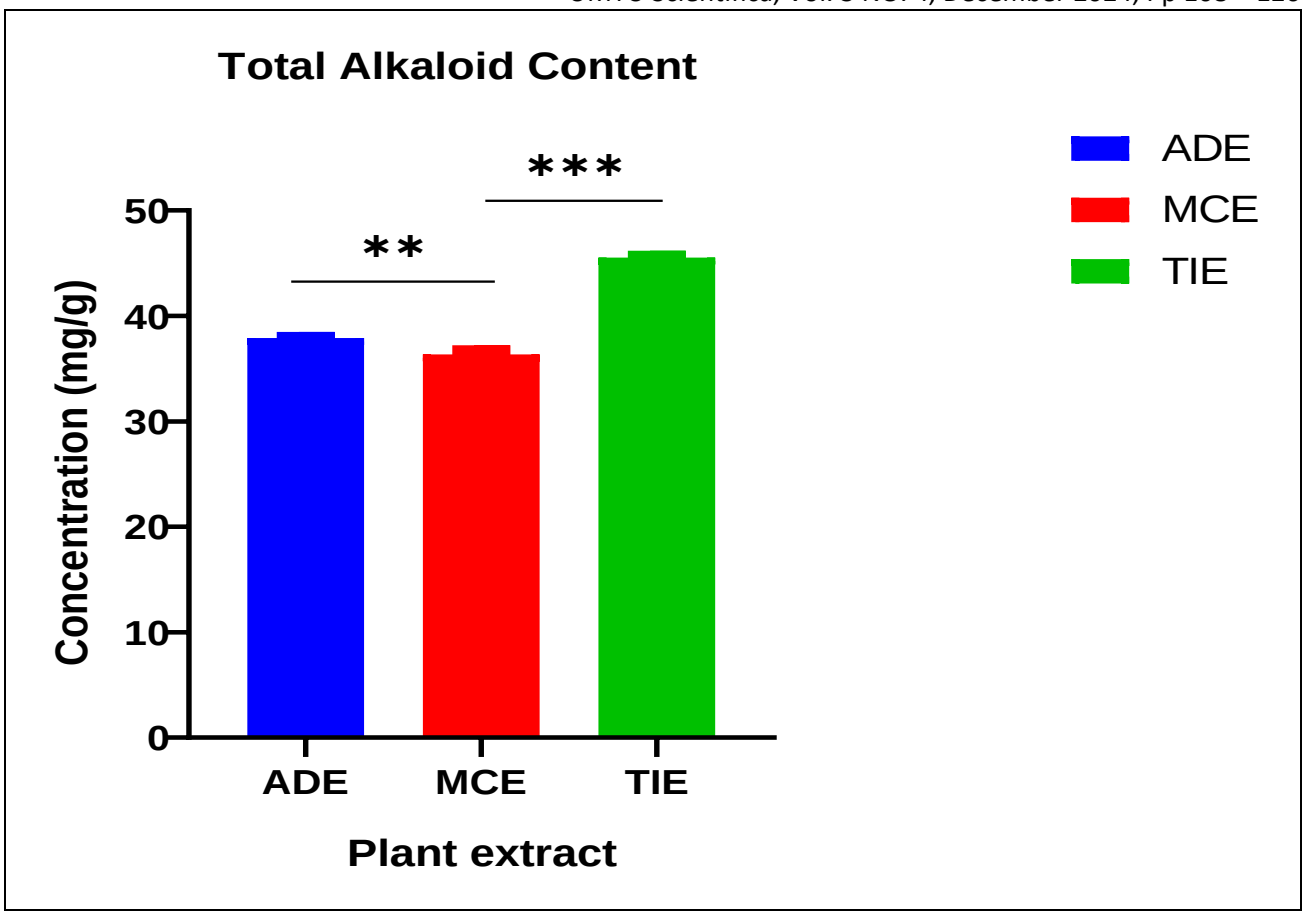

Figure 2: Total alkaloid content of *A. digitata, M.citrifolia* and *T. indica*
** indicates a statistically significant difference ($p < 0.05$) at a 95% confidence interval*

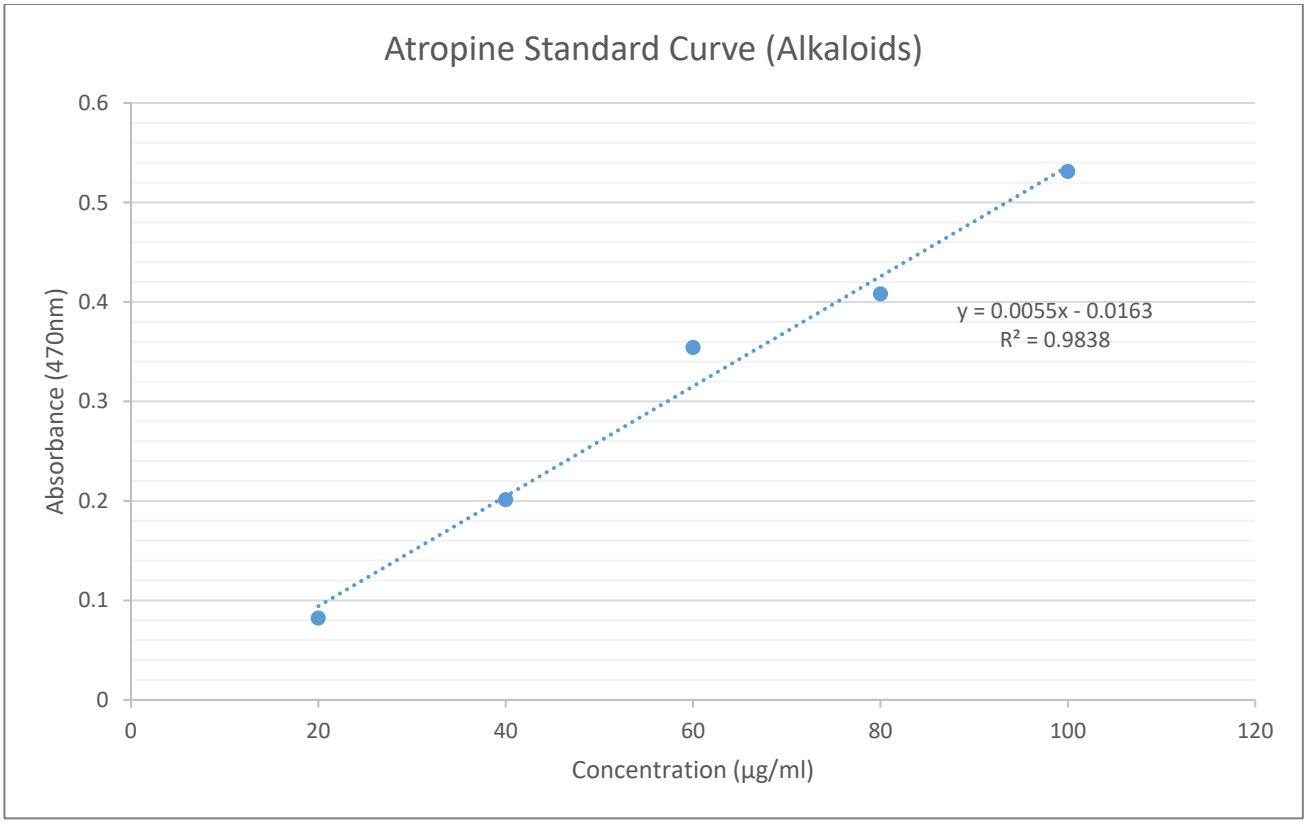

Figure 3: Atropine standard curve for calibration used for total alkaloid estimation

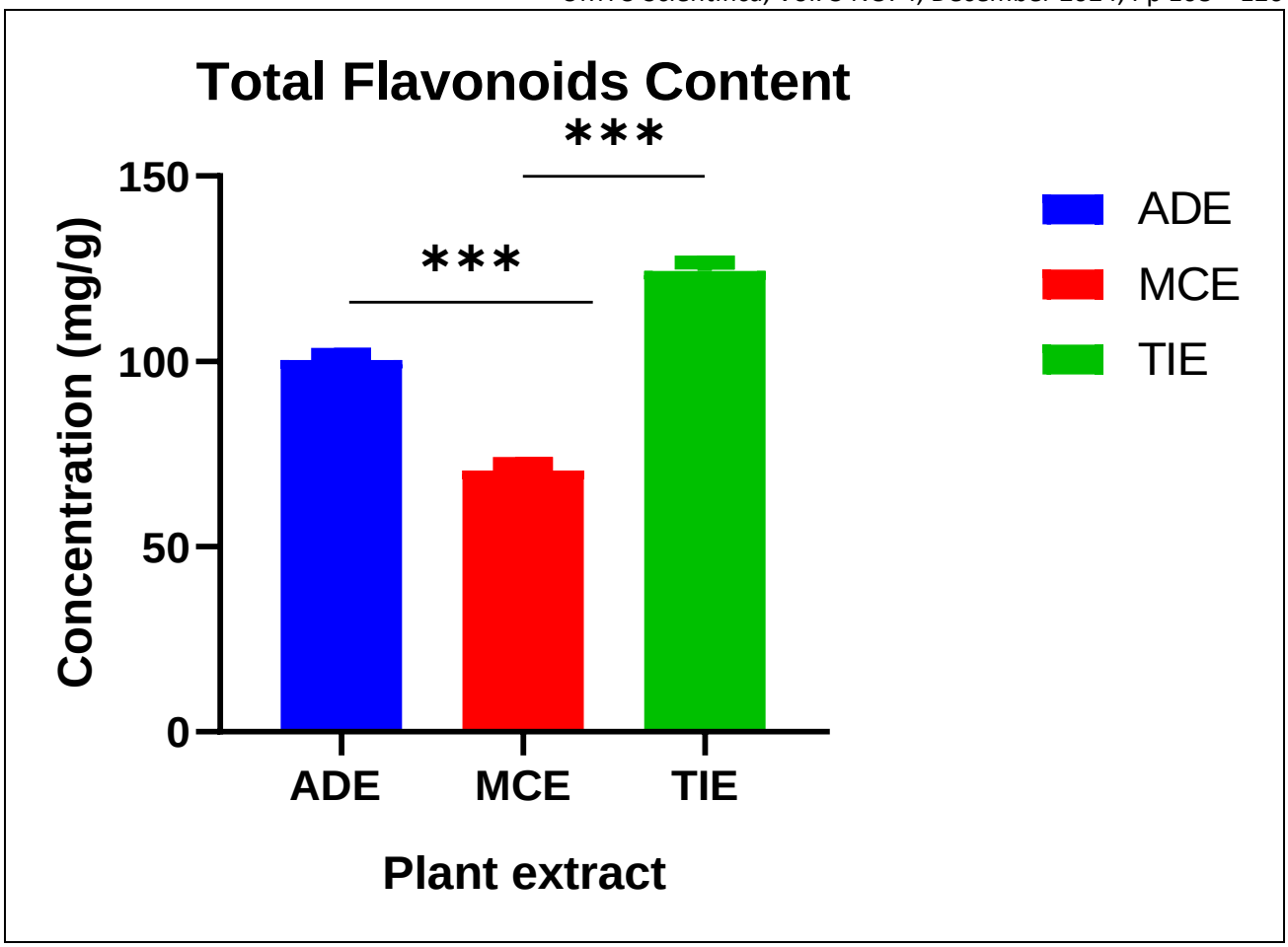

Figure 4: Total flavonoid content of *A. digitata, M.citrifolia* and *T. indica*
** indicates a statistically significant difference ($p < 0.05$) at a 95% confidence interval*

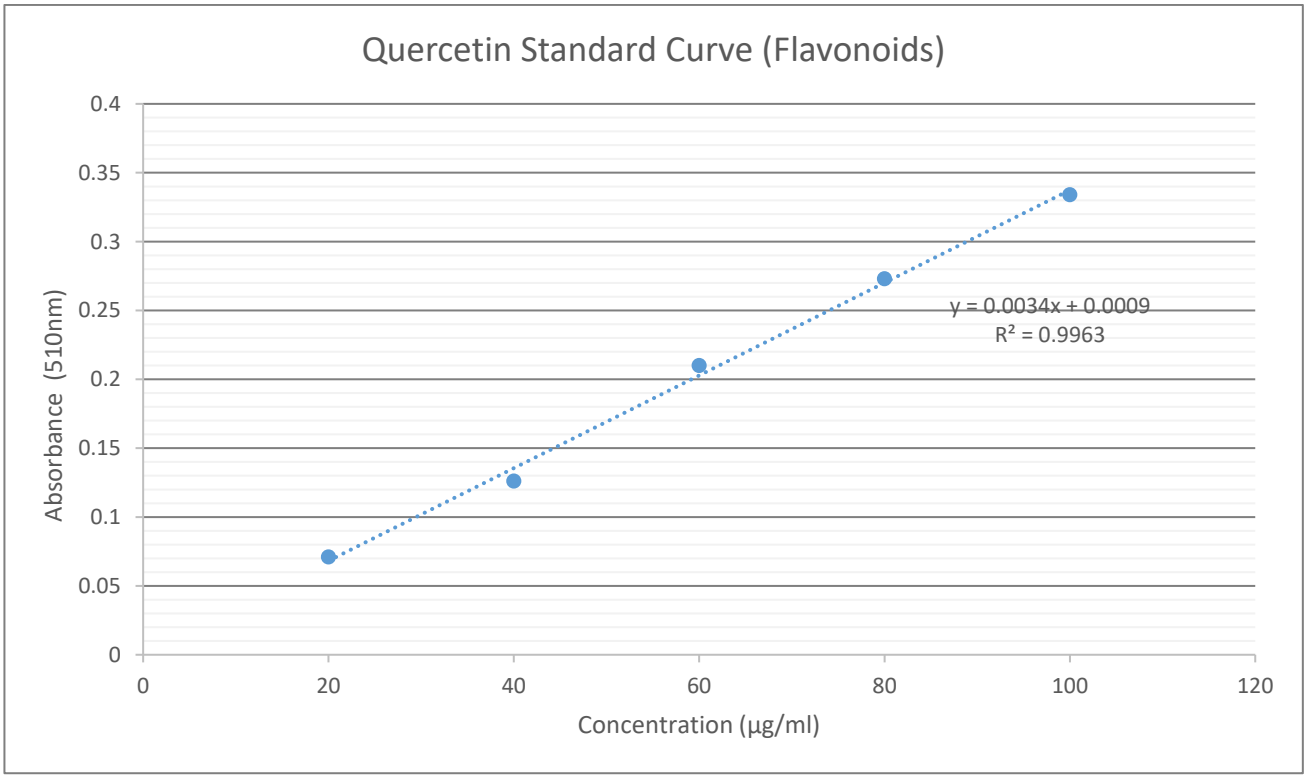

Figure 5: Quercetin standard curve for calibration used for total flavonoid estimation

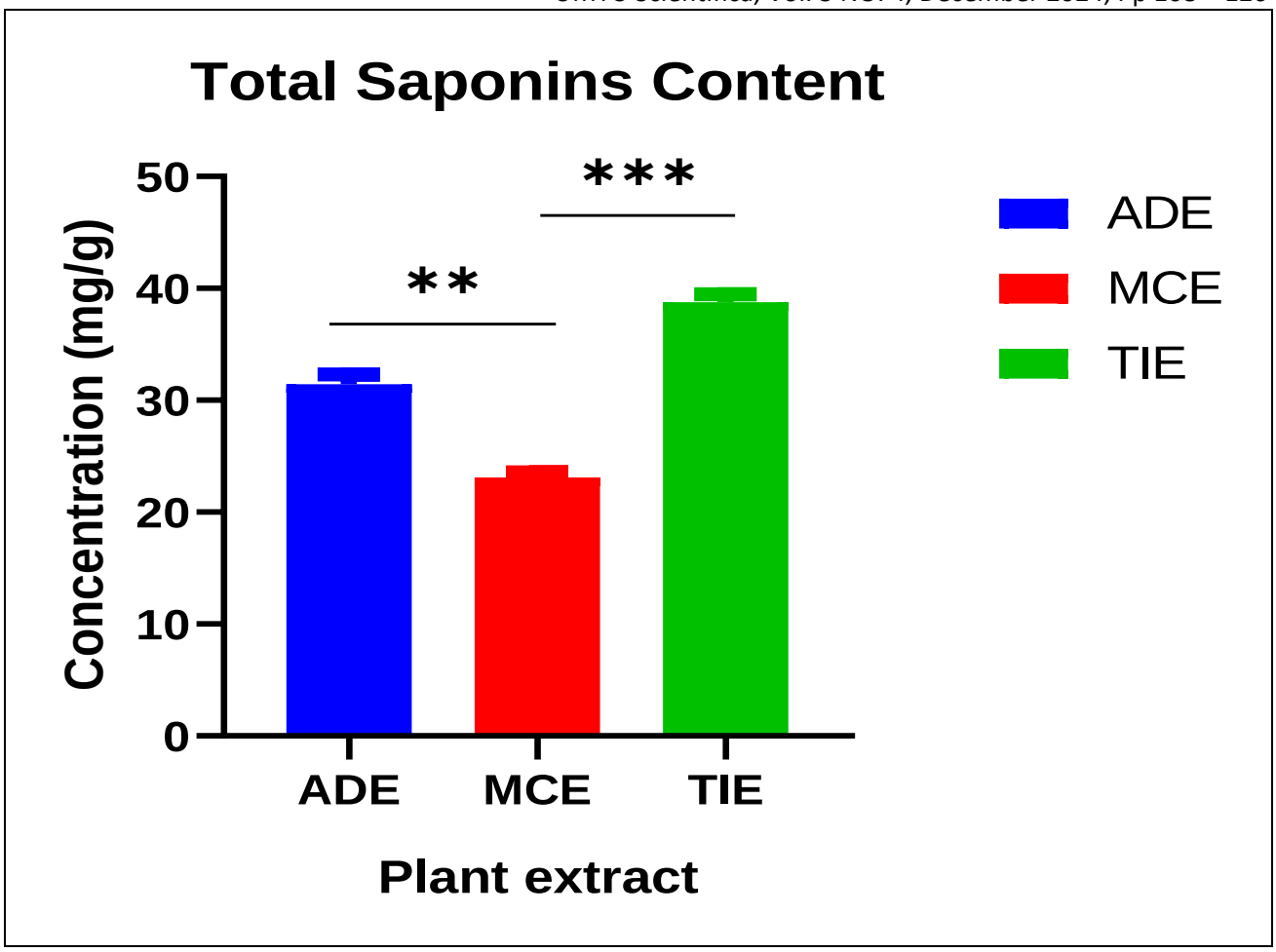

Figure 6: Total saponin content of *A. digitata, M.citrifolia* and *T. indica*
** indicates a statistically significant difference ($p < 0.05$) at a 95% confidence interval*

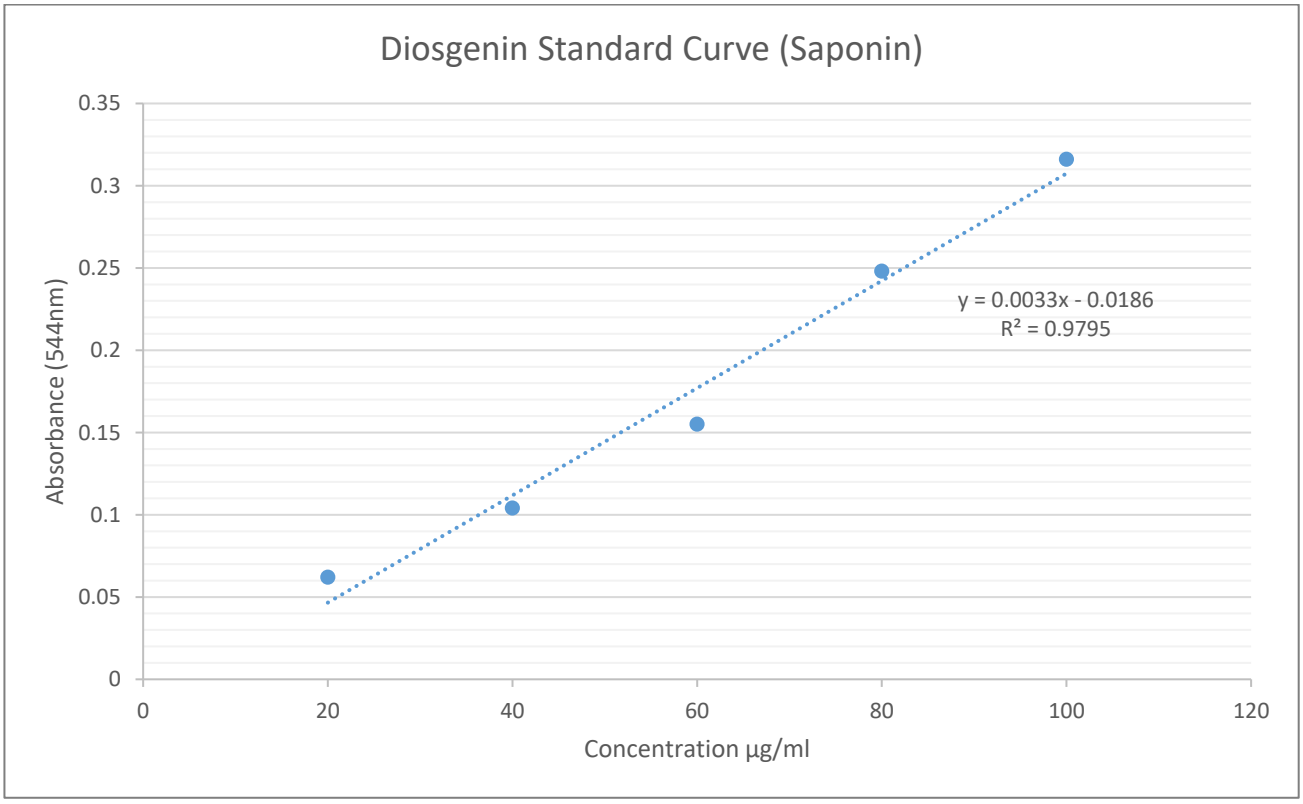

Figure 7: Diosgenin standard curve for calibration used for total saponin estimation

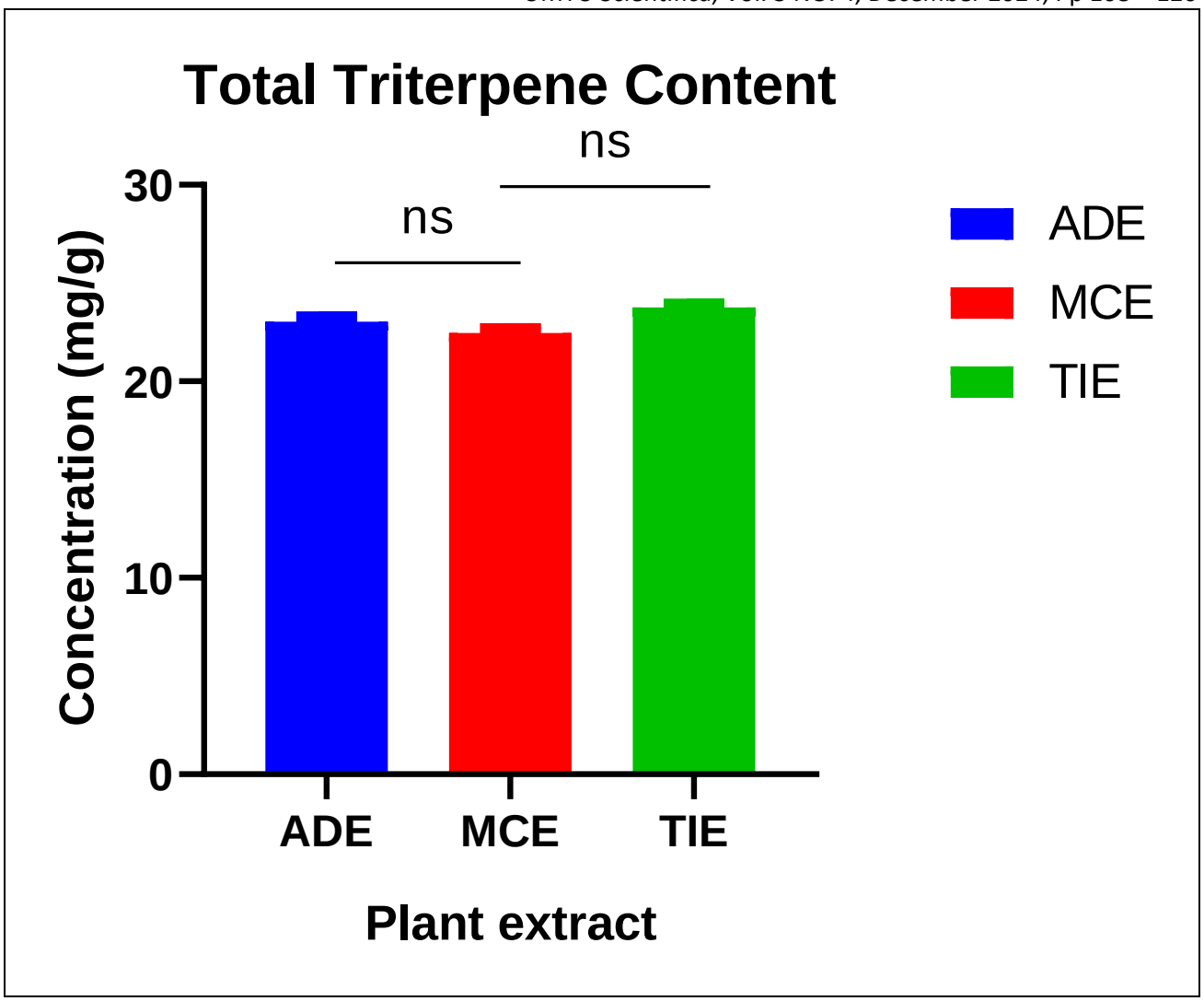

Figure 8: Total triterpene content of *A. digitata, M.citrifolia* and *T. indica*
ns indicates no statistically significant difference ($p < 0.05$) at a 95% confidence interval

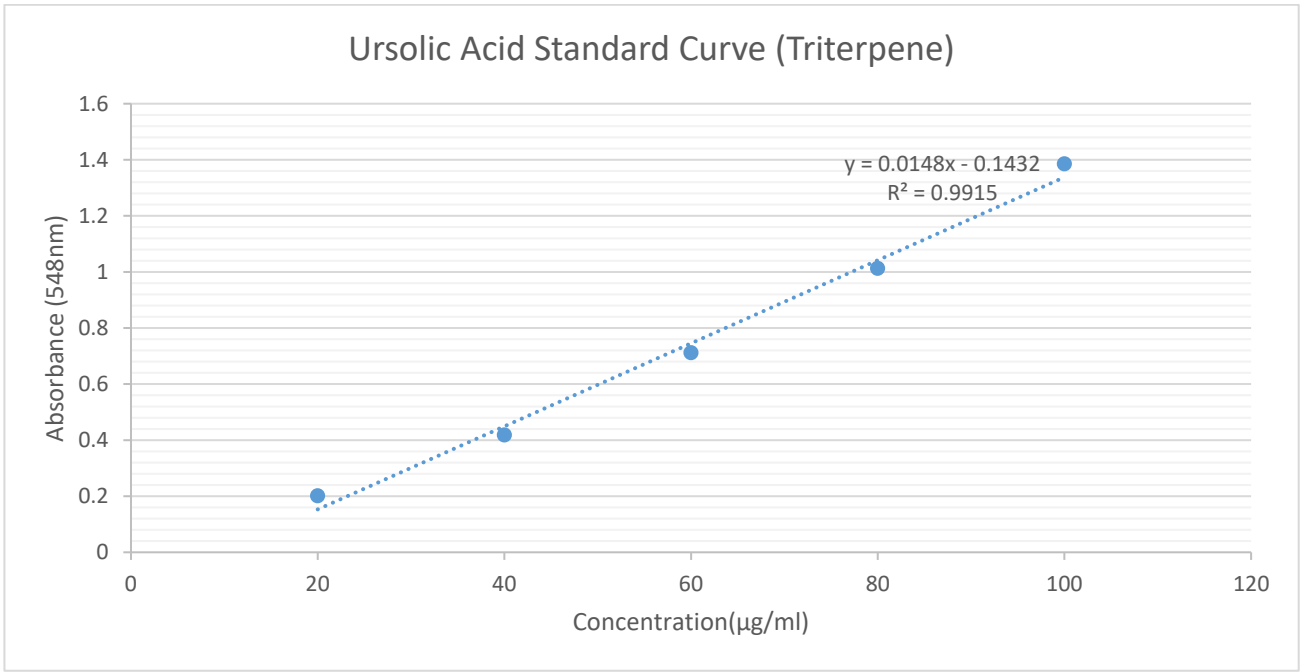

Figure 9: Ursolic acid standard curve for calibration used for total triterpene estimation

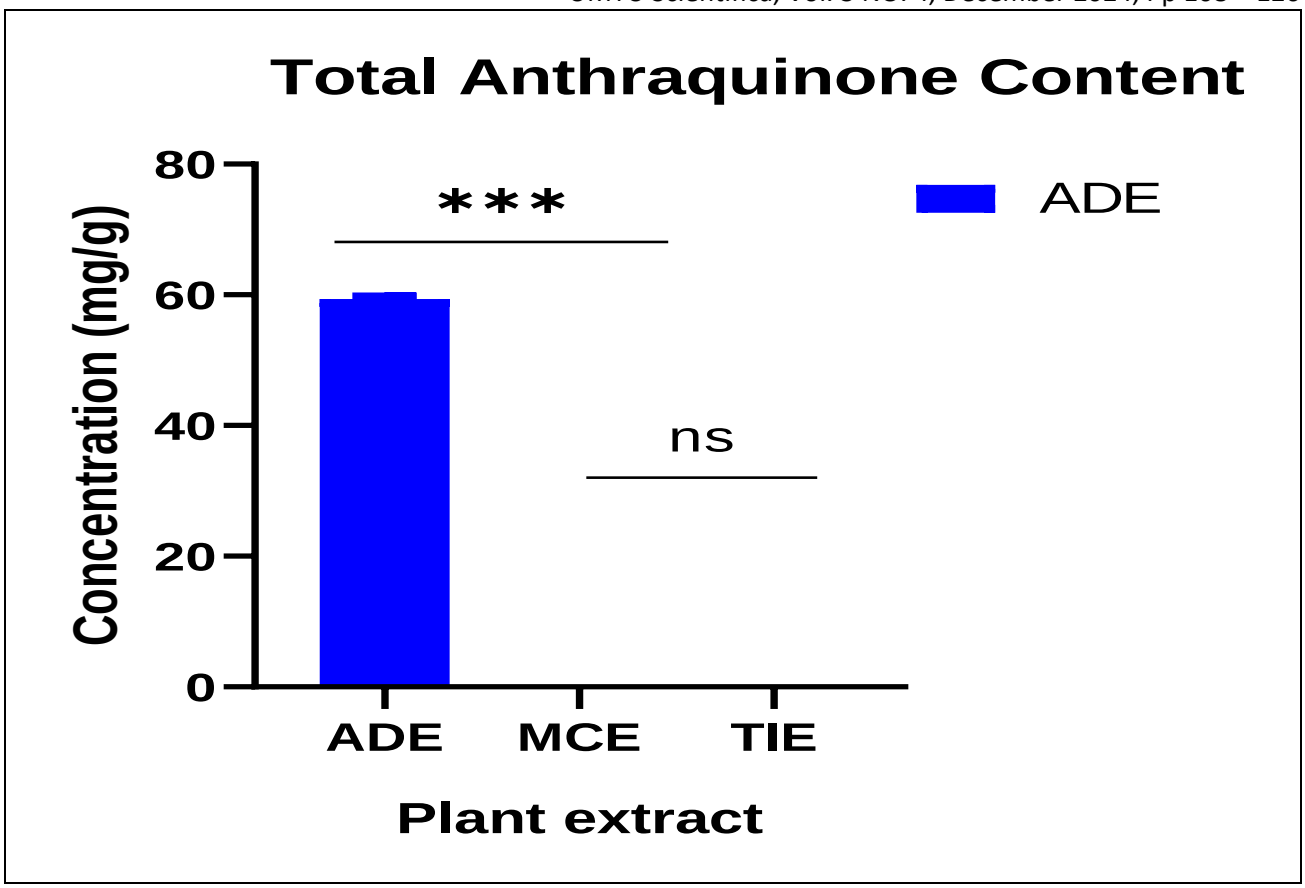

Figure 10: Total anthraquinone content of *A. digitata, M.citrifolia* and *T. indica*
** indicates a statistically significant difference ($p < 0.05$) at a 95% confidence interval*

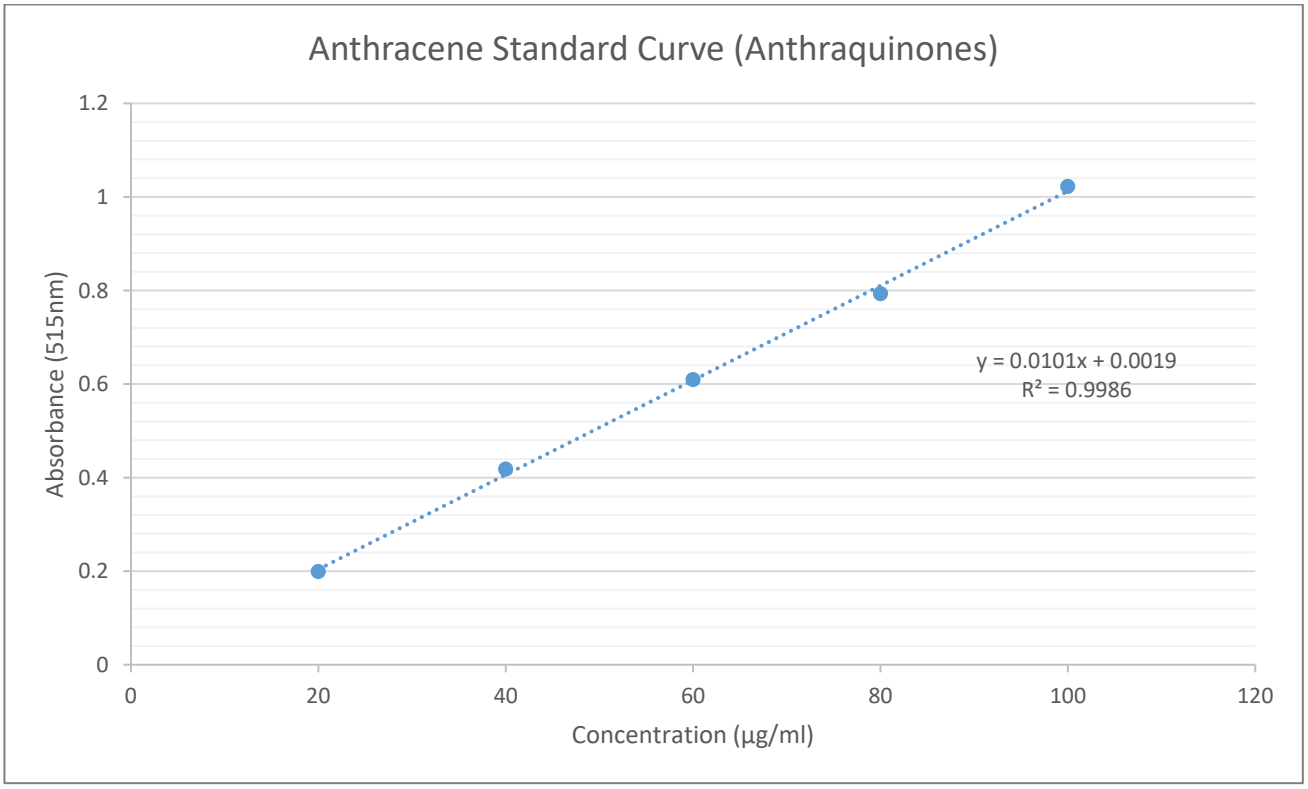

Figure 11: Anthracene standard curve for calibration used for total anthraquinone estimation

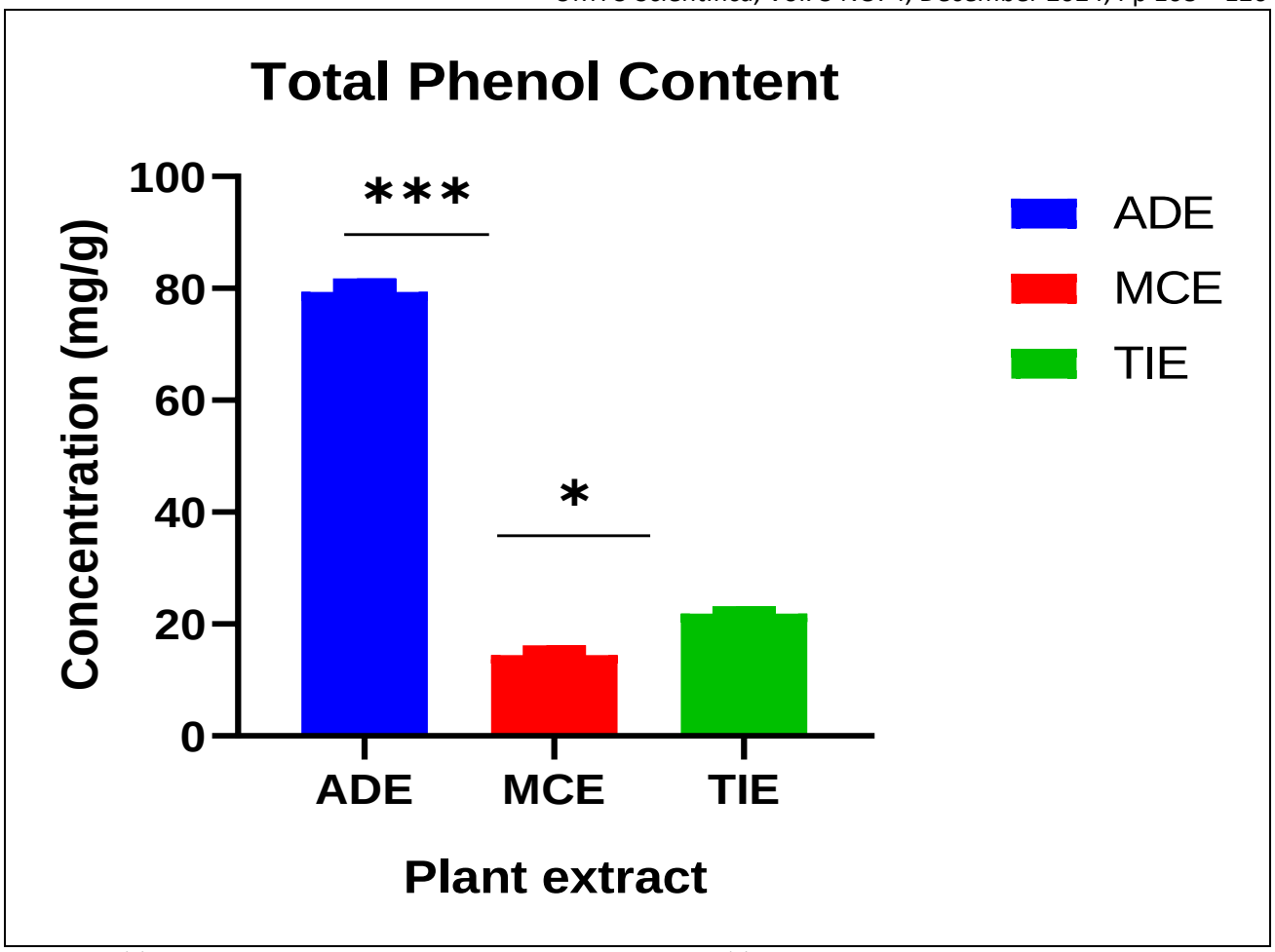

Figure 12: Total phenol content of *A. digitata, M.citrifolia* and *T. indica*
** indicates a statistically significant difference ($p < 0.05$) at a 95% confidence interval*

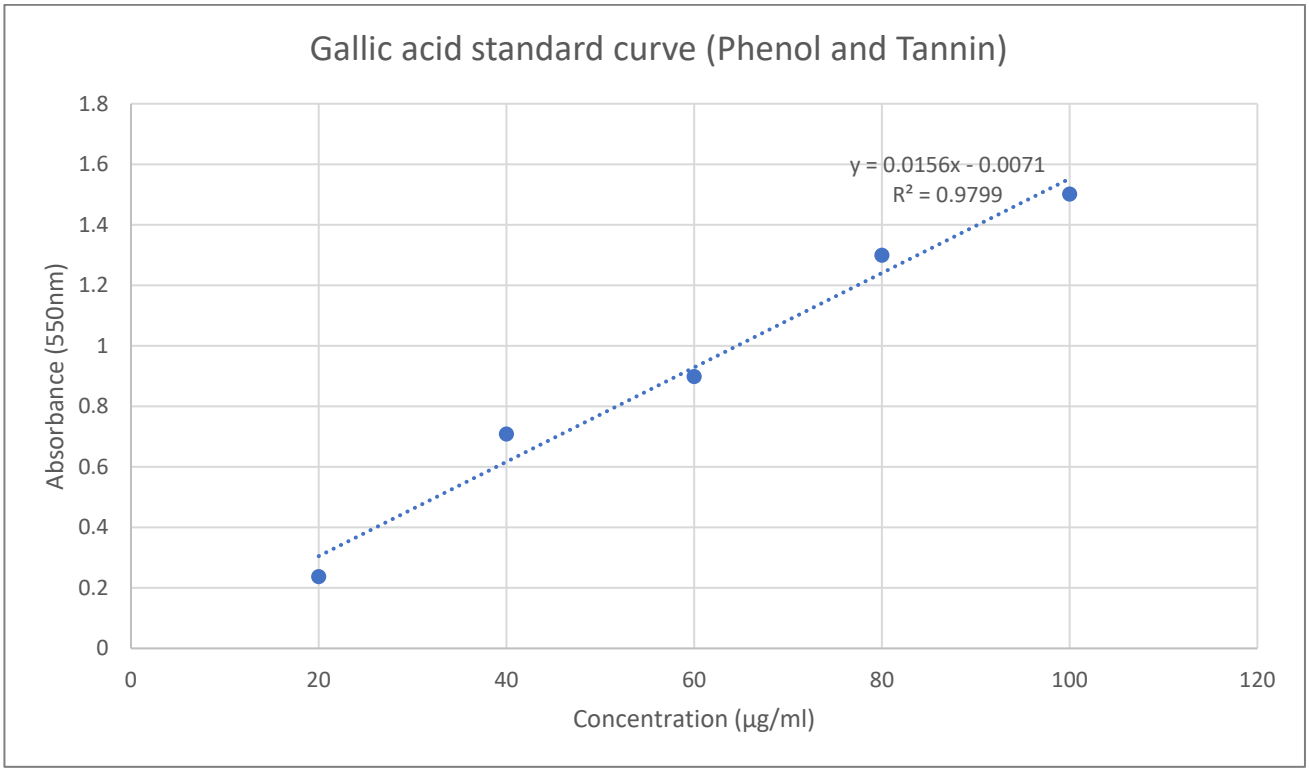

Figure 13: Gallic acid standard curve for calibration used for total phenol and tannin estimation

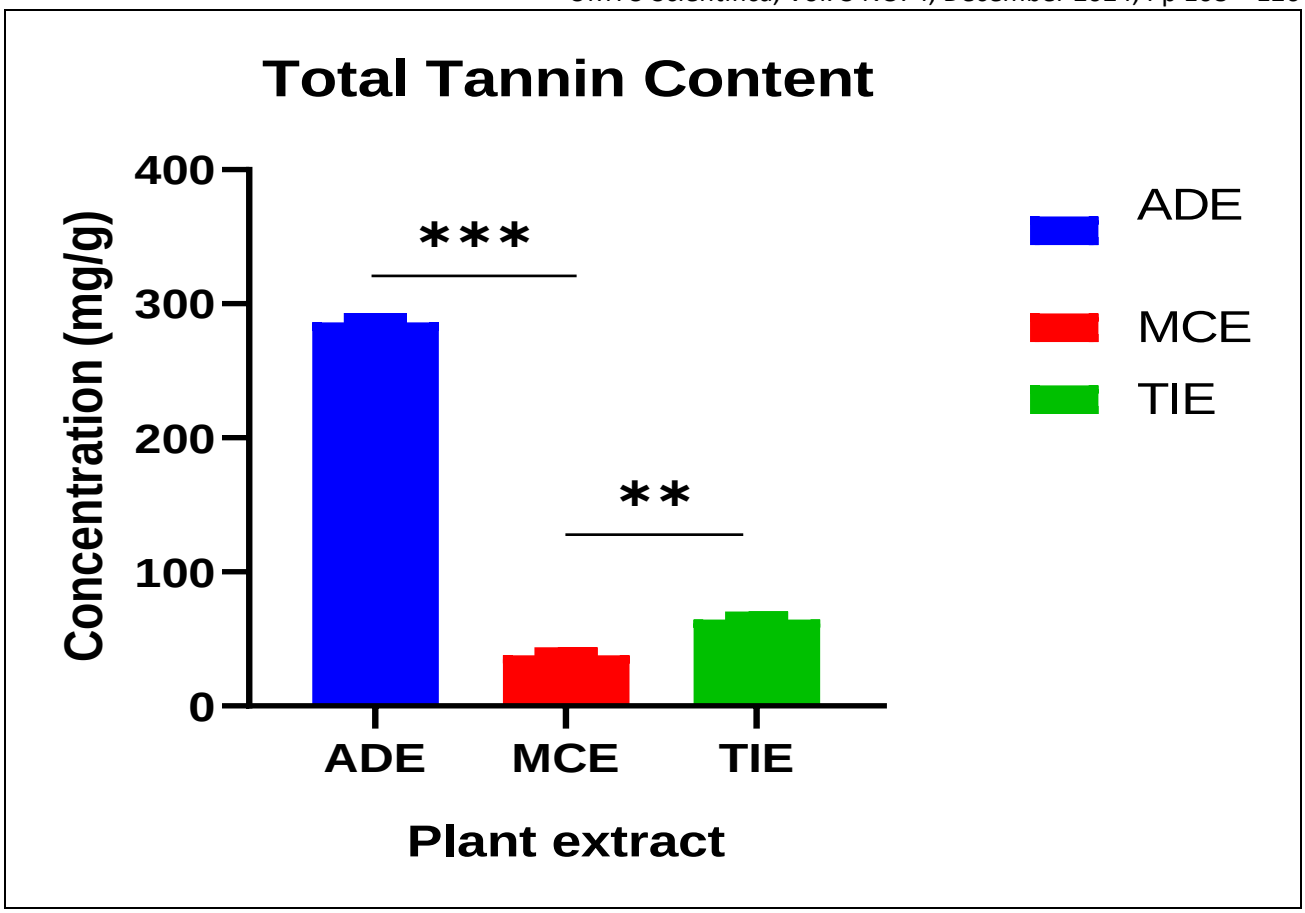

Figure 14: Total tannin content of *A. digitata, M.citrifolia* and *T. indica*
** indicates a statistically significant difference (P < 0.05) at a 95% confidence interval*

DISCUSSION

Since ancient times, researchers have used qualitative and quantitative phytochemical screening to identify the primary physiologically active components of plants to investigate their potential medical uses. Herbal medicinal plants are a rich source of secondary bioactive compounds, known as phytochemicals, which possess therapeutic potential. These compounds, including antioxidants, anti-inflammatory agents, and anti-microbial substances, can improve health and reduce disease risk (Basit *et al.*, 2023).

With a proportion of 23.6%, the *M. citrifolia* crude ethanolic leaf extract yielded the highest percentage. This could be because of the significant amount of chlorophyll that is stored in the leaves and its high solubility. In a related investigation, *M. citrifolia* crude leaf extract exhibited the highest percentage (10.92%), exceeding that of the crude stem (3.30%) and root extract (7.96%) (Samang *et al.*, 2020). Furthermore, percentage yields of 9.3% and 11.3% were reported by Chaniad et al. (2022) and Roka et al. (2020), respectively. The crude leaf extracts of *T. indica* yielded 15.8% in this study, which aligns with the findings of Ayat *et al.* (2019), who found that *T. indica* yielded a percentage of 14.5%. The percentage yield of the *A. digitata* stem bark extract was 5.6%, which was comparable to the 4.2% yield that Gaffo *et al.* (2022) reported. Ayat *et al.* (2019) did, however, report a lower yield of 1.9%. Several factors can influence the variations observed in the yield of plant extracts under study, including the quality of the plant material used, the extraction technique employed, the solvent chosen, the length of the extraction process, the environment, plant variability, and storage conditions (Zeroual *et al.*, 2021).

A qualitative phytochemical screening was conducted to determine the types of secondary metabolites present in the selected plants utilizing chemical reactions and color tests. Phytochemicals are constituents of plants that may play a variety of significant roles in the science of medicine. Depending on how these components are synthesized and accumulated in plant cells, they can be present in various amounts. According to Aondoackaa *et al.* (2024), the active phytochemical elements of a medicinal plant are directly related to its curative impact. The qualitative phytochemical analysis of *A. digitata* revealed the presence of a diverse array of secondary metabolites, including anthraquinones, reducing sugars, anthocyanins, flavonoids, terpenoids, alkaloids, phenolic compounds, tannins, cardiac glycosides, saponins, steroids, as well as carbohydrates. These findings are consistent with previously published research by Ajiboye *et al.* (2020) and Manuel *et al.* (2020). However, research by Daniel *et al.* (2024) revealed that the crude ethanolic stem bark extracts of *A. digitata* were devoid of terpenoids,

steroids, and tannins. Numerous secondary metabolites found in this study are known to offer defense against plant diseases and insect attacks. According to Ayat *et al.* (2019), the results further support the usage of *A. digitata* in African meals because it contains chemicals such as flavonoids, phenols, saponins, and alkaloids.

The qualitative phytochemical profile of *M. citrifolia* corresponds with the outcomes of Haruna *et al.* (2020) and Chaniad *et al.* (2022). However, research by Ene *et al.* (2021) and Sangavai (2024) showed the presence of anthraquinones. These compounds encourage the continuous usage of *M. citrifolia* leaf extracts in traditional medicine to cure various ailments (Shettima *et al.*, 2023).

Findings of *T. indica's* qualitative phytochemical screening were comparable to those of Kagoro (2022), Sookying *et al.* (2022), and Mohamadou *et al.* (2024), who found that reducing sugars were absent, but alkaloids, phenolic compounds, flavonoids, terpenoids, steroids, tannins, coumarins, anthocyanins, and saponins were present. However, Daniel *et al.* (2024) noted that terpenoids and steroids were absent. Furthermore, Alrasheid *et al.*, (2019) as well noted that flavonoids and triterpenes were absent. Steroids in plants serve a variety of functional purposes. The lipid bilayer that surrounds plant cell membranes and regulates its fluidity and permeability is made up primarily of phytosterols.

The UV/VIS spectrophotometric quantitative phytochemical analysis of *A. digitata, M. citrifolia,* and *T. indica* indicated variable amounts of the different secondary metabolites. Statistically significant differences ($p < 0.05$) were observed in the levels of all phytocompounds examined across the plant extracts, except for anthraquinones, which were absent from both *M. citrifolia* and *T. indica.* The present study's quantitative analysis of *A. digitata*'s stem bark revealed a significantly high ($p < 0.05$) concentration of phenols and tannins. The high phenol content indicates the antioxidant activity,anti-carcinogenic, and anti-inflammatory potential of the plants (Ajiboye et al., 2020), while the high tannin content could be attributed to its role in protecting the plant from herbivores, pathogens, UV radiation, and other environmental stresses. Tannins are also used as antiseptics and are crucial for infection prevention and wound healing. The phenolic group's presence is what causes this action. Enteric illnesses, including diarrhea, have been treated using ayurvedic preparations derived from plants high in tannin (Fraga-Corral *et al.*, 2021). This is consistent with the findings of Gaffo *et al.* (2022), who observed total tannin and phenol contents of 68.09 ± 0.17 mg/g and 68.09 ± 2.44 mg/g, respectively. In contrast, however, Daniel *et al.* (2024) reported phenols, tannins, alkaloids, saponins, and flavonoids concentrations of 0.33 ± 0.03 mg/g, 2.16 ± 0.11 mg/g, 1.74 ± 0.46 mg/g, 2.56 ± 0.33 mg/g, and 0.44 ± 0.03 mg/g, respectively. This discrepancy could be the result of the plant's geographic origin, differences in soil properties and climates in different ecosystems, the solvent employed, and the extraction technique.

Numerous phytochemical elements, such as alkaloids and terpenoids, though not in high concentrations when compared *to A.* digitata and *T. indica,* were found in varying concentrations in the crude ethanol leaf extract of *M. citrifolia* following its quantitative analysis. Alkaloids have a variety of pharmacological properties, like anti-malarial and anti-bacterial qualities (Daniel *et al.*, 2024). Terpenoids function as antifungals, and they also have some anti-cancer activity (Ra *et al.*, 2020), as well as anti-parasitic and anti-inflammatory in addition to anti-viral outcomes (Damilola *et al.*, 2024). Ene *et al.* (2021) found that the percentage concentrations of alkaloids, saponins, tannins, and flavonoids were 3.0%, 12.5%, 3.5%, and 5.8%, respectively. Similarly, Sam-ang *et al.* (2020) reported a total phenolic concentration of 12.61 ± 0.38 mg/g. In another study, the total phenols content of *M.citrifolia* leaves was estimated to be 1.80mg/g, which differs from the findings of the current investigation; yet, the total flavonoid concentration in the same study was found to be 69.30 mg/g, which is highly similar to the results of this investigation (Sookying *et al.*, 2022a).

The crude ethanol leaf extract of *T. indica* in this investigation yielded substantially higher ($p < 0.05$) levels of alkaloids, flavonoids, and saponins. Flavonoids, which are a prominent and widespread class of plant phenolic compounds, are found throughout plants, especially in photosynthesizing cells. These compounds serve as the primary pigments in flowers, often occurring in the form of glycosides, which are sugar molecules with one or more phenolic hydroxyl groups attached (Chaniad *et al.*, 2022). Research has demonstrated the anti-cancer, anti-bacterial, and antioxidant qualities of flavonoids, effectively protecting cells from oxidative damage (Ra *et al.*, 2020). Saponins exhibit antitussive, expectorant, analgesic, immunomodulatory, and cytoprotective properties (Shettima *et al.*, 2023), as well as anti-microbial and immune-stimulating effects (Daniel *et al.*, 2024). Similarly, alkaloids act as stimulants, antibiotics, antifungals, and pest-control agents (Ajiboye *et al.*, 2020). The findings in the present study align with the total flavonoid content of 130 mg/g reported by Mohamadou *et al.* (2024). This could be explained by the fact that leaves contain larger amounts of plant metabolites, followed by roots and then stem bark (Ra *et al.*, 2020). However, flavonoids were not detected in studies conducted by Hassan *et al.* (2020), while according to Sookying *et al.* (2022b), the total concentration of flavonoids and phenols was 69.30 mg/g and 1.80 mg/g, respectively.

Despite having the highest percentage yield of extract compared to *A. digitata* and *T. indica, M. citrifolia* exhibited the lowest content of the detected phytochemicals. This observation can be attributed to the inherent variability in phytochemical content among different plant species. Some plants naturally possess high concentrations of phytochemicals, which can result in lower extract yields

but higher concentrations of the desired compounds. Biologically active compounds typically occur in low concentrations within plants (Dhanani *et al*., 2017).

CONCLUSION

In conclusion, the ethanolic crude extracts *of A. digitata, T. indica*, and *M. citrifolia* exhibited a broad variety of biologically active components, such as flavonoids, alkaloids, phenolic compounds, saponins as well as terpenoids, as demonstrated by the qualitative and quantitative phytochemical analysis. *T. indica* had the highest alkaloid, flavonoid, and saponin concentrations, while anthraquinones were present only in *A. digitata,* which also had the highest phenol and tannin concentrations. These findings suggest that these plants could be explored for their anti-microbial, anti-inflammatory, anti-cancer, and antioxidant properties. To fully understand the unique bioactivities and therapeutic potentials of these phytoconstituents, more research is necessary. This could result in the development of innovative natural products with a range of uses in pharmacology, nutrition, and medicine. The results of this analysis will highlight the importance of these plants as valuable sources of bioactive chemicals with potential health-promoting qualities and add to the expanding body of information on the phytochemistry of these plants.

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