Phytochemical, Antioxidant, Anti-diabetic and Anti-inflammatory activities of *Costus speciosus* (J. Koenig) Sm. and *Tinospora cordifolia* (Wild.) Hook. and Thoms.

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, **"Phytochemical, Antioxidant, Anti-diabetic, and Anti-inflammatory activities of *Costus speciosus* (J. Koenig) Sm. and *Tinospora cordifolia* (Wild.) Hook. and Thoms."** is based on the results of investigations carried out by me in the Botany Discipline at the School of Biological Sciences and Biotechnology, Goa University under the supervision of Prof. S. Krishnan and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report, "**Phytochemical, Antioxidant, Anti-diabetic, andAnti-inflammatory Activities of *Costus speciosus* (J. Koenig) Sm. and *Tinospora cordifolia* (Wild.) Hook. and Thoms.**" is a bonafide work carried out by **Ms. Saiksha Sudesh Kuttiker** under my supervision in partial fulfilment of the requirements for the award of the degree of Masters of Science in the Discipline Botany at the School of Biological Sciences and Biotechnology, Goa University.

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PREFACE

Medicinal plants are traditionally used by the local people to treat various diseases. People usually prefer medicinal plants for the treatment because they are safe, cost-effective, and readily available. Ethnobotany deals with the interaction between people and plants. Ethnobotanical data collection helps preserve and document traditional knowledge and is a precursor for developing new drugs. The present study includes ethnobotanical data collection. The plants were selected after performing a thorough literature review. The phytochemical analysis was carried out to check the different phytoconstituents in plants. These phytochemical compounds help prevent various diseases. The phytochemicals are responsible for exhibiting properties such as antioxidant activity, anti-diabetic, anti-inflammatory, etc. The biological activities of the selected plants were studied for antioxidant, anti-diabetic, and anti-inflammatory activities.

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ABBREVIATIONS

Entity	Abbreviation
Chloroform Extract	C.E
gram	g
Methanol Extract	M.E
micro liter	µl
mili gram	mg
mili liter	ml
Water Extract	W. E

ABSTRACT

Costus speciosus and *Tinospora cordifolia* were screened for preliminary qualitative and quantitative phytochemical analysis, antioxidant, anti-diabetic, and anti-inflammatory activities. Initially ethnobotanical data was collected from Usgao village of Ponda Taluka, to gain knowledge about the plants used for treating diabetes. The plants were selected and the extracts were used for the study which includes methanol, chloroform, and water extracts of leaves of *Costus speciosus* and stem of *Tinospora cordifolia*. The preliminary qualitative phytochemical analysis was carried out using standard methods. Quantitative analysis was carried out for flavonoids by aluminium chloride colorimetric method, phenol by Folin – Ciocalteau's method and determination of alkaloid according to bismuth nitrate method. Antioxidant activity was determined by DPPH free radical scavenging activity. Anti-diabetic activity was determined by starch-iodine alpha amylase inhibition method and the anti-inflammatory activity by egg albumin protein denaturation method. Phytochemical screening of *Costus speciosus* shows presence of alkaloids, proteins, terpenoids, flavonoids, and saponins. *Tinospora cordifolia* showed presence of alkaloids, carbohydrates, saponins, proteins, phenolic compounds, terpenoids, and flavonoids. Quantitative analysis revealed high amount of flavonoid content in the methanol extract of *Costus speciosus* which was found to be 828.08 mg of quercetin/g of plant extract followed by alkaloid content reported high in methanol extract of *Tinospora cordifolia* as 89.67 mg bismuth nitrate per gram of plant extract. The maximum amount of phenolic content was found to be 80.47 mg Gallic acid/g of plant extract in methanolic extract of *Tinospora cordifolia*. Total tannin content was found to be high in methanol extract of *Tinospora cordifolia* which was 56 mg tannic acid/g of plant extract. Antioxidant activity suggests chloroform extract of *Costus speciosus* as having better activity with IC_{50} value of 176.55 µg/ml. The water extract of *Tinospora*

cordifolia showed good anti-diabetic activity as compared to chloroform and methanol extracts. The water extract of *Tinospora cordifolia* exhibited maximum anti-inflammatory activity with IC_{50} value as 10 µg/ml in comparison to standard Diclofenac sodium which suggests that the plant possesses good anti-inflammatory activity.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Since ancient times, people utilize plants for medicinal purposes and this practice still continues today (Grover *et al*., 2002). People turn to medicinal plants, as they are safer to human and are cost-effective and the most important aspect is they prevent the negative effects of chemical pharmaceuticals and injury to human health (Jayakumar *et al*., 2007). Plant plays a pivotal role in human life. Humans depends on them for essential nutrients like carbohydrates, proteins, vitamins, and cholesterol lowering other important compounds (Balamurugan *et al*., 2019).

Medicinal plants are common sources of medicine. Solid evidences can be cited in favor of herbs being used for the treatment of diseases and for restoring and fortifying body systems in ancient systems of medicine such as Ayurvedic, Unani, and Chinese traditional medicines. The purpose of the use of herbs in medication was to obtain a positive interaction with body chemistry (Spinella 2001). They possess antioxidants, antiviral, anticancer, antimicrobial, antifungal, and anti-parasitic properties. Flavonoids, phenolics, anthocyanins, and vitamins are among the free radical-scavenging compounds found in plants which have antioxidant-like properties (Chopra *et al*., 2002). Plants can be used medicinally for a variety of purposes, including the administration of their seeds, roots, barks, stems, leaves, extracts, and decoctions (Ogbulie *et al*., 2004). In addition to being useful as therapeutics, medicinal plants can provide a wealth of knowledge on a wide range of chemical ingredients that may be used to create precisely selective pharmaceuticals. These are the sources of chemical compounds with the potential to be useful, which could provide fresh leads and hints for the drug creation (Vijayalakshmi *et al*., 2012).

1.1. Ethnobotany

Ethnobotany is the study of traditional plant knowledge gathered from local communities. It encompasses skills and practices based on theories, beliefs, and experiences unique to their cultures, as well as the application of plants in disease prevention (Prance *et al*., 1991). The main task of ethnobotany is documentation, preservation of plant species, preservation of local botanical knowledge, claim for local and indigenous people's rights, improved nutrition, food security and medical care, and finally the creation of novel drugs (Gerique 2006).

While certain ethnobotanical research can be carried out in an herbarium, botanical, archeological, and linguistic labs. The majority of ethnobotanists agree that, collaborating with individuals on a personal and community level is crucial and important aspect of their field (Nolan and Turner 2011). Ethnobotany offers several interrelated and multidisciplinary subjects while interacting with traditional practitioners including ethnomedicine, ethnoarchaeology, ethno-bryology, ethnoecology, ethno-agriculture, ethno-narcotics, ethnopharmacology etc. (Pandey and Tripathi 2017).

The development of the traditional medicinal system with safety will not help only to preserve the knowledge, but also will help in raising the use of natural products in healthcare system (Mehrotra and Mehrotra 2005). Research on medicinal plants using bioprospecting, based on an ethnobotanical approach may result in the creation and development of beneficial healthcare items like food supplements and phytomedicines (Eldeen *et al*., 2016).

1.2.Selected plants for the study

***Costus speciosus* (J. Koenig) Sm.**

Taxonomic Classification (Pawar and Pawar 2010)

Kingdom- Plantae

Sub Kingdom- Tracheobinota

Super division- Spermatophyta

Division- Mangoliophyta

Class- Liliopsida

Sub class- Zingiberidae

Order- Zingiberales

Family- Costaceae

Genus- *Costus*

Species- *speciosus*

Description

The perennial rhizomatous herb *Costus speciosus* has either erect or spreading stems (Gupta 2010). It has simple, smooth, persistent leaves that are spirally arranged around the stem. There are dark green leaves that are elliptic or obovate in shape having spike inflorescence. A large bract in sub terminal position makes it around 10 cm long. The bracts have an oblong shape and a vivid red hue. The flowers are white with a cup-shaped labellum and yellow stamens. The fruit is a capsule with a red hue. The seeds have a white fleshy aril and are black (Stone and Benjamin 1970). Flowers look like crepe paper, and thus commonly called as crepe ginger. After

the flowers fade away, the attractive red cone-shaped bracts remain. Sprouting of stems takes place in the month of April. The flowering appears during middle of November after which the leaves shed and majority of canes start drying up. The rhizome remains dormant from December to March or even up to April (Sarin *et al*., 1982). *Costus speciosus* is usually found in fertile, organic, moist, and well-drained soils. Propagation is done through the use of rhizomes. The ideal conditions for its cultivation are humid tropical climate with a minimum temperature of 13ºC (Pawar and Pawar 2010).

Medicinal uses

Costus speciosus exhibits high medicinal properties such as anti-inflammatory, analgesic, antipyretic, antibacterial, antifungal, anti-helminthic, anticancer, anti-diabetic, and antioxidant activities (Maji *et al*., 2020). The plant is also used mainly in healing burning wounds, constipation, skin diseases, and worm infection (Veeramuthu *et al*., 2012). Rhizome is used for dizziness, diarrhea, eye and nose pain, snake bites, jaundice, bronchitis, rashes, anemia and to stop vomiting (Vijayalakshmi *et al*., 1997). It is also used as estrogenic, diuretic and hepatoprotective (Choudhary *et al*., 2012). The leaves of the plant are used in case of scabies and in stomach ailments (Bhuyan and Zaman 2008).

***Tinospora cordifolia* (Wild.) Hook. and Thoms.**

Taxonomic classification (Bharti *et al*., 2012).

Kingdom - Plantae

Sub kingdom - Tracheophyta

Super division - Spermatophyta

Division - Magnoliophyta

Class - Magnoliopsia

Sub class - Polypetalae

Order - Ranunculales

Family - Menisparmaceae

Genus - *Tinospora*

Species - *cordifolia*

Description

Tinospora cordifolia is a large, deciduous, and extensively spreading shrub. It has several elongated, and twinning branches. The roots are aerial, thread-like, long filiform, and arise from mature branches. Sometimes the stem reaches the ground (Spandana *et al*., 2013). There is a light grey-brown or creamy white color to the aerial roots. It has a round shape and a slight twist to it. The parenchyma contains starch. The stem is succulent, fleshy, and climbs. The outer bark is brittle (Bishayi *et al*., 2002).

The leaves are simple, round, heart shaped, and have a long petiole around 15 cm long. Half-way around, it is twisted. They are intensely green in color, but the over mature leaves are yellowish green to yellow. They are sour and have a vague odor (Nasreen 2010). The flowers are small and pale yellow. Terminal racemes or panicles are the male flowers. Female flowers are usually solitary (Sinha 2004). The fruit is oval and red in color, with a thick stalk (Kirtikar and Basu 2005).

Medicinal uses

Tinospora cordifolia is widely used medicinal plant with various biological properties such as anti-inflammatory, anti-diabetic, anti-spasmodic, anti-neoplastic, anti-stress, anti-leprotic, anti-malarial, hepato-protective, anti-allergic, and anti-arthritic properties (Bharti *et al*., 2012). It is used for bile juice secretion. The stem is used to prevent vomiting and treat fever. It is used to get rid of jaundice and treat diabetes (Mishra *et al*., 2014). The plant is also used to treat fever, dysentery, urinary diseases, jaundice, skin infections and asthma (Chintalwar *et al*., 1999).

1.3. Phytochemical analysis

Phytochemistry deals with the study of phytochemicals. Phytochemicals are the naturally occurring compounds present in leaves, stem, root, flower (Sabovljevic and Sabovljevic 2008). These compounds are referred to as secondary plant metabolites which possess biological properties such as antioxidant activity, antimicrobial effect, anticancer activity, etc. The purpose of these substances is to defend itself but the researchers have demonstrated that they have the ability to protect humans from illness (Rao 2003).

The phytochemical analysis reveals the presence of various compounds present in plants. Both the selected plants were screened for various phytochemical compounds like alkaloids, carbohydrates, glycosides, saponins, proteins, fats and oils, phenolic compounds, tannins, terpenoids, and flavonoids. Alkaloids are found in many different plants and are one of the biggest groups of secondary metabolites in plants. They are nitrogen-containing compounds with a low molecular weight. Because they have a heterocyclic ring containing a nitrogen atom, they are naturally alkaline (Yang and Stockgit 2010). Alkaloids include neurotransmitters like

nicotine and caffeine as well as life-saving medications like emetine that prevent oral intoxication (Matsura and Neto 2015).

Carbohydrates are essential macromolecules present in all plants. It is made up of sugar molecules and plays an important role in the energy production. Glycosides are a broad class of secondary plant metabolites that are found in many different parts of plants. Glycosides have a variety of structural forms, and because of their established bioactivities and historical applications, they are crucial to the pharmacognosy regime (Bartnik and Facey 2024). Saponins are secondary plant metabolites found in many different parts of the plant. In the plant defense system, it functions as a chemical barrier or shield to fight against infections and herbivores (Augustin *et al*., 2011). Saponins have a wealth of medicinal qualities, and in the recent past, a lot of research has concentrated on their capacity to boost immunological responses (Katselis *et al*., 2007). The biggest class of naturally occurring compounds is flavonoids found in various plant components (John *et al*., 2013). Most of the antioxidant activity found in plants or plant-derived products is attributed to phenolics, the biggest class of phytochemicals and it has several biological activities (Hsu *et al*., 2008).

1.4. Antioxidant activity

Biological oxidation causes our bodies to produce free radicals, also known as reactive oxygen species (ROS). An organism's natural technique for producing energy to power biological cycles is oxidation. On the other hand, unchecked generation of free radicals derived from oxygen, damages the body and contributes to oxidative stress (Thomson 1995). Generation of reactive oxygen species leads to extensive damage to DNA, proteins, and lipids which contributes to diseases (Lee *et al*., 2000). The antioxidant defense system is the most efficient way to stop and reduce the effects of free radicals. Antioxidants are substances that stop or slow

down a molecule's oxidation by preventing an oxidative chain reaction from starting or continuing (Kaneria *et al*., 2009).

Numerous plants include compounds known as antioxidant which protects the cells from the damaging effects of reactive oxygen species such as superoxide anion, hydrogen peroxide, and hydrogen radicals. Natural antioxidants like tocopherols and polyphenols, which are plentiful in spices, herbs, fruits, vegetables, cereals, grains, seeds, teas, and oils, are abundant in higher plants. The antioxidants from marine sources have also been taken into consideration such as algae, fish/shellfish, and marine microorganisms (Shahidi and Zhong 2015). Although the human body has antioxidant defense, natural sources of antioxidants are always needed (Rimbach *et al*., 2005). The antioxidant activity can be determined by DPPH free radical scavenging activity, ABTS scavenging activity, scavenging of H_2O_2 radicals, FRAP, Oxygen radical absorbance capacity (ORAC) and several other assays (Moharram and Youssef 2014).

1.5. Anti-diabetic activity

Diabetes Mellitus (DM) is a widespread condition that is rapidly increasing throughout the world and has significant social, health, and economic ramifications (Kaul *et al*., 2013). A malfunction in either insulin secretion, insulin action, or both can cause diabetes mellitus, a metabolic disease. Chronic hyperglycemia brought on by an insulin shortage is accompanied by abnormalities in the metabolism of proteins, fats, and carbohydrates. Vascular or tissue damage increases when the condition worsens, increasing the risk of developing serious diabetes consequences including retinopathy, neuropathy, nephropathy, and cardiovascular problems (Bastaki 2005). Diabetes mellitus can be classified into the following categories:

Diabetes mellitus type 1- an insulin-dependent condition. The disease is also known as juvenile onset diabetes. 5-10% of people, regardless of age, are affected by it (Dowarah and Singh 2020).

The condition is characterized by insulin deficiency due to pancreatic cell loss and leads to hyperglycemia (Katsarou *et al*., 2017).

Non-insulin-dependent diabetes mellitus- called as type 2 diabetes. It is the most prevalent, affecting between 85 and 90% of people. High levels of blood glucose are a result of insulin resistance and relative insulin deficiency (Ginter and Simko 2013). The production of insulin takes place in the cells of the pancreas, but insulin receptors or insulin-responsive cells in the cell membrane do not respond normally to insulin.

Gestational diabetes- a different kind of disease that only affects pregnant women, usually in the later stages of pregnancy. Treatments for gestational diabetes include special dietary plans and regular physical activity (Dowarah and Singh 2020).

The antidiabetic activity can be determined using alpha amylase inhibition by DNSA method. 3, 5-dinitrosalicylic acid (DNSA) method is the most widely used method. The oxidation of glucose converts DNSA to 3-amino-5-nitrosalicylic acid which changes colour from yellow to orange which can be measured at 456 nm (Miller 1959). The enzyme alpha amylase breaks the O-glycosidic bonds in starch molecules (Hussain *et al*., 2013). In a short amount of time, the starch iodine method is utilized to screen many possible candidate alpha amylase inhibitors (Oliveira *et al*., 2019). It is based on the starch-iodine complex's declining blue color. This alteration, which is indicative of starch endocleavage, can be routinely employed to measure alpha amylase activity. A technique for tracking enzyme activity is utilized when researching the suppression of the amylase enzyme (Ononamdu *et al*., 2020). Acarbose was the first alpha-glucosidase inhibitor to be made commercially available for the treatment of diabetes. (Agarwal and Gupta 2016).

1.6. Anti-inflammatory activity

Higher organisms have evolved the defensive mechanism of inflammation in response to harmful stimuli including microbial infection and tissue damage. It is a crucial immune system reaction that aids in both tissue repair and the elimination of damaging stimuli (Medzhitov 2008). Inflammation is a process in which body reacts to infection, irritation or injury and the key features are redness, warmth, swelling, and pain (Srdan and Stankov 2012).

There are mainly two types of inflammation, they are acute inflammation and chronic inflammation. Acute inflammation includes emigration of leukocytes, capillary infiltration, and enhanced vascular permeability. Chronic inflammation involves fibroblast activation, proliferation (angiogenesis), fibrosis, and the infiltration of mononuclear immune cells, macrophages, monocytes, and neutrophils (Kumar *et al*., 2013).

The appropriative administration and dosages of anti-inflammatory medications can normalize the disorganized and protracted inflammatory response based on the degrees of the inflammation on reaction for long term injuries. Non-steroidal Anti-inflammatory drug (**NSAIDAs)** are typically the first choice for therapeutic use since they can effectively reduce inflammation by up-regulating anti-inflammatory molecules and blocking inflammatory pathways for example Aspirin (Zejun *et al*., 2021).

1.7. The aim and objectives of the present study are:

1. To collect plant samples, specifically leaves of *Costus speciosus* and stem of *Tinospora cordifolia*, from Usgao village within Ponda Taluka.
2. Preliminary qualitative and quantitative phytochemical analysis.
3. *In-vitro* determination of Antioxidant activity by DPPH free radical scavenging activity.
4. *In-vitro* determination of Anti-diabetic activity by Starch-iodine alpha-amylase inhibition method.
5. *In-vitro* determination of Anti-inflammatory activity by Egg albumin protein denaturation method.

1.8. Hypothesis

Ethnobotanical study helps in documentation and preservation of traditional knowledge. The two plants, *Costus speciosus* and *Tinospora cordifolia* were selected based on the ethnobotanical study. *Costus speciosus* contains wide range of phytochemical such as tannins, phenols, flavonoids, alkaloids, saponins, steroids, and terpenoids whereas, *Tinospora cordifolia* contains glycosides, terpenoids, alkaloids, carbohydrates, flavonoids, phenols, steroids, and tannins. Both the selected plants possess biological activities such as antioxidant, anti-diabetic and anti-inflammatory activities. These biological activities may be attributed to the higher concentration of secondary metabolites in both the selected species.

1.9. Scope

Based on the ethnobotanical knowledge available for the selected plants, and also the knowledge gathered from locals, the scientific evidence for the aforementioned biological activities such as anti-diabetic, radical scavenging effects, antimicrobial, etc, are scarce. Therefore, the purview of research utilizing the selected plants is vast. Hence, keeping this

background knowledge in mind specific objectives were proposed for the work to evaluate the biological activities using standard scientific procedures.

CHAPTER 2
REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The relevant literature on the present study has been briefly reviewed to understand the different parameters of the study based on the specific objectives.

Jayakumar *et al.* (2008) carried out ethnobotanical survey of the plants which are used for treating diabetes. This article mainly deals with the plants and their folk knowledge for treating diabetes. People mostly use plant therapy in villages instead of chemical drugs. This may be because of the low cost and plant based natural products are most suitable for the human body. The documentation and preservation of such knowledge is essential, therefore many plants with their local names and their remedies have been documented.

Binny *et al*. (2010) carried out study on the anti-inflammatory and anti-pyretic properties of the rhizome of *Costus speciosus* with ethanol extract. The anti-inflammatory activity was carried out in carageenan induced paw edema and cotton pellet granuloma formation in rats. At 800 mg/kg good anti-inflammatory activity was found against carageenan induced edema formation in rat and at 400 mg/kg and 800 mg/kg against cotton pellet granuloma formation. There was minimal antipyretic activity observed only at 800 mg/kg dose which was carried out in yeast-induced pyrexia in rats.

The study conducted by Jain *et al*. (2010) compared the antioxidant potential of two species of *Tinospora*, namely *Tinospora sinensis* and *Tinospora cordifolia*. Aqueous and ethanolic extracts of both plants were tested using *in-vitro* antioxidant activity screening models, including total antioxidant assay, lipid peroxidation inhibition, ferric ion reduction, nitric oxide, and superoxide radical scavenging activity, DPPH and ABTS assays. Both plants had almost similar activity in every model examined, DPPH activity showed 97.68±1.08% free radical scavenging activity in aqueous extract and 106.86±0.80% in ethanol extract of *Tinospora*

cordifolia. The aqueous extract of *Tinospora sinensis* showed 100.46±0.36 % and ethanol extract reported 94.66±0.049 %, ABTS assay of *Tinospora cordifolia* reported 94.25±0.84% and 85.48±0.52% in aqueous and ethanol extract respectively. *Tinospora sinensis* also showed 104.75±0.64% and 90.44±0.36% in aqueous and ethanol extracts supporting *Tinospora sinensis*'*s* claims which have not received much research, and adding to the current medical system.

Jha *et al*. (2010) studied *in-vitro* antioxidant and cytotoxic potential of *Costus speciosus* with methanolic extract of rhizome. Antioxidant activity was carried out using total antioxidant, DPPH, and nitric oxide scavenging assays, together with total phenolic and flavonoid contents. The total phenolic and flavonoid content was found to be 93.95 mg gallic acid/g plant extract and 9.32 mg quercetin/g plant extract respectively. The total antioxidant activity was reported to be 82.88%. Based on the concentration, plant extract showed effective antioxidant activity. Rhizome extract also showed strong cytotoxic activity against brine shrimp with an IC_{50} value 31.55 µg/ml.

Choudhary *et al*. (2013) analyzed *Tinospora cordifolia* through ethnobotanical and phytochemical studies. The ethnobotanical study revealed that *Tinospora* has significant role in medical science. The plant exhibits immunomodulatory, hypoglycemic, antioxidant, antihyperglycemic, anti-allergic, anti-inflammatory, and anti-diabetic properties. Alkaloids, glycosides, steroids, diterpenoids, lactones, sesquiterpenoid, aliphatic compounds, and other miscellaneous compounds were found to be present during the phytochemical analysis. The study also showed that this plant also plays an important role in pharmacology.

Singh *et al*. (2013) studied the anti-diabetic activity of ethanolic extract of *Tinospora cordifolia*. The leaf extract was provided in different dosages to streptozotocin induced diabetic

albino rats for 10 to 30 days. It is more likely that *Tinospora cordifolia* works as an anti-diabetic medication through a peripheral mechanism such as enhancing liver storage of glycogen by inhibiting release of glucose, rather than through insulin secretion. The outcome clearly demonstrated that, in comparison to insulin, *Tinospora* had 50-80 % more anti-diabetic efficacy.

Upadhyay *et al*. (2013) studied the antioxidant effect of *Tinospora cordifolia* stem using the methanol and ethanol extracts. Antioxidant assay was carried out using DPPH radical scavenging activity. Ethanolic extract showed better activity of 56.35%. Determination of total phenolic content was also performed by Folin Ciocalteu reagent method, where ethanolic extract showed highest phenolic content of 66.28 ± 0.82 mg gallic acid/g plant extract.

Mishra *et al*. (2014) worked on *Tinospora cordifolia*, stem for phytochemistry and *in-vitro* antibacterial activity with different strains of bacteria by cup plate method. The obtained results were compared against Streptomycin. While the hydro-methanolic extract shows an inhibition zone on a few species, including *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, and Staphylococcus epidermidis,* the ethanolic extract exhibits effective antibacterial activity against all organisms except for *E. coli*, *Proteus vulgaris,* and *Pseudomonas aeruginosa.*

Revathy *et al*. (2014) determined the anti-diabetic activity of ethanolic rhizome extract of *Costus speciosus* in Alloxan-induced albino rats. The secondary metabolites were identified and quantified by HPLC analysis. Antioxidant activity was also determined by hydrogen peroxide and phosphomolybdate methods. Anti-diabetic activity determined by subjecting ethanol extract to rats orally and blood samples were measured using auto-analyzer. Phytochemical screening revealed alkaloids, flavonoids, tannins, phenols, steroids, carbohydrates, amino acids, glycosides, proteins, ascorbic acid, coumarins, and quinines. The anti-diabetic activity suggested that the

ethanolic extract enhances insulin secretion by islets of langerhans and enhances peripheral glucose utilization and increases serum protein level.

Ramya and Dharmotharan (2015), studied the qualitative and quantitative aspects of *Costus speciosus*. The leaf extract was prepared using various solvents and exposed to phytochemical screening. The ethanolic extract showed better results as compared to other solvents and reveals the presence of chemical constituents like alkaloids, tannins, saponins, steroid, terpenoids, flavonoid, and total phenols. The ethanolic leaf extract of *Costus speciosus* contained the highest percent crude yield of tannins, phenols, flavonoids, alkaloids, saponins, steroids, and terpenoids. Quantitative analysis of the above active compounds was also conducted in the ethanol leaf extract which reported high number of total phenols 25.4 mg gallic acid/g plant extract. The results suggest that the presence of bioactive constituents may be used for curing various ailments and possesses potential antioxidant, antimicrobial, anti-inflammatory, and anti-diabetic activities.

Sonkamble and Kamble (2015), worked on the anti-diabetic potential and identification of phytochemicals from *Tinospora cordifolia*. Determination of total phenols, thin layer chromatography and alpha- amylase inhibition assay was also performed. Extracts were analyzed for LCMS and data was processed using online databases. The highest phenolic content was observed in the ethanolic extract with 0.244 mg/ml and highest alpha-amylase inhibition of 64.04% was observed in the ethyl acetate extract. They determined seven compounds responsible for the anti-diabetic activity.

The evaluation of anti-inflammatory and analgesic property of the seeds of *Costus speciosus* with methanol extract was done by Ansari *et al*. (2016). *In-vitro* anti-inflammatory activity was determined by membrane stability testing and protein denaturation inhibition

method. *In-vivo* determination was carried out by formalin induced paw edema. Maximum inhibition with extract on paw was at 500mg/kg and 50% inhibition was shown after 1 hour of carrageenan administration. Through this study they concluded that the plant part has the capacity to reduce pain and inflammation.

Bindu *et al*. (2016) carried out the anti-diabetic study of *Costus speciosus* using mouse. The plant extract likely exerts anti-diabetic effects by encouraging adipocytes to absorb glucose while significantly inhibiting adipogenesis. This indicates that the extract's relative potency to commercial insulin is consistent. The plant extract from *Costus speciosus* has been found to have a substantial relative potency when compared to commercial insulin in this preliminary *in-vitro* study. This finding may have applications in the treatment of type II diabetes. After carefully planned preclinical and clinical research, this plant source may prove to be a viable, sustainable, natural anti-diabetic medication.

Tinospora cordifolia was examined by Kaur *et al*. (2016) for phytochemicals following extraction using the Soxhlet equipment. Qualitative phytochemical analyses were performed on a number of solvent components. The flavonoids and phenols underwent quantitative analysis. The DPPH radical scavenging activity, reducing power assay, and hydrogen peroxide scavenging assays have been used to measure the antioxidant activity. Thin layer chromatographic investigations and the agar well diffusion method for antibacterial research were also conducted. A preliminary examination of the phytochemical composition of the stem revealed the presence of amino acids, carbohydrates, glycosides, flavonoids, phenols, and tannins. The methanolic extract displayed superior antibacterial activity among the extracts when tested *in-vitro* against both gram-positive and gram-negative bacteria. The amount of phenolic content in the ethyl acetate extract was also good, while the amount of flavonoids in the methanolic extract was

superior. In terms of antioxidant activity, the percentage inhibition increased along with the concentration.

Kumar *et al*. (2018) carried out study on the hydro-ethanolic extract of *Tinospora cordifolia* for phytochemicals, antioxidant, anti-inflammatory, and antimicrobial activity. Phytochemical screening revealed the presence of glycosides, terpenoids, alkaloids, carbohydrates, flavonoids, phenols, steroids, and tannins. Total phenolic content showed 2.38±0.15 mg/g and total flavonoid content was found to be 18.91±0.21 mg/g. They observed linear correlation between flavonoid, phenols, and antioxidant studies, which revealed that the plant has good medicinal properties. The antioxidant studies were carried out with different methods such as DPPH, FRAP, MC, NO and SA. The best antioxidant activity was observed in nitrous oxide assay. Anti-inflammatory activity was determined by bovine serum albumin method, which showed that the extract has good inhibition activity. Antimicrobial activity was performed with three fungal and bacterial strains. The maximum inhibition was found in the *Staphylococcus aureus* followed by three strains.

Garg and Garg (2018), worked on the qualitative and quantitative analysis of the leaves and stem of *Tinospora cordifolia* with ethanol and methanol extract. Quantitative analysis was carried out using total flavonoid content by aluminium chloride colorimetric method. Flavonoids, amino acids, diterpenes, proteins, saponins and carbohydrates reported during qualitative phytochemical analysis. Ethanolic leaf extract showed higher total phenolic content with 1.820 mg gallic acid/g plant extract than methanolic extract with 1.813 mg gallic acid/g plant extract. Whereas the stem shows better result in methanolic extract with 2.14 mg gallic acid/g plant extract than ethanolic extract which was found to be 1.75 mg gallic acid/g plant extract.

Behera *et al*. (2020), carried out phytochemical analysis and antioxidant potential of *Costus speciosus* leaf, stem, and rhizome with different solvent extracts. Phytochemical analysis revealed tannins, flavonoids, phenolic compounds, saponins, terpenoids, glycosides, and reducing sugars. Rhizome extract showed good scavenging activity with DPPH assay of methanol and acetone extract exhibiting 62.05±0.03% and 69.21±0.30 % respectively. The metal chelating assay reported 61.40±0.26 % and 66.20±0.50 % in methanol and ethanol extract.

Comparative study of the solvent polarity on qualitative and quantitative phytochemical analysis and antioxidant activities of *Costus speciosus* were carried out with root extract by Garwal *et al*. (2021). Accordingly, *Costus speciosus* was found to have several pharmacological properties like antibacterial, antifungal, antioxidant. Qualitative phytochemical screening showed presence of carbohydrates, reducing sugars, amino acids and flavonoids. The quantitative phytochemical analysis was carried out for total phenolic content, revealed ethanol extract with high phenol content of 99.70 mg GAE/g followed by hexane and methanol. Antioxidant activity was determined by DPPH free radical scavenging activity, which showed Vitamin C with highest activity and among the extracts, ethyl acetate extract showed good antioxidant activity.

Shaikh *et al*.(2021) investigated the leaf and rhizome of *Costus speciosus* and *Costus pictus* for phytochemical, histochemical, and *in-vitro* antimicrobial study by agar well diffusion and disc diffusion methods using three different solvents. Phytochemical analysis showed the presence of alkaloids, flavonoids, quinines, and saponins in both the plant extracts. Histochemical analysis resulted in the presence of alkaloids, proteins, and phenols in vascular bundles, cortex and epidermis of stem, roots, and leaves. Methanol and hexane extract showed inhibition zone which indicated better antimicrobial activity.

Tinospora cordifolia was studied by Siddiqui *et al*. (2022) for total phenolic content and antioxidant activity. The Folin-Ciocalteu's reagent was used to quantitatively assess the total phenolic content in aqueous, ethanolic, and hydroalcoholic extracts. DPPH radical scavenging was used to measure the *in-vitro* antioxidant activity with gallic acid as standard. It was discovered that the total phenolic content of the ethanolic, hydroalcoholic, and aqueous extracts was, respectively, 13.09, 41.50, and 14.0 mg/g GAE. In comparison to aqueous and hydroalcoholic extracts, ethanolic extract demonstrated strong antioxidant activity with a low IC_{50} value of 67.47±0.034 µg/ml ($p<0.01$). They confirmed their investigation with the Unani description of the test drug Giloe (*Tinospora cordifolia*), including its general tonic properties and resolvent/anti-inflammatory properties. The current research indicates that it may be utilized as an effective therapeutic agent of the next generation to treat a variety of illnesses linked to free radicals, including arthritis, cancer, neurological disorders, cardiovascular diseases, etc.

The anti-inflammatory properties of flower of *Costus speciosus* were examined by Fathirah *et al*. (2023). The purpose of their study was to determine the anti-inflammatory activity of the ethyl acetate, methanol, and water extracts. Sodium diclofenac was employed as a positive control, and bovine serum albumin was to suppress protein denaturation. Ethyl acetate extract contains quinines and steroids. Flavonoids and phenols were present in methanol and water extract. IC_{50} values of ethyl acetate were more as compared to methanol such as 80.178 and 14.9772 mg/L respectively. Thus, the flower with methanol and ethyl acetate extract may have anti-inflammatory properties.

Siriwardhene *et al*. (2023) studied the hypoglycemic effect of leaves of *Costus speciosus* in both normal and alloxan-induced male Wistar diabetic rat models. Methanolic extract used for phytochemical screening rats weighing 150-220 g to check anti-diabetic activity. Student t-test

was performed to determine the statistical significance. 80% of methanol extract exerted overall hypoglycemic effect with normal rats at 90 min. The same showed improvement in glucose tolerance of alloxan-induced diabetic rats, reducing the blood glucose at 90 min by 60% when compared with the control. These effects were found to be comparable with glipizide, a synthetic drug.

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Collection of Plant Material

Ethnobotanical data was collected in the month of July 2023. The knowledge about plants was gained from the local people of Ponda Taluka. Data was collected from basic oral interview and the information was carefully noted down. Healthy mature leaves of *Costus speciosus***(Plate 4.1)** and stem of *Tinospora cordifolia***(Plate 4.3)** were collected**(Table 4.2)**and washed properly with distilled water and shade dried for 14 days at room temperature (25±1ºC). The dried leaves and stems of the respective plants were grounded using a mechanical blender and the fine powder was stored in an air tight container at room temperature (25±1ºC). The powder was used for preparation of extracts using different solvents.

3.2. Preparation of plant extracts

The solvents used to prepare the extracts included methanol, chloroform, and distilled water. The extracts were prepared by maceration method.10 g of shade dried powder was mixed with 100 ml of the respective solvents and kept at room temperature (25±1ºC) for 3 days and were stirred for every 4 to 5 hours. On the fourth day the extracts were filtered using Whatman filter paper No1. The filtrate was poured on a large petriplate and were dried on a water bath around 40ºC till it gets dried. The extract was scraped with spatula and stored in a glass vial in the refrigerator and used for further studies. The stock solution of plant extract was prepared by dissolving 10mg of extract in 10 ml of the respective solvents. The stock solution was used for all the studies (**Plate 4.3 and 4.4**).

3.3. Phytochemical analysis

The preliminary qualitative and quantitative phytochemical analysis were carried out using plant extract with different solvents.

3.3.1. Qualitative Phytochemical Analysis

The qualitative phytochemical analysis of plant extracts was carried out using the standard protocol by Raman 2006.

1. Test for Alkaloids

50 mg of extract was stirred with few ml of dilute hydrochloric acid and filtered. Using the filtrate the following tests were performed.

Mayer's Test: one drop of Mayer's reagent was added to 2ml of filtrate. A white or creamy precipitate reveals the presence of alkaloids.

2.Test for Carbohydrates

100 mg of extract was dissolved in 5ml of water, filtered and used for the following tests.

Benedict's Test: 0.5ml of Benedict's reagent was added to 0.5ml of filtrate and heated in a boiling water bath for 2 minutes. A brown colour precipitate reveals the presence of sugars.

3.Tests for Glycosides

50mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and used for the following test.

Borntrager's Test: 3ml of chloroform was added to 2ml of hydrolysate and mixed. The chloroform layer gets separated and 10% ammonia solution was added to it. A pink colour reveals the presence of glycosides.

4.Test for Saponins

50mg of extract was diluted with 20ml distilled water, the suspension was mixed in a graduated cylinder for 15 minutes. A 2cm layer of foam reveals the presence of saponins.

5.Test for Proteins

100mg of extract was dissolved in 10ml of distilled water and filtered. This was used for the further test.

Ninhydrin Test: two drops of Ninhydrin solution was added to 2ml of aqueous filtrate. Purple colour reveals the presence of proteins.

6. Test for Fats and Oils

4 to 5 drops of 0.5N Alcoholic potassium hydroxide was added to a small quantity of extract and a drop of phenolphthalein. The mixture was heated in a water bath for 2 hours. Foam formation reveals presence of fats and oils.

7. Test for phenolic compounds and Tannins

Ferric chloride Test: 50mg of extract was dissolved in 5ml of distilled water. Few drops of 5% ferric chloride solution were added to it. White precipitate revealed the presence of phenolic compounds.

8. Test for Terpenoids

2ml of chloroform and 3ml of sulphuric acid was added in 2ml of extract. Reddish-brown colouration reveals the presence of terpenoids.

3.3.2. Quantitative phytochemical analysis

The quantitative phytochemical analysis of plant extracts was carried out for Flavonoids, Tannins, Phenols, and Alkaloids using standard methods with slight modifications.

1. Determination of Total Flavonoids by $AlCl_3$ Colorimetric Method

The Total Flavonoid Content was determined by $AlCl_3$ colorimetric method according to Chang *et al*., 2002.

To the 0.5 ml of plant extract of serial dilutions of 200, 400, 600, 800, and 1000 µl, 1.5 ml of methanol and 0.1 ml of 10% $AlCl_3$ was added. To this 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water was added and kept at room temperature for 30 mins. The absorbance of reaction mixture was measured at 415 nm.

The calibration curve was prepared using quercetin in methanol and results were expressed as amount of flavonoid content. 10 mg of quercetin in 10 ml of 80% methanol and diluted to 200, 400, 600, 800, and 1000 µl. Further they were treated same like extracts. Blank was prepared by substituting distilled water in place of extracts. The test was performed in triplicates. The concentration of total flavonoid content was calculated as mg quercetin equivalent to plant extract using quercetin calibration curve.

2. Quantification of Tannin

The Total Tannin Content (TTC) was determined according to Amorim *et al*., 2009. To 1 ml of plant extract 0.5 ml of Folin's reagent was added. To this 7.5 ml of demineralized water and 1 ml of 35% sodium carbonate was added and mixed well. The solution was incubated for 30 mins at room temperature and the absorbance was taken at 725 nm.

The calibration curve was determined using Tannic acid as standard. The stock solution of tannic acid was prepared by dissolving 10 mg tannic acid in 10 ml distilled water. The same above procedure was followed for this. The concentration of total tannins was calculated as mg tannic acid equivalent of extract using tannic acid calibration curve.

3. Total Phenol Estimation

The Total Phenolic Content (TPC) was determined by Folin Ciocalteu's method according to Dewanto *et al*., 2002. Gallic acid was used as a standard for determining the calibration curve. The stock solution of gallic acid was prepared by mixing 10 mg of gallic acid in 10 ml of distilled water. The calibration curve was plotted by mixing 100 µl aliquots of 20, 40, 60, 80 and 100 µl of gallic acid solution. The final volume was adjusted to 500 µl with distilled water. 150 µl of 10% Folin's reagent was added and vortexed. The mixture was maintained at room temperature for 10 mins. 500 µl of 7.5% sodium carbonate was added and incubated in dark for 1 hour. The absorbance was measured at 760 nm. The blank was prepared by adding 100 µl of distilled water instead of gallic acid. The same procedure was followed for all the plant extracts. The concentration of total phenolic content was calculated as mg gallic acid by using gallic acid calibration curve.

4. Quantification of Alkaloid

The total alkaloid content was determined according to Sreevidya and Mehrota 2003. 5 ml of plant extract was taken and maintained at a pH of 2 to 2.5 with dil. HCl. 2 ml of Dragendroff's reagent was added to it and centrifuged at 5000 rpm for 10 min. The supernatant was decanted carefully. The precipitate was washed with alcohol and the filtrate was discarded and the residue was treated with 2 ml of disodium sulfide and dissolved in 2 ml conc. nitric acid with slight warming. The solution was diluted to 10 ml in a standard flask with distilled water. 1 ml of this solution was pipetted and 5ml of thiourea solution was added to it. The absorbance was measured at 435nm against blank containing nitric acid and thiourea.

Bismuth nitrate was used for calibration curve. The stock solution was prepared using 10 mg bismuth nitrate into 10 ml distilled water. The serial dilution of stock was made from 1ml-

9ml making final volume as 10 ml. From this 1 ml was taken and 5 ml of 3% thiourea was added and the absorbance was measured at 435 nm. The amount of bismuth presents in a solution calculated by multiplying absorbance value taking into consideration the dilution factor obtained from the standard curve which is constant for different concentration.

3.4. Antioxidant Activity

The antioxidant activity of the plant extracts were carried out by DPPH radical scavenging activity according to Blois 1958, Madhu *et al*., 2020. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable organic nitrogen radical characterized by a characteristic deep violet colour. It shows a maximum absorbance at 517 nm. DPPH changes its violet colour to yellow when it gets scavenged. This method's foundation is the reduction of DPPH in an alcoholic solution when an antioxidant that donates hydrogen is present since this reaction produces the non-radical form DPPH-H˙. DPPH radicals are scavenged and the absorbance is decreased when they encounter a proton donor substrate, such as an antioxidant (Blois 1958; Elmastas *et al*., 2006).

Preparation of DPPH stock solution: 24 mg of DPPH with 100 ml ethanol and then stored in amber colored bottle at -20ºC. The working solution was prepared by diluting 10 ml stock solution using 45 ml ethanol.

Preparation of L-Ascorbic acid stock solution: 10 mg of ascorbic acid was dissolved in 10 ml of distilled water

Preparation of plant extract: 10 mg of plant extract was dissolved in respective solvents. The ascorbic acid was used as a standard. The series were prepared in the range of 12.5, 25, 50, 100 and 200 µg/ml. From these series 1ml was pipette out in test tube and 3ml of DPPH was added and incubated in dark for 30 min. The absorbance was taken at 517 nm. Control was prepared by

adding distilled water instead of ascorbic acid. Ethanol was used as a blank. For plant extracts the same procedure was followed.

The percent inhibition was calculated by the following formula:

$$\textbf{Inhibition \%} = \frac{\textbf{Absorbance}_{\textbf{control}} - \textbf{Absorbance}_{\textbf{sample}} \times \textbf{100}}{\textbf{Absorbance}_{\text{control}}}$$

The antioxidant assay was performed in triplicates, and mean values were expressed. IC_{50} values were calculated by plotting percent (%) inhibition against the concentration (µg/mL) of the extract.

3.5. Anti-diabetic activity

The Anti-diabetic activity was carried out using starch-iodine alpha amylase method to detect inhibition of the enzyme according to Xiao *et al*., 2017. The blue color indicates presence of starch. It is based on the starch-iodine complex's declining blue color. This alteration, which is indicative of starch endocleavage, can be routinely employed to measure alpha amylase activity. A technique for tracking enzyme activity is utilized when researching the suppression of the amylase enzyme (Chimaobi *et al*., 2020). Acarbose was used as standard to detect inhibition activity of enzyme.

Stock solution of Acarbose: 10 mg of acarbose was dissolved in 10 ml of distilled water. The series were prepared using stock solution as 20, 40, 60, 80 and 100 µg/ml.

Alpha amylase stock solution: 0.5 mg alpha- amylase was dissolved in 10 ml distilled water. To 500 µl of acarbose from series, 500 µl of alpha-amylase and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) were added. The mixture was incubated at 37ºC for 10 min. Then 500 µl of 1 % starch were added and again incubated at 37ºC for 15 min. 250 µl of HCl and 200 µl of iodine reagent was added. The absorbance was

measured at 620 nm. The assay was performed in triplicates and mean was taken. The IC_{50} values were calculated by plotting % inhibition of enzyme against concentration (µg/ml).

3.6. Anti-inflammatory Activity

The anti-inflammatory activity was detected by egg albumin protein denaturation method according to Samaweera 2023. Anti-inflammatory drugs have the capacity to prevent protein denaturation. Diclofenac sodium, an anti-inflammatory drug has been used as standard in this assay. The protein denaturation was observed through heating. The prominent white colour indicates that there is complete denaturation of proteins. As the concentration increases the colour decreases which indicate that the inhibition of inflammation increases (Samaraweera *et al*., 2023).

Preparation of standard concentration and screening of the egg albumin denaturation method: The stock solution of Diclofenac sodium was prepared by dissolving 10 mg Diclofenac sodium in 10 ml distilled water. The series were prepared in 20, 40, 60, 80 and 100 µg/ml. Diclofenac sodium was used as a positive control. To the2.8 ml of Phosphate buffer saline (pH 6.4) 0.2 ml of egg albumin and 0.2 ml of diclofenac sodium were added. The mixture was incubated at 37ºC in a water bath for 15 min. The samples were heated at 70ºC for 5 min. The samples were cooled for 10 min. The absorbance was measured at 660 nm.

The % inhibition of protein denaturation was calculated by the following formula:

$$\textbf{Inhibition \%} = \frac{\textbf{Absorbance}_{\textbf{control}} - \textbf{Absorbance}_{\textbf{sample}} \times \textbf{100}}{\textbf{Absorbance}_{\textbf{sample}}}$$

The tests were performed in triplicates and mean values were taken. The IC_{50} value was calculated by plotting graph of % inhibition v/s concentration (µg/ml).

CHAPTER 4

RESULTS AND DISCUSSION

4.RESULTS AND DISCUSSION

4.1. Ethnobotanical data collection

Ethnobotany is a multidisciplinary rapidly growing science, which attracts people based on their interests. It requires variety of skills such as training for the identification and preservation of plant specimens, to understand the cultural concepts and linguistic training. It provides wealth of information about the past and present relationships between plants and people and it has been proven that ethnobotany plays an important role in development of new drugs in pharmaceuticals (Pandey and Tripathy 2017).

The ethnobotanical data was collected from local practitioners of Usgao village of Ponda Taluka. The knowledge and data was collected mainly for the plants which are used for treating Diabetes Mellitus. The names of the plants are noted down in **Table 4.1**. From the collected data it was noted that there were 9 plants that were used for the treatment belonging to the families such as Apocynaceae, Costaceae, Lauraceae, Malvaceae, Meliaceae, Menispermaceae, Myrtaceae and Nyctaginaceae. It was noted that the two Apocynaceae plants were being used as medication for treating diabetes. It was also observed that the leaves were mostly used for the treatment and the way of administration of the medication was is in the form of decoction.

4.2. Extract yield

Extraction is the process of extracting medicinally active compounds present in plants with selected solvents (Pandey and Tripathy 2014). The extract yield is the weight of the dried extract obtained after the extraction method per weight of plant material multiplied by 100. Based on the chemical nature, phytochemicals are extracted using solvents of

Plate 4.1.*Costus speciosus* a. Habit b. Leaf (adaxial) c. Leaf (abaxial) d. Flower.

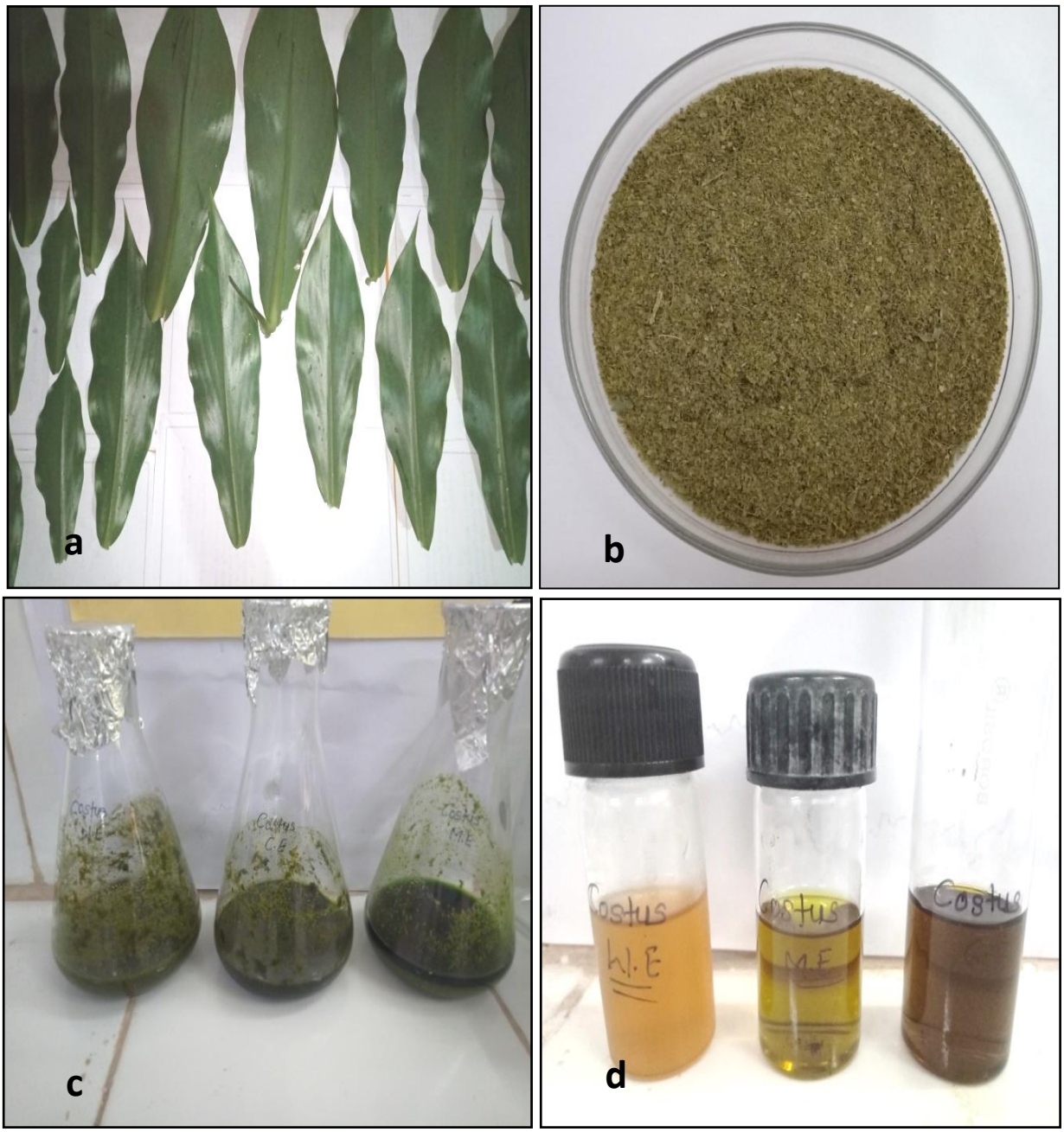


Plate 4.2. Extraction of leaves of *Costus speciosus.* a. Drying of leavesb. Powder c. Extraction by maceration method d. Stock solution of extracts.

Plate 4.3.*Tinospora cordifolia* a. Habit b. Enlarged view of stem c. stem.

Plate 4.4. Extraction of the stem of *Tinospora cordifolia.* a. Drying of stemb. Powder c. Extraction by maceration method d. Extracts stock solution.

Table no. 4.1. The List of the plants, their local name, plant parts and methods used for treating diabetes.

Sr. no	Plant name	Local name	Parts used	Methods
1	*Azadirachta indica* A. Juss.	Kadulimbu	Leaves	Decoction
2	*Boerrhavia diffusa* Linn.	Punarnava	Leaves	Decoction
3	*Calotropis gigantea* (L.) W. T. Aiton	Rui	Leaves	Leaves are tied at the bottom of the legs
4	*Catharanthus roseus* (L.) G. Don	Sadafuli	Leaves	Decoction
5	*Cinnamomum verum* (cinnamon)	Tikhi	Leaves and Bark	Decoction
6	*Costus speciosus* **(**J.Koenig) Sm.	Bonkalo	Leaves and Rhizome	Leaves are eaten
7	*Sterculia urens* Robx.	Arjun	Bark	Decoction
8	*Syzygium cumini* (L.) Skeels.	Jambla	Fruits and Seeds	Raw fruits are eaten, Powder of dried seeds are mixed in cup of milk and drank
9	*Tinospora cordifolia*(Wild.)	Amrutvel	Stem	Chewing of stem

	Hook. and Thoms.			

different polarity as single solvent may not be able to extract all phytochemicals present in plants (Lapornik *et al*., 2005; Assanga *et al*., 2015).

The choice of solvents plays an important role in determining the phytochemicals. For the study three solvents were used viz., methanol, chloroform and water based on their polarity indices as shown in **Table 4.3**. According to the literature, methanol gives better results as compared to water because of its ability to extract higher amounts of polyphenols due to its polarity index of 5.1. Methanol is mostly used for extraction of phenolic compounds. On the other hand, chloroform is less polar with 4.1 as polarity index and is mainly used for the extraction of terpenoids and tannins. Water is a common solvent having 10.2 polarity index which mainly extracts polar substances and is usually used for plant extracts with anti-microbial activity (Balamurugan *et al*., 2019).

Maceration technique was used as the extraction method to extract phytoconstituents from the leaf of *Costus speciosus* and stem of *Tinospora cordifolia*. This is an old method used mainly for the medicinal preparation. The method involves conversion of plant material into powder form by grinding, which helps in establishing good contact between solvent and plant material followed by mixing of solvent and material and need to be kept in closed vessels. The occasional shaking helps in increasing diffusion and helps in obtaining more extract yield (Azmir *et al*., 2013). This method is simple, does not require any complicated equipment and skilled operator. It is an energy saving process and suitable method for less potent and cost-effective drugs. But it is a very slow process, duration of extraction time is longer and requirement of solvent is also more (Rasul 2018).

Table no. 4.2. Names of the collected plant and their location.

Sr. No.	Name of the Plant	Part Used	Location	Coordinates
1	*Costus speciosus*	Leaf	Dhaushire, Ponda	Lat-15.423668º Lon-74.072318º
2	*Tinospora cordifolia*	Stem	Dhaushire, Ponda	Lat-15.423699º Lon-74.072618º

Table no. 4.3. Polarity indices of solvents.

Sr. No.	Name of solvent	Polarity indices
1	Water	10.2
2	Methanol	5.2
3	Chloroform	4.1

Table no. 4.4. Yield of the plant extracts with different solvents.

Sr. No	Name of plant	Type of extract	Extract yield (%)
1	*Costus speciosus*	Methanol	3.73 ± 0.05
		Chloroform	4.85 ± 0.21
		Water	5.20 ± 0.19
2	*Tinospora cordifolia*	Methanol	2.64 ± 0.23
		Chloroform	3.09 ± 0.24
		Water	7.15 ± 0.15

In our study, water extract of both the plants prepared using the leaves of *Costus speciosus* and stem of *Tinospora cordifolia* showed maximum amount of yield, as compared to the methanol and chloroform extracts. This may be attributed to the high polarity index of water thus, making it suitable to extract more phytochemical compounds. The water extract of *Tinospora cordifolia* showed 7.15 % yield whereas, *Costus speciosus* showed a yield of 5.20%, followed by chloroform and methanol extracts as shown in **Table 4.4** and **figure 4.1**. Since, traditional medicinal systems use water for the preparations of medicines for the treatments, it contains better capacity of extracting phytochemicals from plants and hence it can be used as a solvent for extracting phytoactive compounds from *Costus speciosus* and *Tinospora cordifolia*.

4.3. Phytochemical analysis

4.3.1. Qualitative phytochemical analysis

Phytochemicals are compounds that protects the plant from diseases, damage caused by pollution, UV, stress, and other environmental factors. These phytochemical compounds also play an important role in protection of human health (Balamurugan *et al*., 2019). The qualitative phytochemical analysis of methanol extract of leaf of *Costus speciosus* represented in **Plate 4.5** and **4.6** revealed the presence of alkaloids, proteins, terpenoids, and flavonoids while, methanol extract of *Tinospora cordifolia* showed the presence of alkaloids, terpenoids, and flavonoids (**Table 4.5** and **4.6**). The chloroform extract of *Costus speciosus* showed only the presence of terpenoids. Chloroform extract of *Tinospora cordifolia* showed presence of alkaloids, carbohydrates, saponins, and flavonoids. The water extract of *Costus speciosus* showed presence of saponins, proteins, terpenoids, and flavonoids. The water extract of *Tinospora cordifolia* showed positive results for alkaloids,

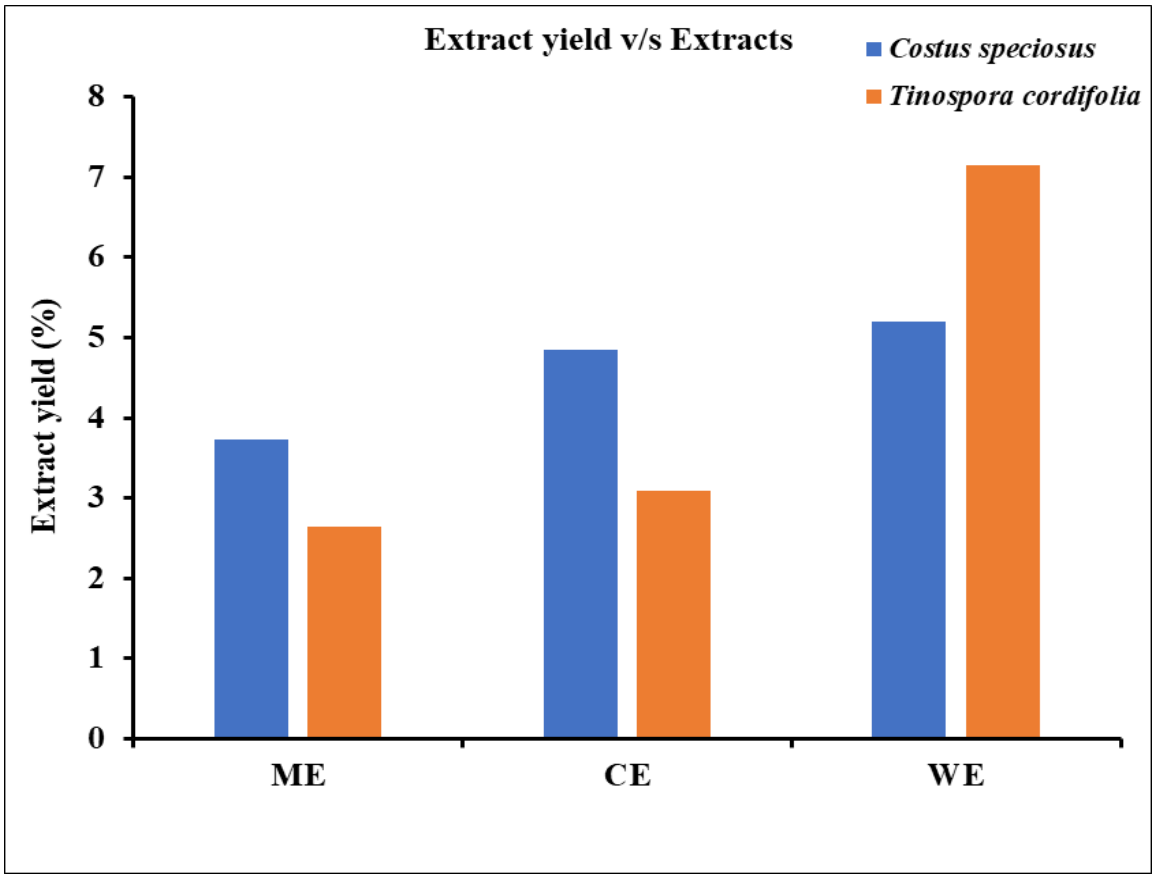


Figure 4.1. Graph of extract yield of *Costus speciosus* and *Tinospora cordifolia* with solvents such as methanol, chloroform, and water.

Plate 4.5. Preliminary qualitative phytochemical analysis of *Costus speciosus*. a. Methanol extract b. Chloroform extract. c. Water extract.

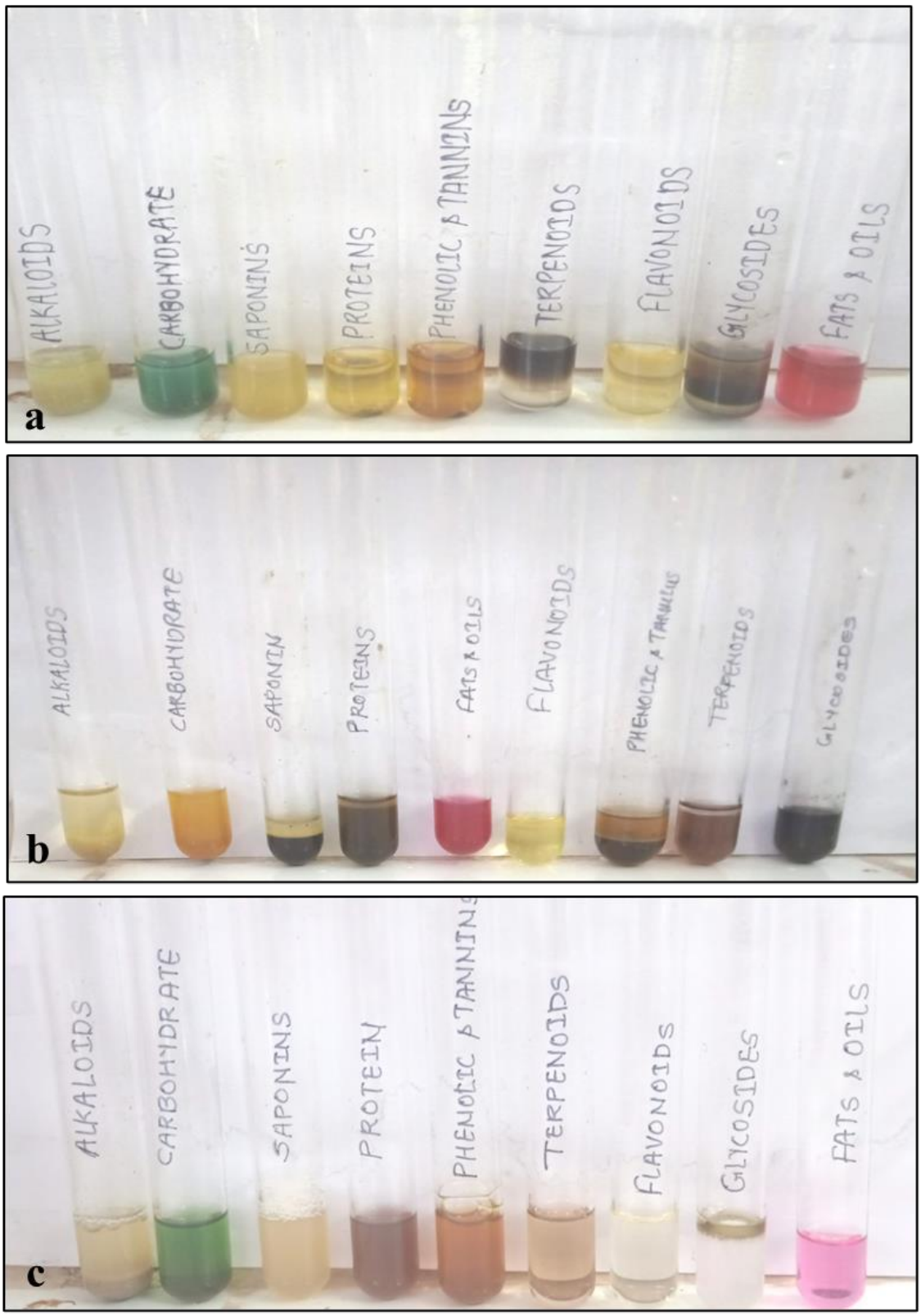


Plate 4.6. Preliminary qualitative phytochemical analysis of *Tinospora cordifolia* of a. Methanol extract b. chloroform extract c. water extract.

Table no. 4.5. Preliminary qualitative phytochemical analysis of *Costus speciosus*.

Tests	Methanol extract	Chloroform extract	Water extract
Test for Alkaloids (Mayer's Test)	+	+	+
Test for Carbohydrate (Benedict's Test)	-	+	-
Test for Saponins	-	+	+
Test for Proteins (Ninhydrin Test)	-	-	+
Test for Phenolic compounds and Tannins (Ferric Chloride Test)	-	-	+
Test for Terpenoids	+	-	+
Test for Flavonoids	+	+	+
Test for Glycosides	-	-	-
Test for Fats and Oils	-	-	-

(+) - Presence (-) - Absence

Table no. 4.6. Preliminary qualitative phytochemical analysis of *Tinospora cordifolia*.

Tests	**Methanol extract**	**Chloroform extract**	**Water extract**
Test for Alkaloids (Mayer's Test)	+	+	+
Test for Carbohydrate (Benedict's Test)	-	+	-
Test for Saponins	-	+	+
Test for Proteins (Ninhydrin Test)	-	-	+
Test for Phenolic compounds and Tannins (Ferric Chloride Test)	-	-	+
Test for Terpenoids	+	-	+
Test for Flavonoids	+	+	+
Test for Glycosides	-	-	-

(+) - Presence (-)- Absence

and phenolic compounds along with saponins, proteins, terpenoids, and flavonoids. Glycosides, fats, and essential oils showed negative results for both the plants. Among all the six extracts, water extract of *Tinospora cordifolia* showed a range of secondary metabolites identified using the standard methods.

The results obtained for phytochemical analysis of *Costus speciosus* were similar as reported for the methanolic and water extract of rhizome of *Costus speciosus* which revealed the presence ofalkaloids, flavonoids, cardiac glycosides, saponins, sterols, and tannins (Saraf 2009). Also, reported by Revathy *et al*. (2014) where, alkaloids, flavonoids, tannins, phenols, steroids, carbohydrates, amino acids, glycosides, proteins, ascorbic acid, coumarins, quinines, were identified in the ethanolic extract of rhizome of *Costus speciosus*. The results obtained for qualitative phytochemical screening of *Tinospora cordifolia* were similar as reported by Kaur *et al*. (2016), where a range of phytochemicals such as carbohydrates, glycosides, amino acids, flavonoids, phenols, and tannins were reported in the methanol extract of stem of the plant.

4.3.2. Quantitative phytochemical analysis

4.3.2.1. Determination of Total Flavonoid

The Total Flavonoid content of methanol extract of *Costus speciosus* was found to be 828.08 mg of quercetin/g of plant extract and that of *Tinospora cordifolia* was 343.91mg quercetin/g plant extract. The chloroform extract of *Costus speciosus* revealed 191.41mg quercetin/g of plant extract while of *Tinospora cordifolia* was 801.41mg of quercetin. Water extract of *Costus speciosus* revealed 1085 mg quercetin/g of plant extract and 159.75 mg quercetin of *Tinospora cordifolia* as shown in **Table 4.7**, **4.8** and **4.9**. The total flavonoid

Table no. 4.7. Preliminary quantitative phytochemical analysis of flavonoids using quercetin.

Quercetin concentration (µg/ml)	Absorbance at 415 nm			Mean	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
200	0.117	0.107	0.107	0.11	0.01
400	0.276	0.203	0.235	0.238	0.04
600	0.361	0.416	0.387	0.288	0.03
800	0.545	0.486	0.483	0.504	0.03
1000	1.425	1.356	1.356	1.379	0.04

Table no. 4.8. Preliminary quantitative phytochemical analysis of flavonoids of *Costus speciosus*.

Extracts (µg/ml)	Absorbance at 415nm			Mean	Total Flavonoid Content (mg quercetin/plant dry extract)
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
M.E	0.756	0.872	0.872	0.833	828.08 ± 0.07
C.E	0.067	0.071	0.069	0.069	191.41 ± 0.01
W.E	0.365	1.526	1.536	1.142	1085 ± 0.7

Table no. 4.9. Preliminary quantitative phytochemical analysis of flavonoids of *Tinospora cordifolia*.

Extracts (µg/ml)	Absorbance at 415nm			Mean	Total Flavonoid Content(mg quercetin/plant dry extract)
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
M.E	0.262	0.247	0.247	0.252	343.91 ± 0.01
C.E	0.301	1.046	1.057	0.801	801.41 ± 0.5
W.E	0.033	0.031	0.031	0.031	159.75 ± 0.01

content was found to be higher in the leaf water extract of *Costus speciosus* than *Tinospora cordifolia* as indicated in Plate **4.7** and **Figure 4.2**.

Previous reports have mentioned the total flavonoid content in the hydroalcoholic extract of rhizome of *Costus speciosus* was 0.647 mg quercetin/g of plant extract (Kujur *et al*., 2019). Also, flavonoid content of the same plant was found to be 9.32 mg quercetin/g of plant extract according to Jha *et al*. (2010). But in our study the results obtained show a very good amount of flavonoid content than the reported ones in the leaves of *Costus speciosus*. Similarly, in *Tinospora cordifolia* high content of total flavonoid in the methanolic stem extract was reported as 4.50 mg quercetin/g of plant extract (Garg and Garg 2018), ethanolic extract of the same plant reported highest flavonoid content of 66.28mg quercetin/g of plant extract (Upadhyay *et al*., 2013). But in our study again the total flavonoid content was higher than the already reported values from the available literatures.

4.3.2.2. Estimation of Tannins

The Total Tannin content of the methanolic extract of *Costus speciosus* was found to be 49 mg tannic acid/g of plant extract and *Tinospora cordifolia* contains maximum number of tannins of 56 mg tannic acid/g of plant extract as shown in **Table 4.10**, **4.11** and **4.12**. The chloroform extract of *Costus speciosus* and *Tinospora cordifolia* contains 0.16 mg and 6.66 mg tannic acid/g of plant extract respectively **(Plate 4.8)**. Water extract of *Costus speciosus* contains 41.16 mg tannic acid/g of plant extract and *Tinospora cordifolia* contain 2 mg tannic acid/g of plant extract **(Figure 4.3)**.

Sivakumar *et al*. (2010) reported tannin contents in the methanolic stem extract of *Tinospora cordifolia* with 8.75 mg tannic acid/g of plant extract whereas, the methanol extract of the same plant showed maximum amount of tannin content in our study.

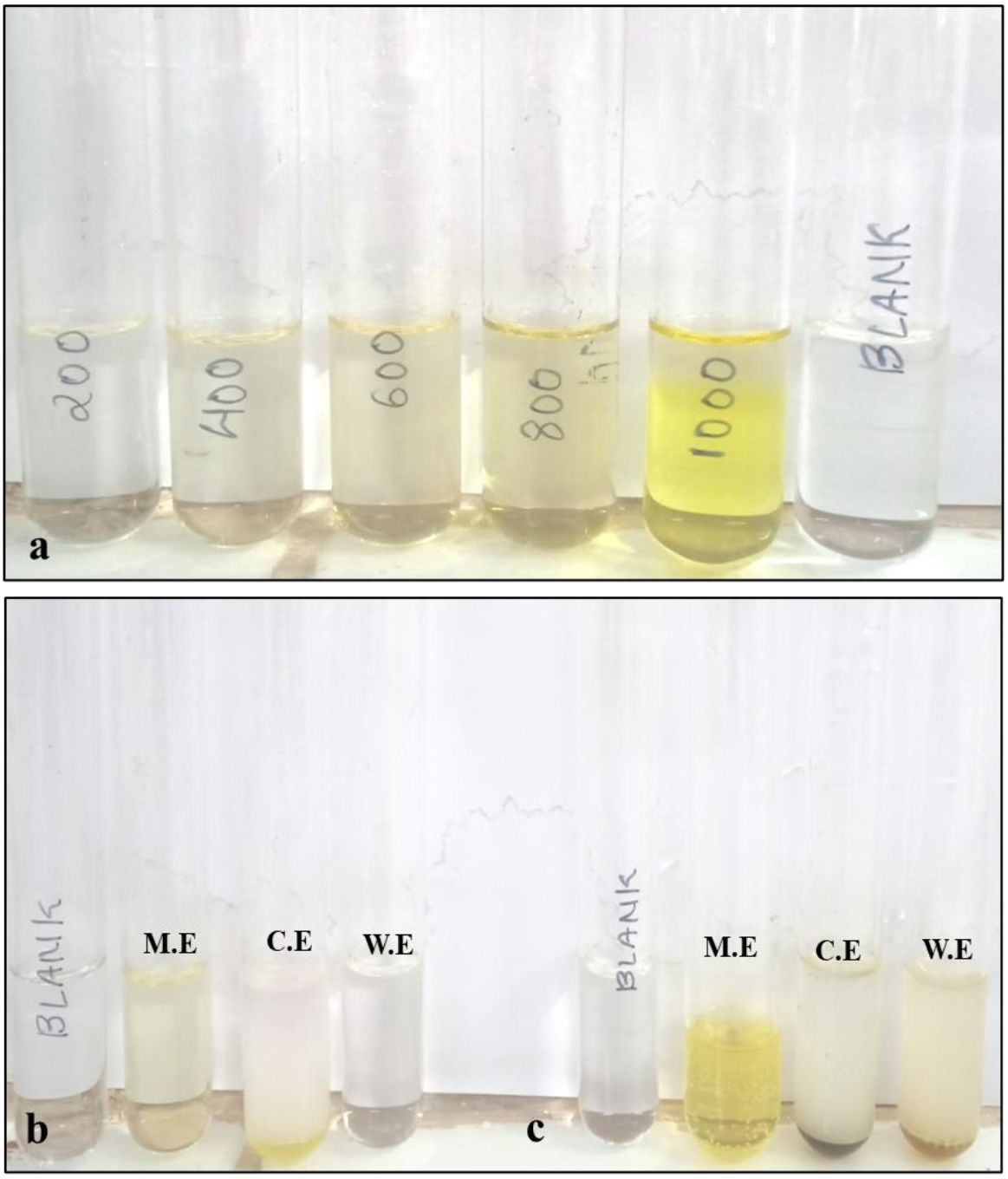
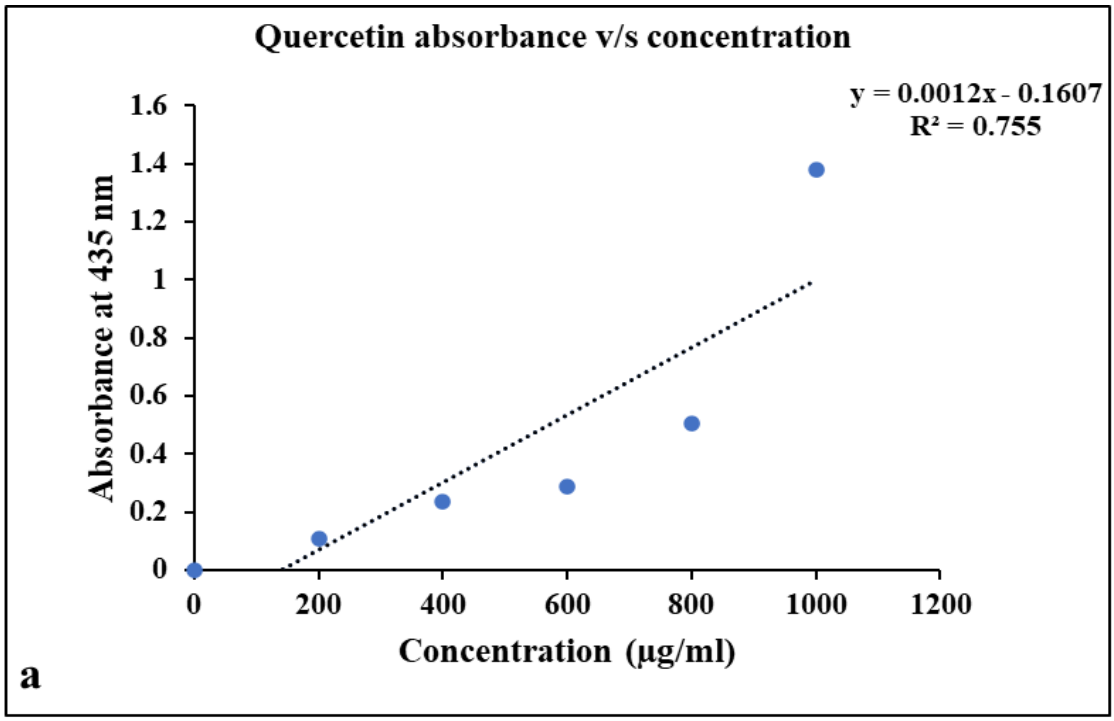


Plate 4.7. Quantitative estimation of flavonoids. a. Quercetin b. Methanol, Chloroform, and Water extract of *Tinospora cordifolia* c. Methanol extract, Chloroform, and Water extract of *Costus speciosus*.



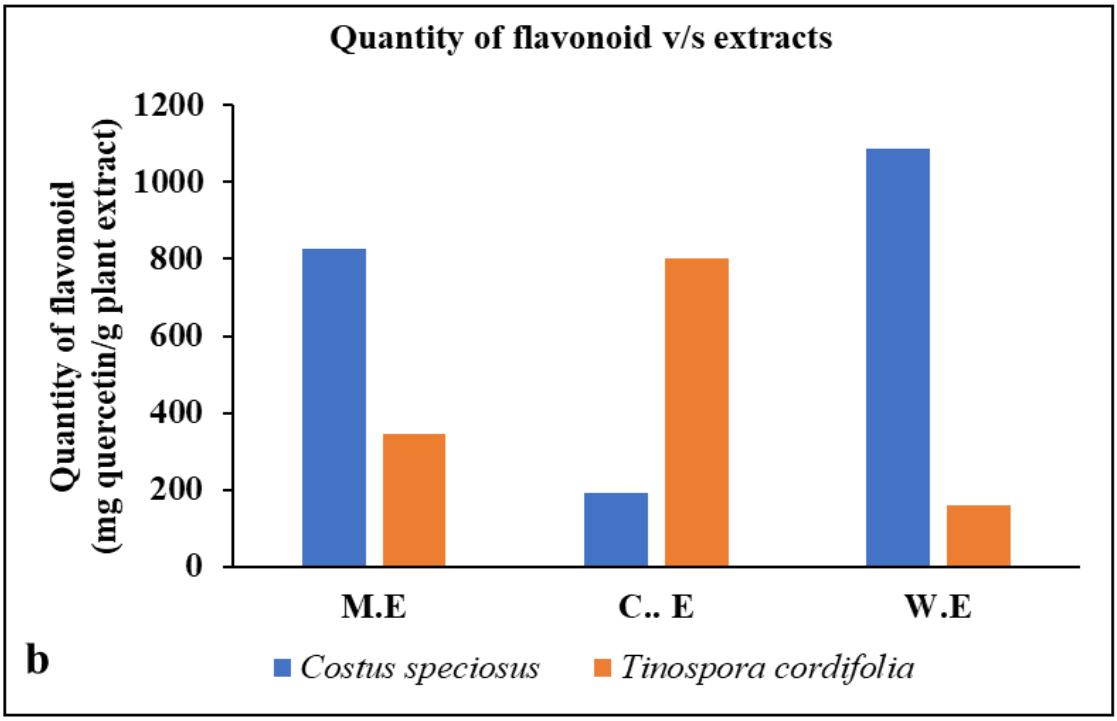


Figure 4.2. Graph representing determination of total flavonoids. a. Quercetin b. Quantity of flavonoids in extracts.

Table no. 4.10. Preliminary quantitative phytochemical analysis of Tannins with tannic acid.

Tannic acid concentration (µg/ml)	Absorbance at 725 nm			Mean	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
20	0.251	0.228	0.228	0.235	0.01
40	0.417	0.392	0.399	0.402	0.01
60	0.527	0.540	0.587	0.534	0.03
80	0.570	0.599	0.584	0.584	0.01
100	0.509	0.650	0.649	0.602	0.08

Table no. 4.11. Preliminary quantitative phytochemical analysis of Tannins of *Costus speciosus.*

Extracts (µg/ml)	Absorbance at 725 nm			Mean	Total Tannic Content (mg tannic acid/plant dry extract)
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
M.E	0.381	0.391	0.389	0.387	49± 0.0052
C.E	0.092	0.094	0.093	0.094	0.16 ± 0.0010
W.E	0.346	0.337	0.337	0.340	41.16 ±0.0051

Table no. 4.12. Preliminary quantitative phytochemical analysis of tannins of *Tinospora cordifolia*.

Extracts (µg/ml)	Absorbance at 725 nm			Mean	Total Tannic Content (mg tannic acid/plant dry extract)
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
M.E	0.420	0.434	0.435	0.429	56 ± 0.0083
C.E	0.096	0.098	0.097	0.097	6.66 ± 0.0010
W.E	0.116	0.100	0.100	0.105	2 ± 0.0092

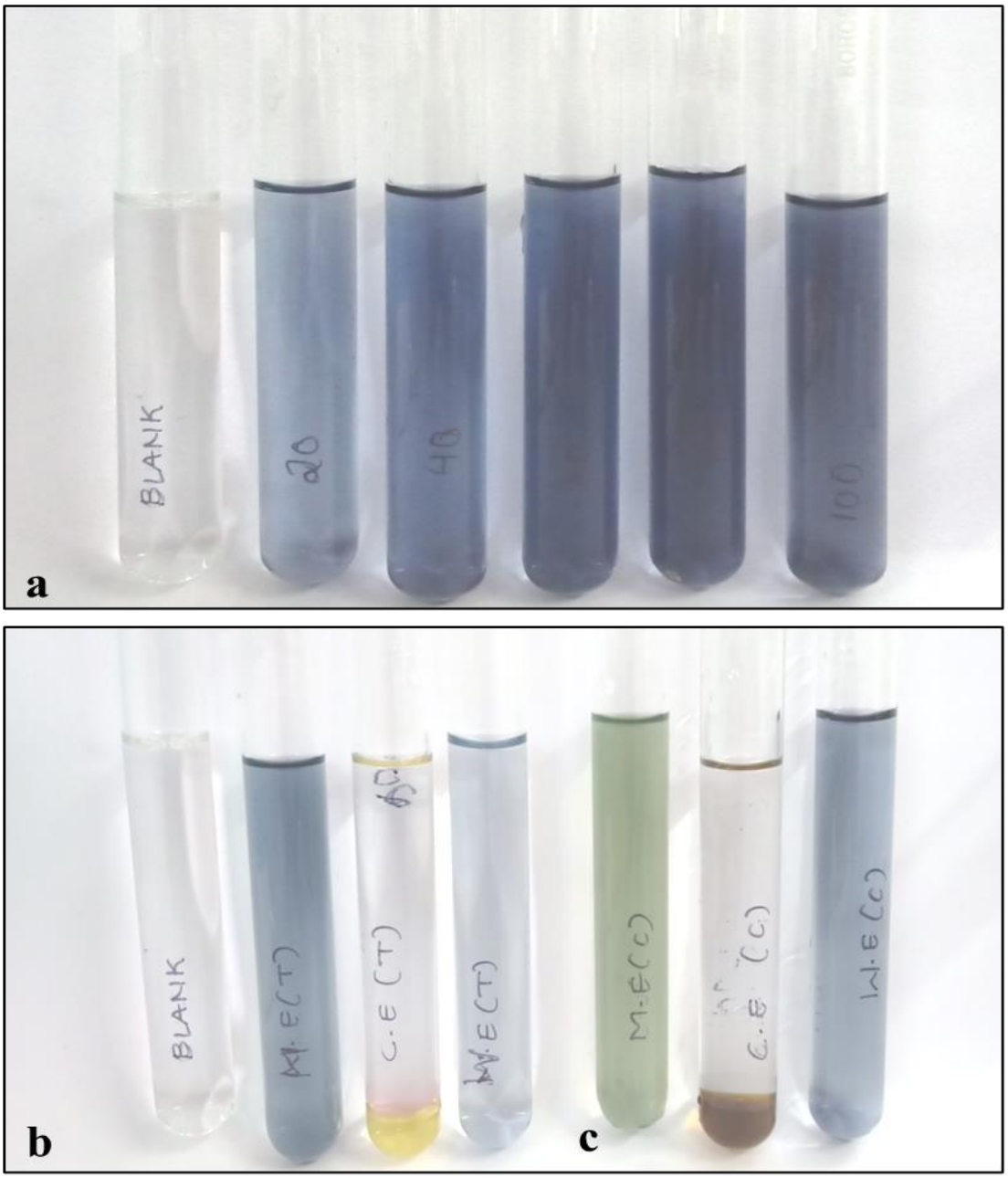


Plate 4.8. Quantification of Tannin. a. Tannic acid b. Methanol, chloroform, and water extract of *Tinospora cordifolia* c. Methanol, chloroform, and water extract of *Costus speciosus*.

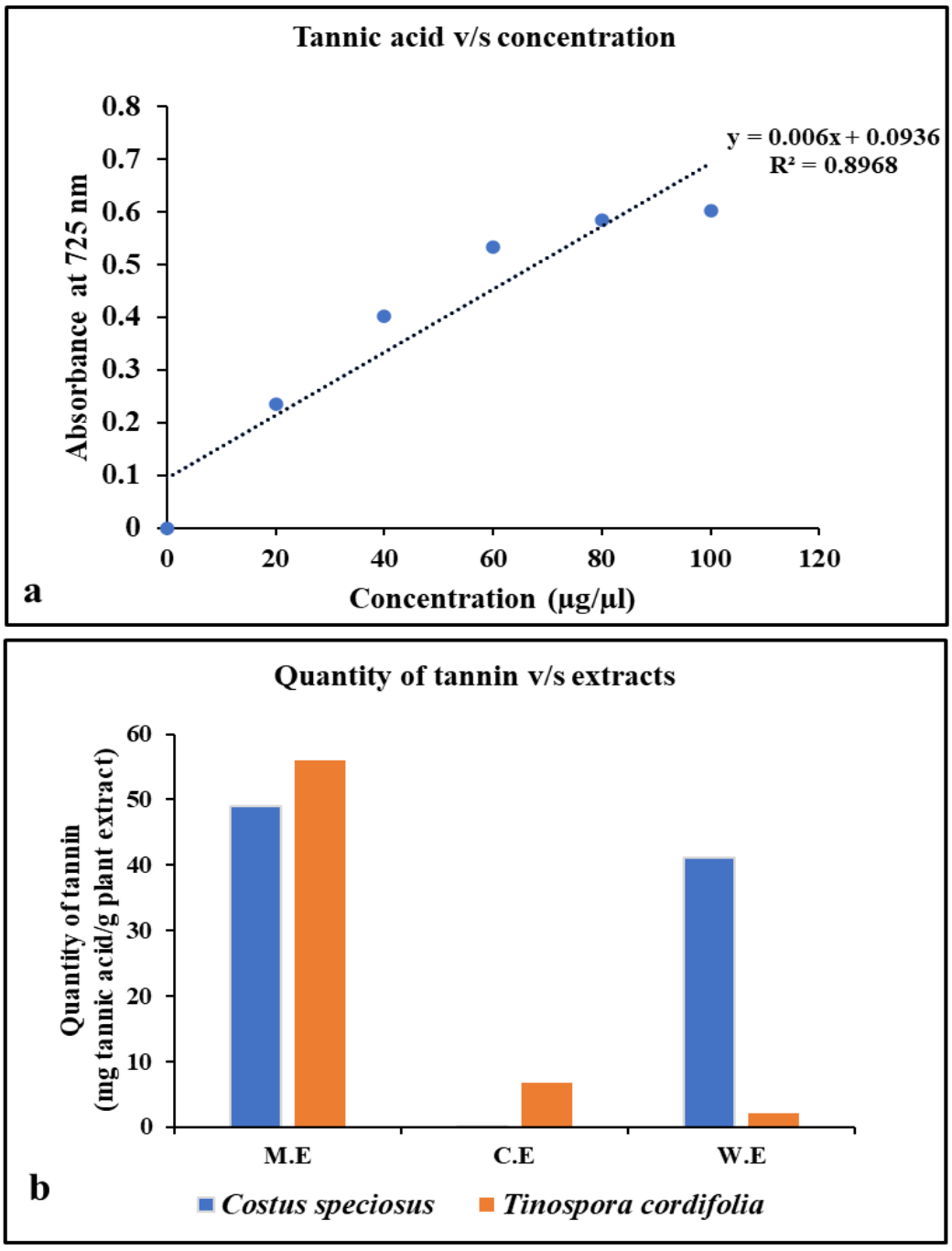


Figure 4.3 Graphs representing tannic acid a. Tannic acid b. Quantity of tannin present in extracts.

4.3.2.3. Determination of Total Phenols

The Total Phenolic content of the extracts was found with the help of Gallic acid as shown in **Table 4.13**, **4.14** and **4.15**, **Plate 4.9** and **Figure 4.4**. The maximum amount of total phenolic content in the methanol extracts of *Costus speciosus* and *Tinospora cordifolia* was found to be 62.10 mg and 80.47 mg Gallic acid/g of plant extract respectively. The chloroform extracts of *Costus speciosus* and *Tinospora cordifolia* was found to contain 52.65 mg and 50.47 mg Gallic acid/g of plant extract respectively. Water extracts of *Costus speciosus* and *Tinospora cordifolia* contains phenolic contents as 74.65mg and 52.47mg Gallic acid/g of plant extract respectively. While, Garwal *et al*. (2021) reported highest phenolic content in the ethanolic extract of 99.70 mg Gallic acid/g of plant extract which was more than the present study. The phenolic content was reported in methanolic extract of stem of *Tinospora cordifolia* as 7.21 mg Gallic acid/g of plant extract (Sivakumar *et al*., 2010).

4.3.2.4. Determination of Total alkaloids

The Total Alkaloid content of the extracts was found with the help of Bismuth nitrate as shown in **Table 4.16**, **4.17** and **4.18**, **Plate 4.10** and **Figure 4.5**. Higher amount of alkaloid content was found in the methanolic extract of *Costus speciosus* which as 85.39 mg bismuth nitrate per gram of plant extract. The least amount of alkaloid content was found in the methanolic extract of *Tinospora cordifolia* such as 9.06 mg bismuth nitrate per gram of plant extract. Chloroform extract of *Costus speciosus* and *Tinospora cordifolia* showed similar and maximum alkaloid content of 89.67 and 84.38mg bismuth nitrate per gram of plant extract. Water extract of the leaf of *Costus speciosus* contains more alkaloid content than the stem of *Tinospora cordifolia* which reported 44.83 and 26.19mg bismuth nitrate per gram of plant extract.

Table no. 4.13. Quantitative phytochemical analysis of phenols using Gallic acid as standard.

Gallic acid concentration (µg/ml)	Absorbance at 760nm			Mean	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
20	0.139	0.134	0.134	0.135	0.01
40	0.301	0.104	0.303	0.236	0.1
60	0.147	0.393	0.352	0.297	0.13
80	0.277	0.386	0.745	00.469	0.24
100	0.312	0.318	1.028	0.552	0.41

Table no. 4.14. Quantitative analysis of phenols of *Costus speciosus*.

Extracts (µg/ml)	Absorbance at 760 nm			Mean	Total Phenol Content (mg gallic acid/plant dry extract)
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
M.E	0.419	0.359	0.360	0.379	62.10 ± 0.03
C.E	0.317	0.328	0.325	0.323	52.65 ± 0.01
W.E	0.449	0.501	0.421	0.457	74.65 ± 0.04

Table no. 4.15. Quantitative analysis of phenols of *Tinospora cordifolia*.

Extracts (µg/ml)	Absorbance at 760 nm			Mean	Total Phenol Content (mg gallic acid/plant dry extract)
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
M.E	0.404	0.393	0.645	0.480	80.47 ± 0.14
C.E	0.291	0.340	0.324	0.318	50.47 ± 0.02
W.E	0.342	0.352	0.302	0.332	52.47 ± 0.02

Plate 4.9. Quantitative estimation of Phenols. a. Gallic acid b. Methanol, chloroform, and water extract of *Tinospora cordifolia* c. Methanol, chloroform, and water extract of *Costus speciosus*.

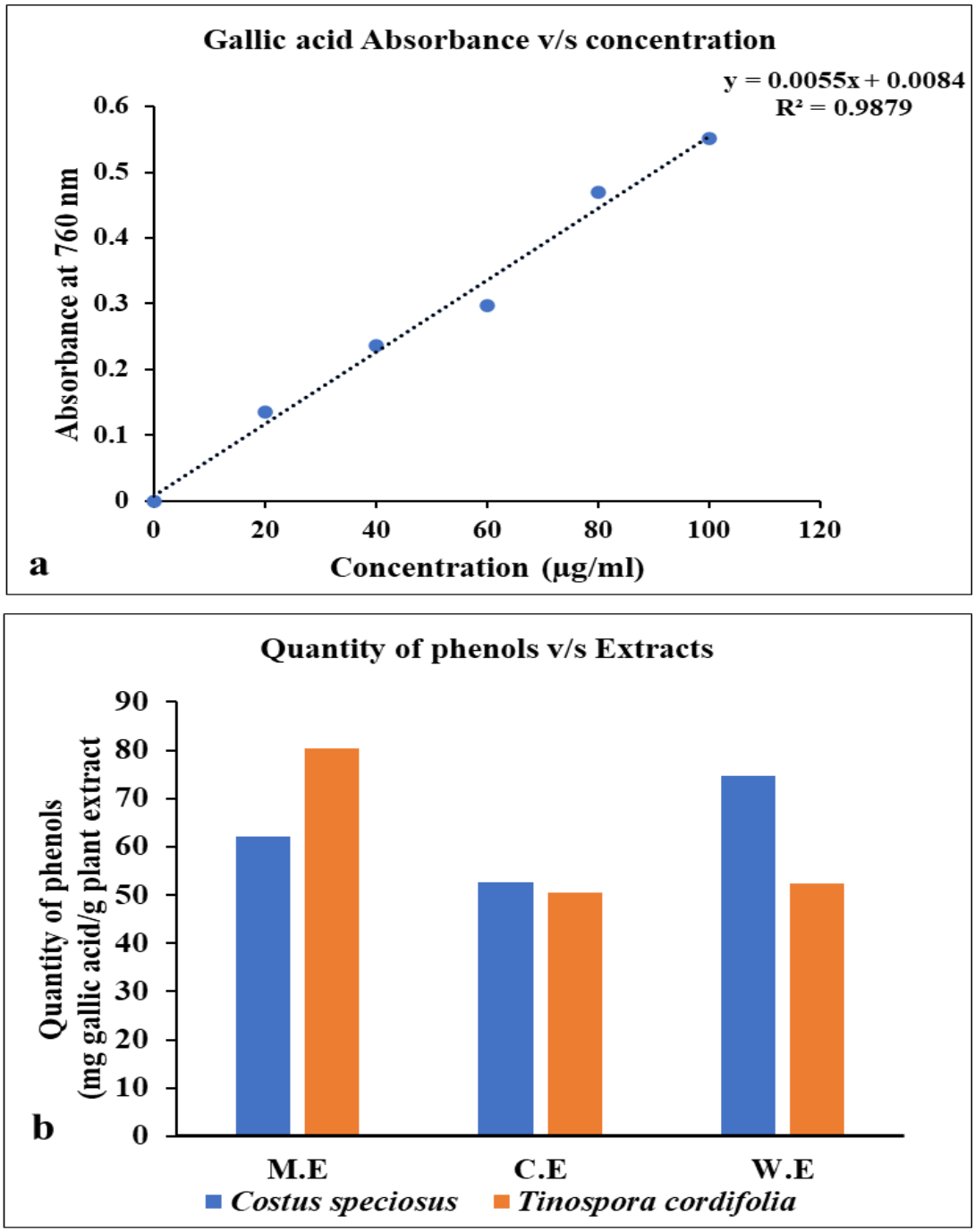


Figure 4.4. Graph representing determination of total phenols a. Gallic acid b. Quantity of phenols present in extracts.

Table no. 4.16. Quantitative phytochemical analysis of Alkaloids using Bismuth nitrate as standard.

Bismuth nitrate concentration (µg/ml)	Absorbance at 435 nm			Mean	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
1	0.031	0.032	0.029	0.030	0.01
2	0.062	0.060	0.083	0.068	0.01
3	0.119	0.119	0.138	0.125	0.01
4	0.148	0.156	0.177	0.160	0.01
5	0.222	0.225	0.225	0.224	0.01
6	0.229	0.229	0.313	0.257	0.04
7	0.252	0.256	0.269	0.259	0.01
8	0.297	0.308	0.307	0.304	0.01
9	0.354	0.356	0.362	0.357	0.01

Table no. 4.17. Quantitative phytochemical analysis of Alkaloids of *Costus speciosus*.

Extracts (µg/ml)	Absorbance at 435 nm			Mean	Total Alkaloid Content (mg bismuths/plant dry extract)
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
M.E	0.328	0.327	0.410	0.355	89.67 ± 0.04
C.E	0.329	0.335	0.351	0.338	85.39 ± 0.01
W.E	0.157	0.175	0.201	0.177	44.83 ± 0.02

Table no. 4.18. Quantitative phytochemical analysis of Alkaloids of *Tinospora cordifolia*.

Extracts (µg/µl)	Absorbance at 435 nm			Mean	Total Alkaloid Content (mg bismuth/plant dry extract)
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
M.E	0.033	0.035	0.038	0.035	9.06 ± 0.01
C.E	0.068	0.149	0.092	0.103	26.19 ± 0.04
W.E	0.324	0.340	0.339	0.334	84.38 ± 0.01

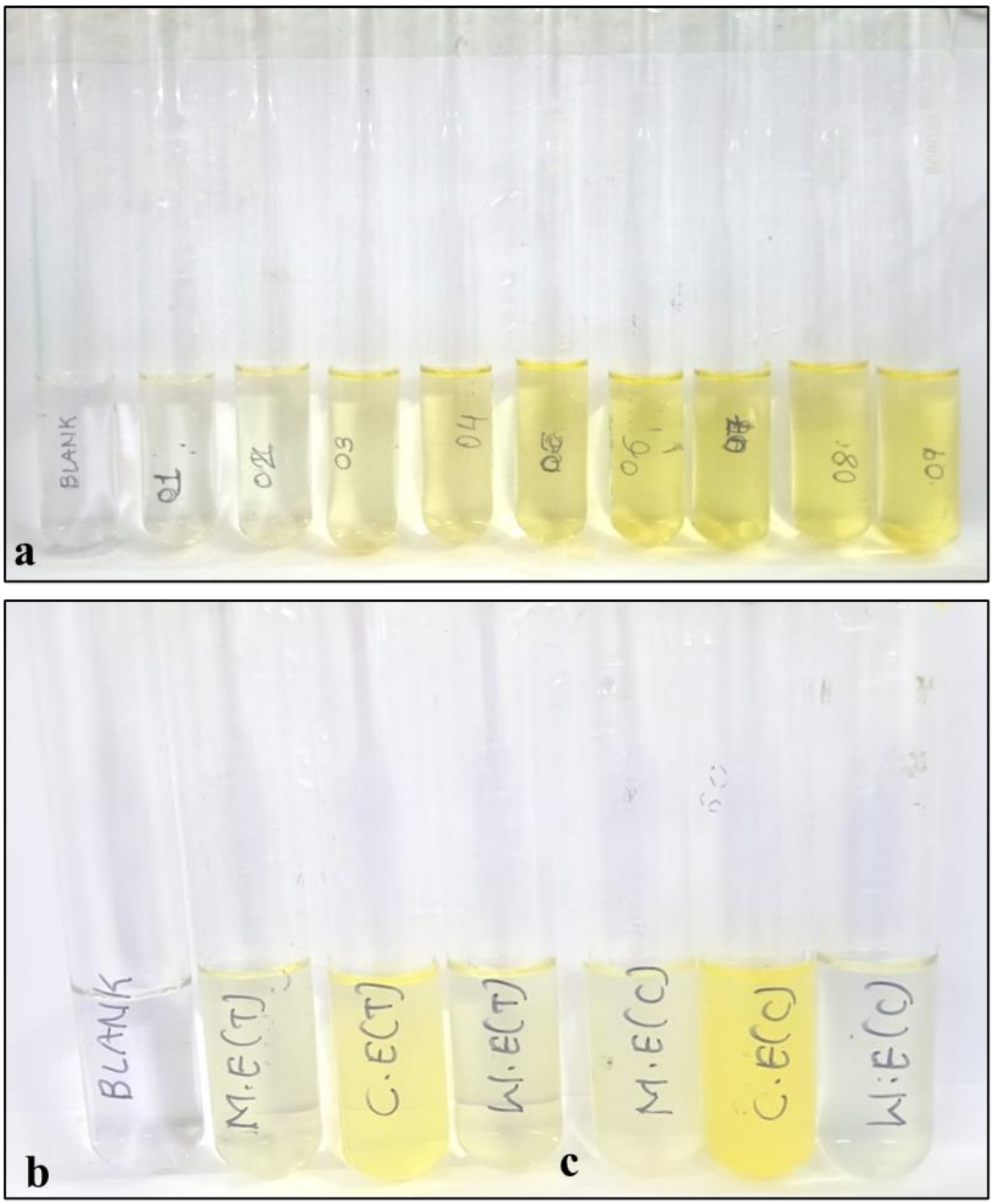


Plate 4.10. Quantitative estimation of alkaloid. A. Bismuth nitrate b. Methanol, chloroform, and water extract of *Tinospora cordifolia* c. Methanol, chloroform, and water extract of *Costus speciosus*.

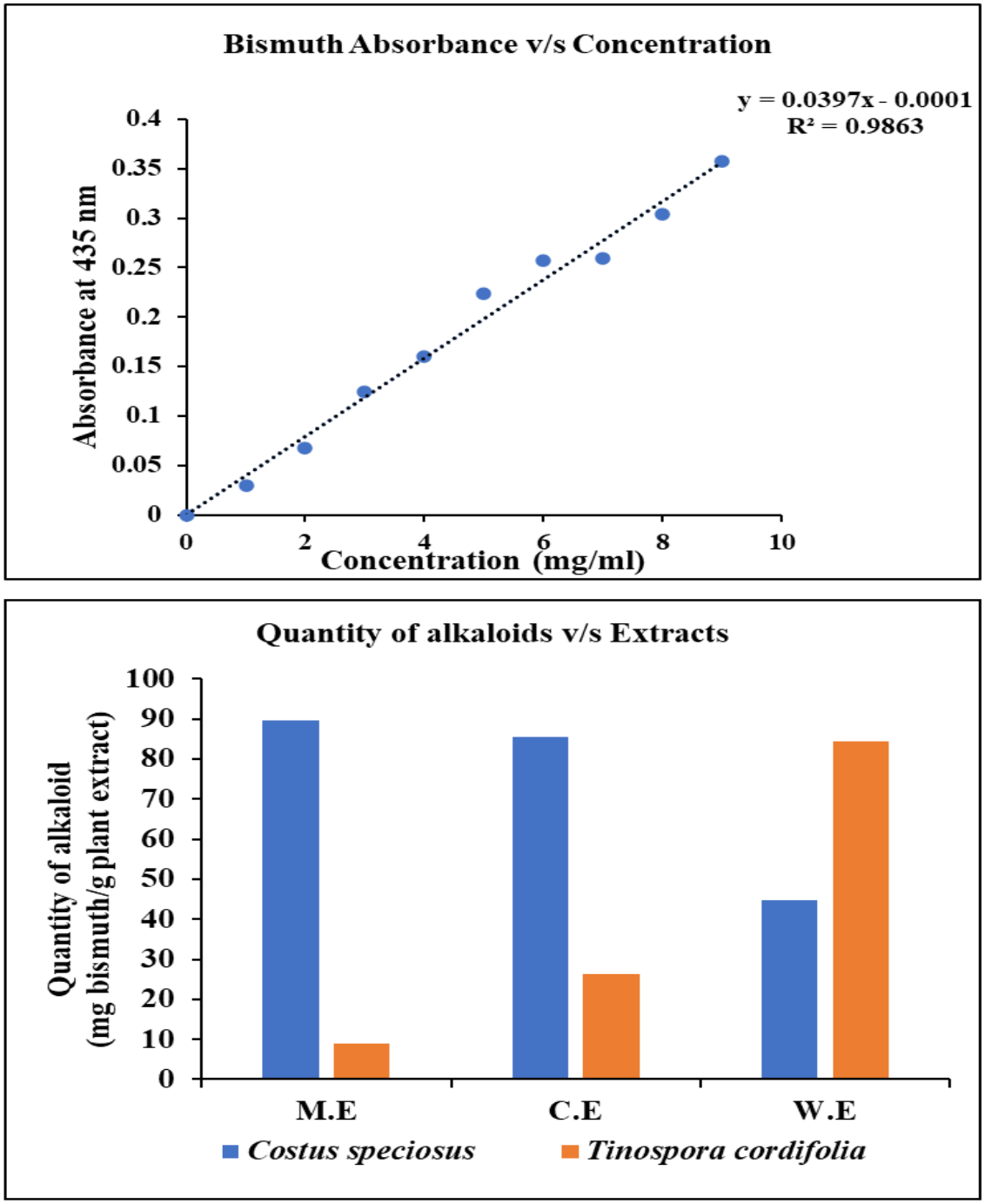


Figure 4.5. Graph representing determination of total alkaloids. a. Bismuth nitrate b. Quantity of alkaloids present in extracts.

4.4. Antioxidant activity

The antioxidant activity of *Costus speciosus* and *Tinospora cordifolia* were determined using the DPPH free radical scavenging activity according to Blois 1958, Madhu *et al*., 2020. DPPH is a stable, dark purple colour antioxidant compound which changes to yellow colour after getting scavenged and measures at 517nm in UV-visible spectrophotometer. Ascorbic acid, the natural antioxidant was used as the standard to determine the antioxidant activity of all the plant extracts.

The antioxidant activity of ascorbic acid showed good activity with an IC_{50} value of 41.55µg/ml as shown in (**Table 4.19**, **4.20**, **4.21**, **Plate 4.11**, **4.12**, **4.13** and **Figure 4.6**, **4.7**,and **4.8**). The methanol extract of *Tinospora cordifolia* showed more antioxidant activity than the methanol extract of *Costus speciosus* with IC_{50} values as 178.45 and 206.50 µg/ml respectively. The antioxidant activity of chloroform extract of *Costus speciosus* shows good scavenging activity as compared to *Tinospora cordifolia* with 176.55 and 211.37 µg/ml IC_{50} values respectively. The water extract of *Tinospora cordifolia* also showed good activity than *Costus speciosus* with IC_{50} values as 182.55 and 207.64 µg/ml respectively. The chloroform extract of *Costus speciosus* exhibits best antioxidant activity, but results shows that *Tinospora cordifolia* possesses good antioxidant activity.

The antioxidant activity of *Costus speciosus* was found to be higher in the methanolic extract as compared to other solvents like water and chloroform (Jagtap and Satpute 2014). But the present study shows that chloroform extract of *Costus speciosus* contains more antioxidant activity. The IC_{50} value of ascorbic acid was 14.11 µg/ml and that of the methanol rhizome extract of *Costus speciosus* was found to be 67.37 µg/ml (Kujur *et al*., 2019). Rhizome extracts

Table no. 4.19. DPPH free radical scavenging assay: % scavenging activity of DPPH by ascorbic acid and methanolic, chloroform and water extract of *Costus speciosus*.

Sr. No	Concentration (µg/ml)	L-Ascorbic acid	*Costus speciosus*		
			M.E	C.E	W.E
1	12.5	18.48 ± 0.10	3.60 ± 0.01	7.85 ± 0.01	2.22 ± 0.01
2	25	66.61 ± 0.23	7.80 ± 0.01	10.44 ± 0.01	3.49 ± 0.01
3	50	85.46 ± 0.01	10.8 ± 0.01	19.51 ± 0.01	13.33 ± 0.01
4	100	89.27 ± 0.03	24.32 ± 0.01	30.89 ± 0.01	33.11 ± 0.01
5	200	94.56 ± 0.01	48.37 ± 0.01	45.79 ± 0.03	53.96 ± 0.01

Table no. 4.20. DPPH free radical scavenging assay: % scavenging activity of DPPH by ascorbic acid and methanolic, chloroform and water extract of *Tinospora cordifolia.*

Sr. No	Concentration (µg/ml)	L-Ascorbic acid	Tinospora cordifolia		
			M.E	C.E	W.E
1	12.5	18.48 ± 0.14	0.54 ± 0.01	0.87 ± 0.01	10.27 ± 0.01
2	25	66.61 ± 0.23	9.45 ± 0.01	2.10 ± 0.01	11.48 ± 0.01
3	50	85.46 ± 0.01	21.45 ±0.01	13.66 ± 0.08	24.77 ± 0.01
4	100	89.27 ± 0.03	30.90 ± 0.01	24.34 ± 0.01	38.36 ± 0.01
5	200	94.56 ± 0.01	54.18 ± 0.01	44.65 ± 0.01	48.94 ± 0.01

Table no. 4.21. IC_{50} values of methanol, chloroform and water extract of *Costus speciosus* and *Tinospora cordifolia*.

Sr. No	Sample	Type of extract	IC_{50} Value
1	L-Ascorbic acid		41.55
2	*Costus speciosus*	Methanol extract	206.50
		Chloroform extract	176.55
		Water extract	207.64
3	*Tinospora cordifolia*	Methanol extract	178.45
		Chloroform extract	211.37
		Water extract	182.55

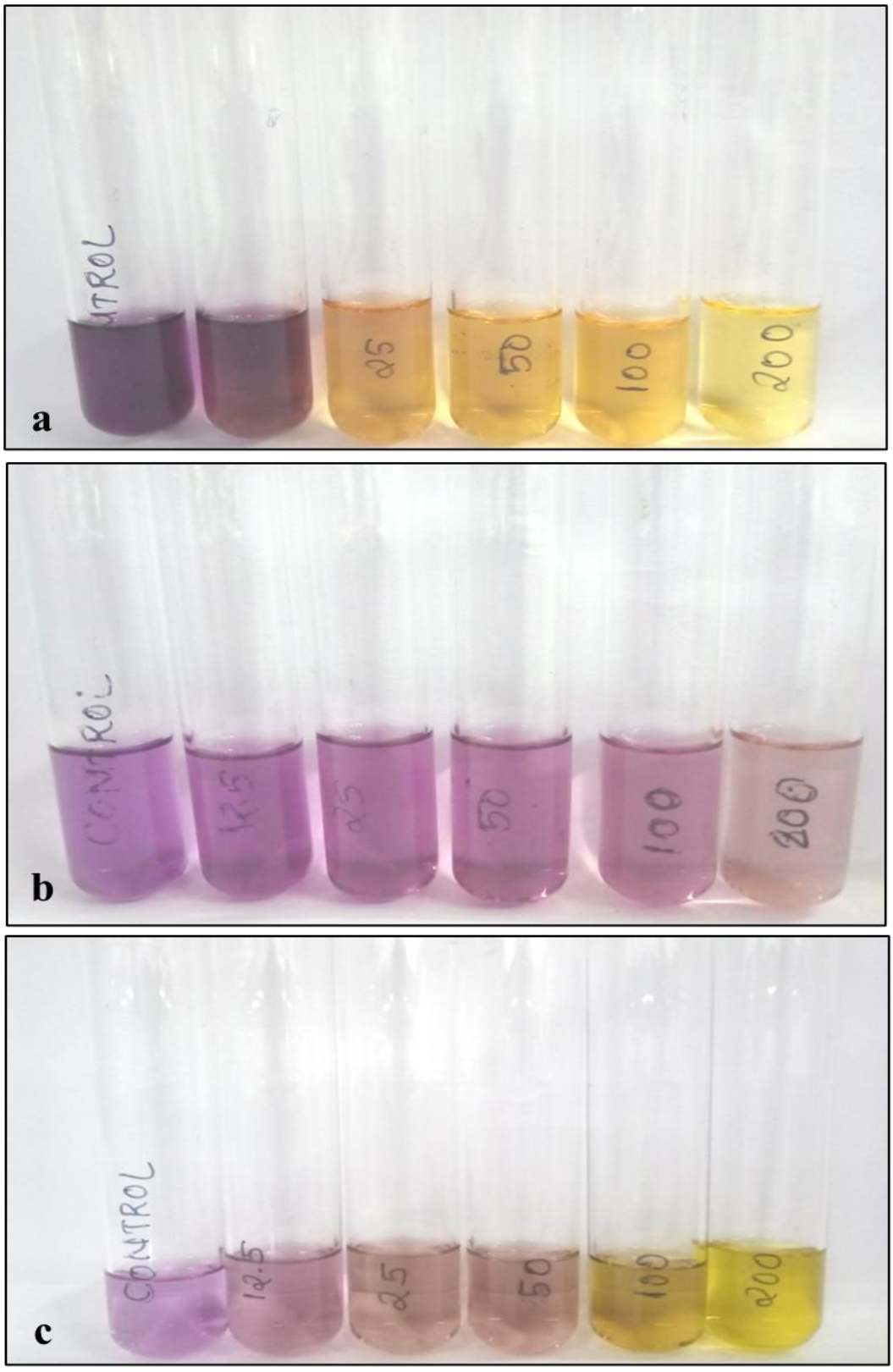


Plate 4.11. Determination of antioxidant activity. a. Ascorbic acid b. Methanol extract of *Costus speciosus* c. Chloroform extract of *Costus speciosus*.

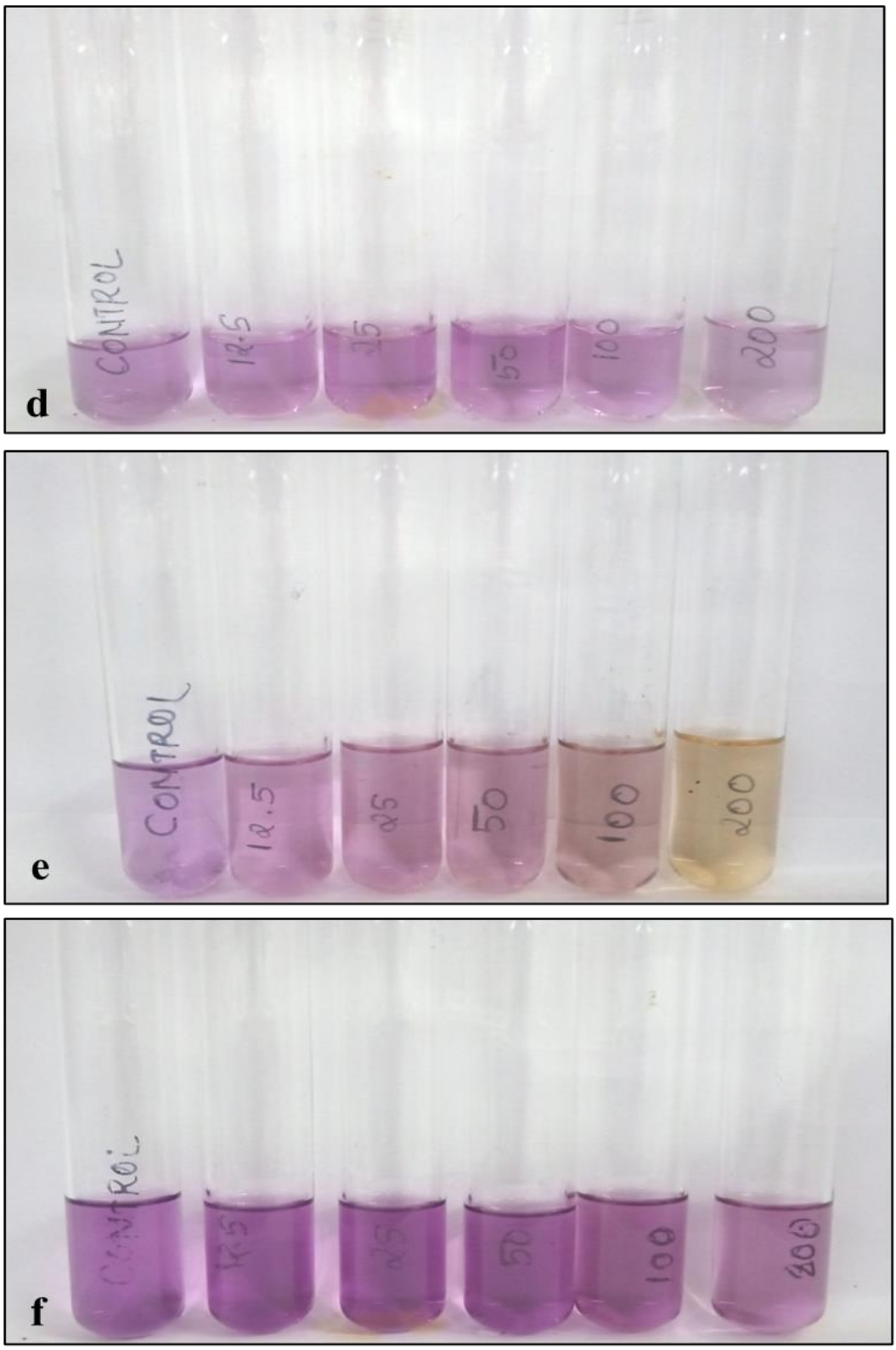


Plate 4.12. Determination of antioxidant activity. d. Water extract of *Costus speciosus* e. Methanol extract of *Tinospora cordifolia* f. Chloroform extract of *Tinospora cordifolia*.

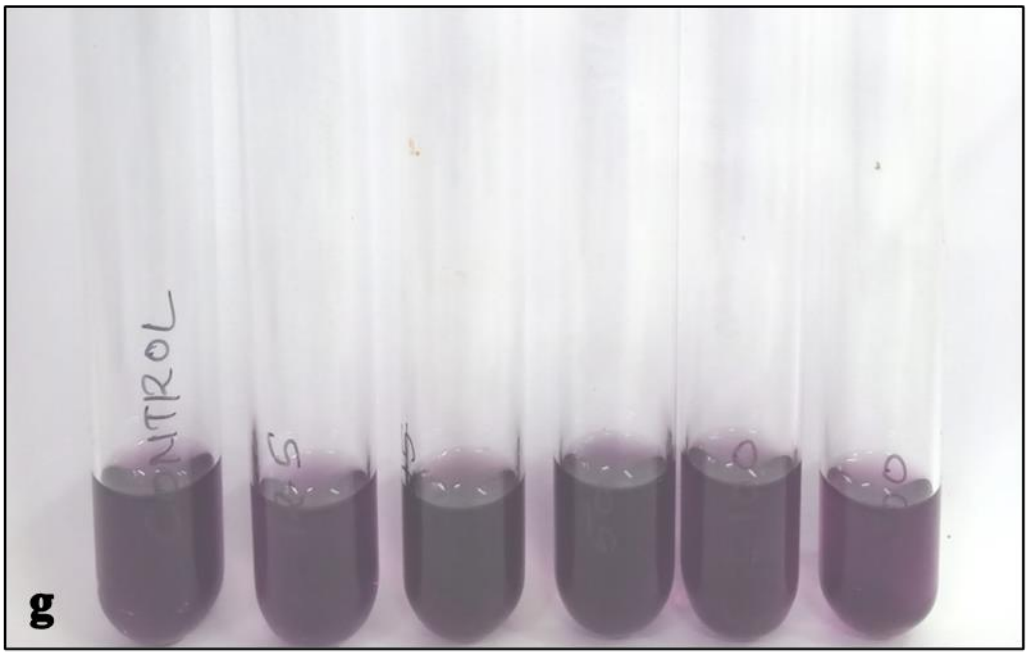


Plate 4.13. Determination of antioxidant activity: g. Water extract of *Tinospora cordifolia*.

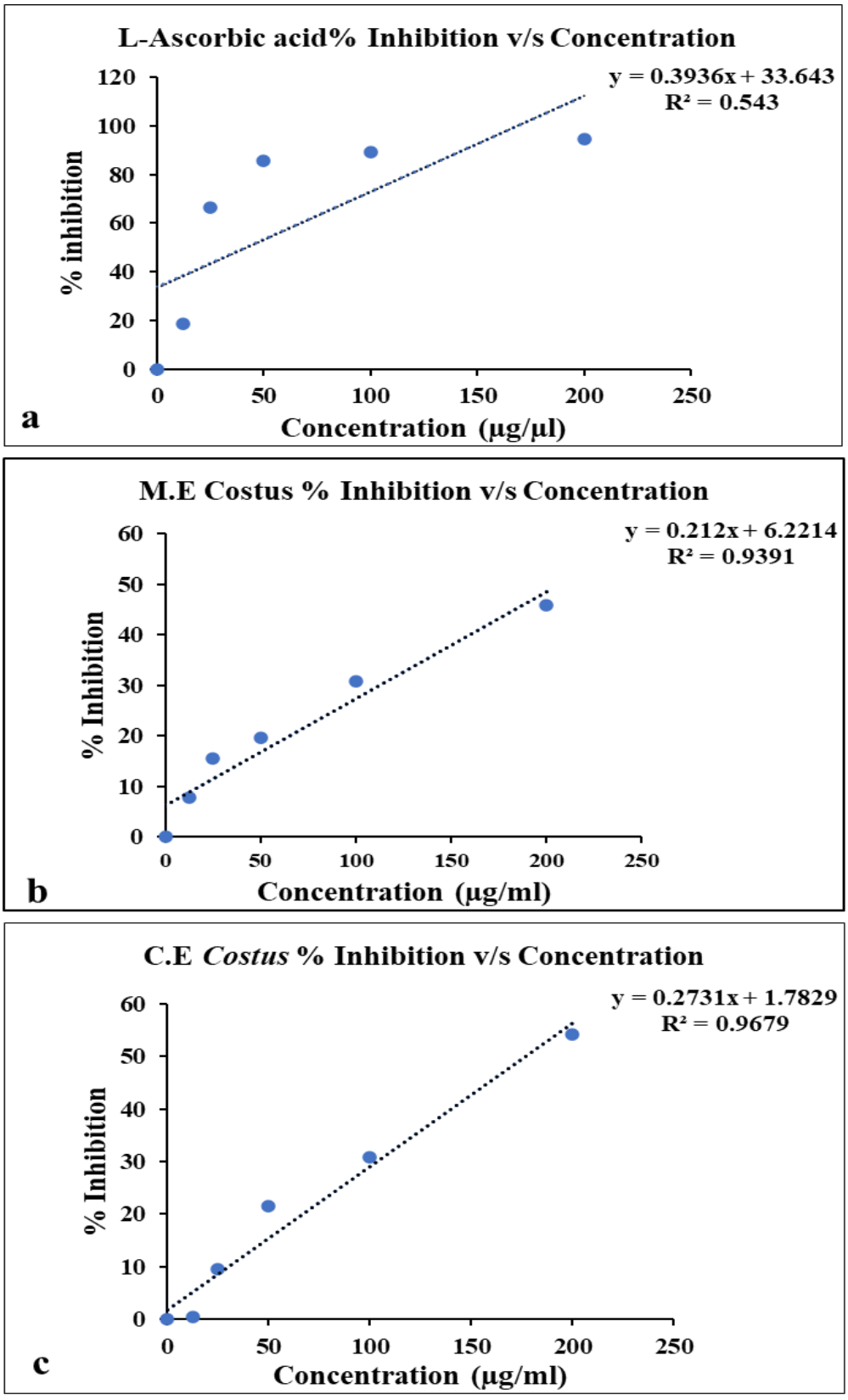


Figure 4.6. Graph of determination of antioxidant activity. a. L-ascorbic acid b. methanol extract of *Costus* c. chloroform extract of *Costus*.

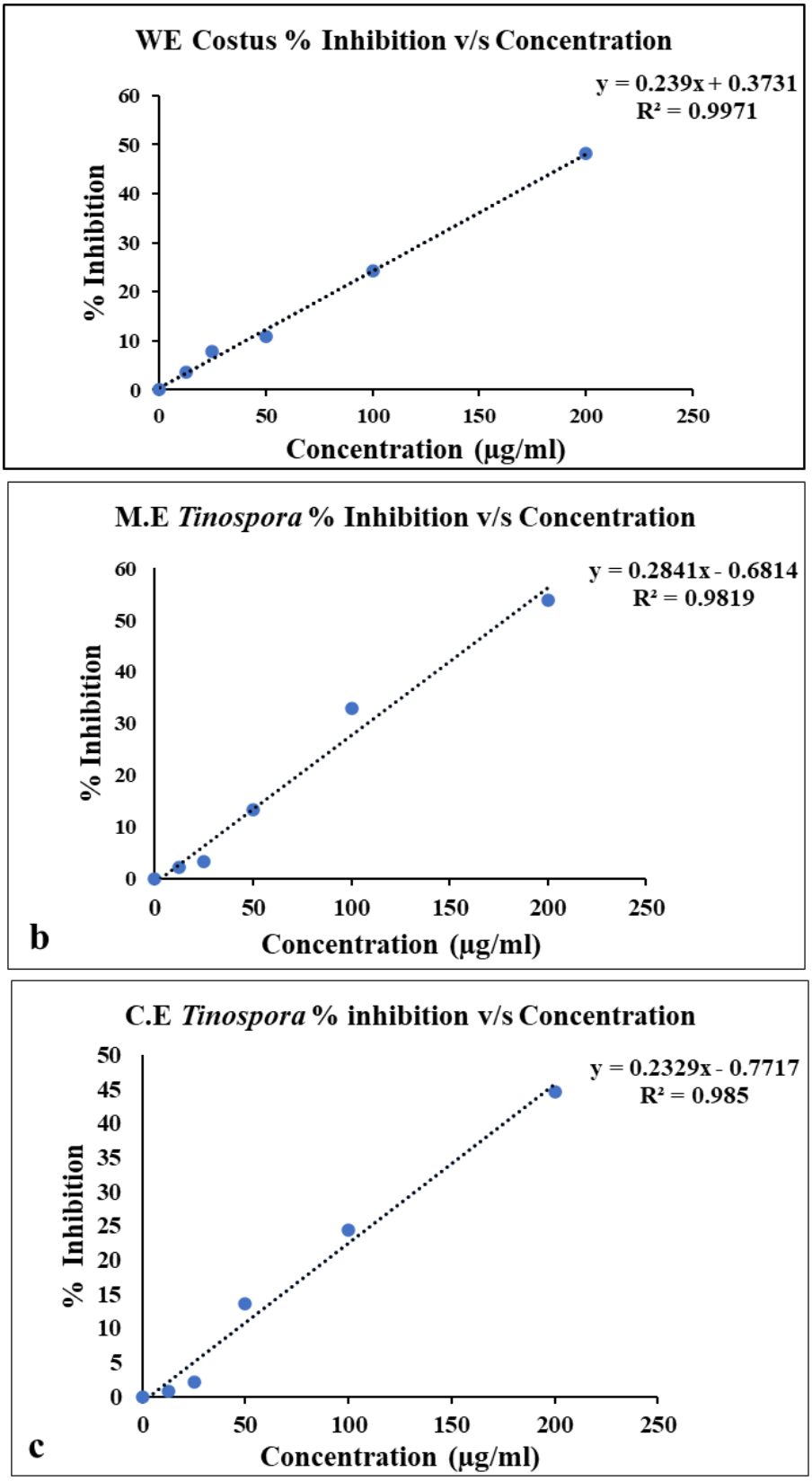
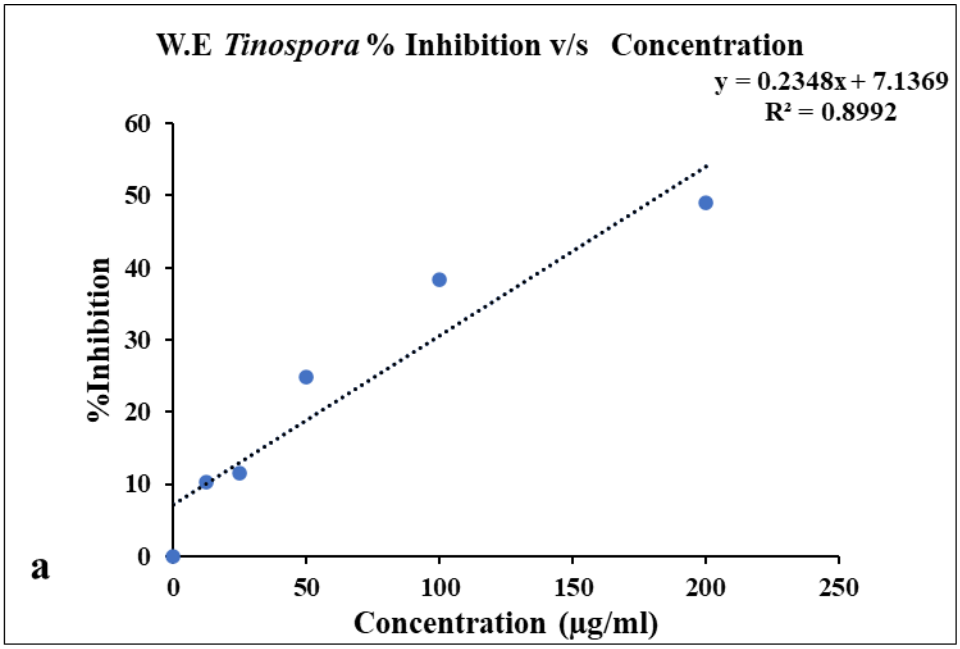


Figure 4.7. Graph of determination of antioxidant activity. a. water extract of *Costus* b. methanol extract of *Tinospora* c. chloroform extract of *Tinospora*.



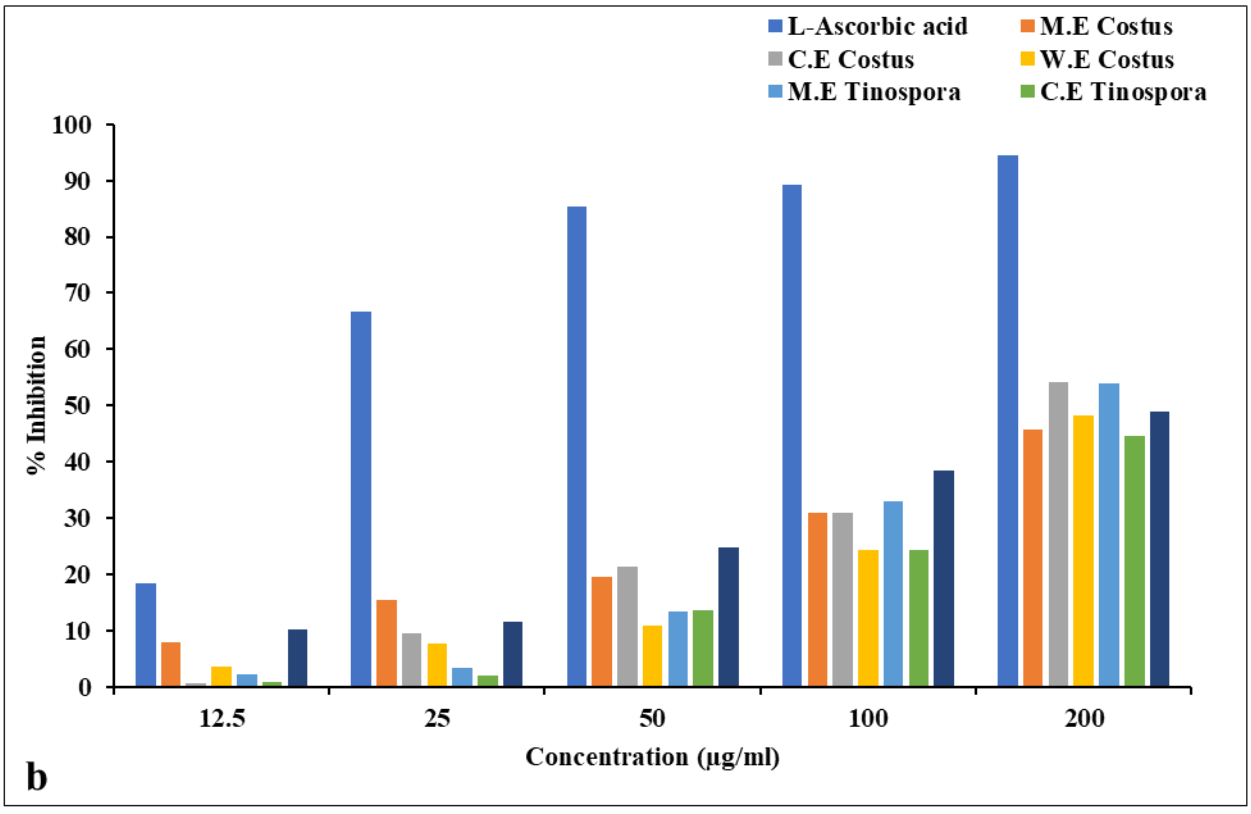


Figure 4.8. Graph of determination of antioxidant activity. a. Water extract of *Tinospora* b. Comparative graph of L-ascorbic acid and the extracts of *Costus* and *Tinospora*.

of *Costus speciosus* with methanol and acetone showed good free radical scavenging activity which are performed by DPPH and metal chelating methods (Behera *et al*., 2020).

Free radical scavenging activity of stem extract of *Tinospora cordifolia* was reported high with ethanol extract as 71.49% (Upadhyay *et al*., 2014). The IC_{50} value of the same plant was found to be 97.68 µg/ml in the aqueous extract and 106.80 µg/ml in the ethanol extract (Jain *et al*., 2010). In the literature it was reported that ethanol and aqueous extracts possess good antioxidant activity and the present study also showed that methanol contains good antioxidant activity.

4.5. Anti-diabetic activity

The anti-diabetic activity was determined by starch iodine alpha amylase inhibition assay. It is based on the reduction of starch iodine complex as the concentration increases, which indicates the breakdown of starch and hence, the activity of alpha amylase can be detected. The complex formed which is blue to dark brown in colour is measured at 620 nm (Chimaobi *et al*., 2020). Acarbose was used as the standard inhibitor to detect the activity of the plant extracts.

It was observed that as the concentration increases the absorbance also increases which shows that alpha amylase attains good inhibition activity at the highest concentration. Acarbose showed good activity as shown in **(Table 4.22**, **4.23**, **4.24**, **4.25**, **4.26**, **4.27** and **4.28)**. At least concentration that is 20 µg/ml the absorbance was 0.589 and 1.11 at the highest concentration of 100 µg/ml. The methanol extract of *Tinospora cordifolia* has good anti-diabetic activity which was observed based on the absorbance at 20 µg/ml as 0.713 and 1.102 at 100 µg/ml. The same extract of *Costus speciosus* showed little less activity observed with absorbance at 20 µg/ml and 100 µg/ml as 0.698 and 0.944 respectively **(Plate 4.14**, **4.15**, **4.16** and **Figure 4.9**, **4.10**,and **4.11)**.

Table no. 4.22. Anti-diabetic activity of Acarbose as standard by Starch -Iodine Alpha Amylase Inhibition.

Sr. No	Concentration (µg/mL)	Absorbance at 620 nm			Mean	Deviation
		Absorbance 1	Absorbance 2	Absorbance 3		
1	Control	1.135	1.134	1.136	1.135	0.01
2	20	0.589	0.590	0.589	0.589	0.01
3	40	0.650	0.652	0.650	0.650	0.01
4	60	0.889	0.892	0.890	0.890	0.01
5	80	0.949	0.951	0.950	0.950	0.01
6	100	1.112	1.108	1.111	1.110	0.01

Table no. 4.23. Anti-diabetic activity of methanol extract of *Costus speciosus* by Starch -Iodine Alpha Amylase Inhibition.

Sr. No	Concentration (µg/mL)	Absorbance at 620 nm			Mean	Deviation
		Absorbance 1	Absorbance 2	Absorbance 3		
1	control	0.987	0.986	0.987	0.987	0.01
2	20	0.696	0.698	0.701	0.698	0.01
3	40	0.721	0.740	0.749	0.736	0.01
4	60	0.792	0.784	0.812	0.796	0.01
5	80	0.843	0.889	0.873	0.868	0.02
6	100	0.923	0.949	0.962	0.944	0.01

Table no. 4.24. Anti-diabetic activity of chloroform extract of *Costus speciosus* by Starch -Iodine Alpha Amylase Inhibition.

Sr. No	Concentration (µg/mL)	Absorbance at 620 nm			Mean	Deviation
		Absorbance 1	Absorbance 2	Absorbance 3		
1	control	1.109	1.100	1.100	1.100	0.01
2	20	0.689	0.812	0.906	0.802	0.10
3	40	0.693	0.832	0.919	0.814	0.11
4	60	0.723	0.942	0.997	0.887	0.14
5	80	0.980	0.942	0.843	0.921	0.07
6	100	1.012	1.212	1.005	1.076	0.11

Table no. 4.25. Anti-diabetic activity of water extract of *Costus speciosus* by Starch -Iodine Alpha Amylase Inhibition.

Sr. No	Concentration (µg/mL)	Absorbance at 620 nm			Mean	Deviation
		Absorbance 1	Absorbance 2	Absorbance 3		
1	control	0.949	0.927	0.932	0.936	0.01
2	20	0.653	0.628	0.701	0.660	0.03
3	40	0.664	0.712	0.721	0.699	0.03
4	60	0.709	0.750	0.762	0.740	0.02
5	80	0.768	0.810	0.824	0.800	0.03
6	100	0.830	0.923	0.919	0.890	0.05

Table no. 4.26. Anti- diabetic activity of methanol extract of *Tinospora cordifolia* by Starch - Iodine Alpha Amylase Inhibition.

Sr. No	Concentration (µg/mL)	Absorbance at 620 nm			Mean	Deviation
		Absorbance 1	Absorbance 2	Absorbance 3		
1	control	1.114	1.115	1.115	1.115	0.01
1	20	0.639	0.646	0.649	0.644	0.01
2	40	0.716	0.718	0.720	0.718	0.01
3	60	0.955	0.776	0.950	0.893	0.10
4	80	0.968	0.964	0.896	0.942	0.04
5	100	1.071	1.073	1.098	1.080	0.01

Table no. 4.27. Anti- diabetic activity of chloroform extract of *Tinospora cordifolia* by Starch - Iodine Alpha Amylase Inhibition.

Sr. No	Concentration (µg/mL)	Absorbance at 620 nm			Average	Deviation
		Absorbance 1	Absorbance 2	Absorbance 3		
1	control	1.022	1.025	1.023	1.023	0.01
2	20	0.783	0.659	0.653	0.698	0.07
3	40	0.831	0.948	0.853	0.877	0.06
4	60	0.876	0.902	0.918	0.898	0.02
5	80	0.989	0.992	1.006	0.995	0.01
6	100	0.984	1.012	1.019	1.005	0.01

Table no. 4.28. Anti- diabetic activity of water extract of *Tinospora cordifolia* by Starch -Iodine Alpha Amylase Inhibition.

Sr. No	Concentration (µg/mL)	Absorbance at 620 nm			Mean	Deviation
		Absorbance 1	Absorbance 2	Absorbance 3		
1	control	1.119	1.121	1.120	1.120	0.01
2	20	0.713	0.716	0.710	0.713	0.01
3	40	0.781	0.786	0.783	0.783	0.01
4	60	1.071	0.955	1.073	1.033	0.06
5	80	1.093	1.099	1.093	1.095	0.01
6	100	1.102	1.103	1.102	1.102	0.01

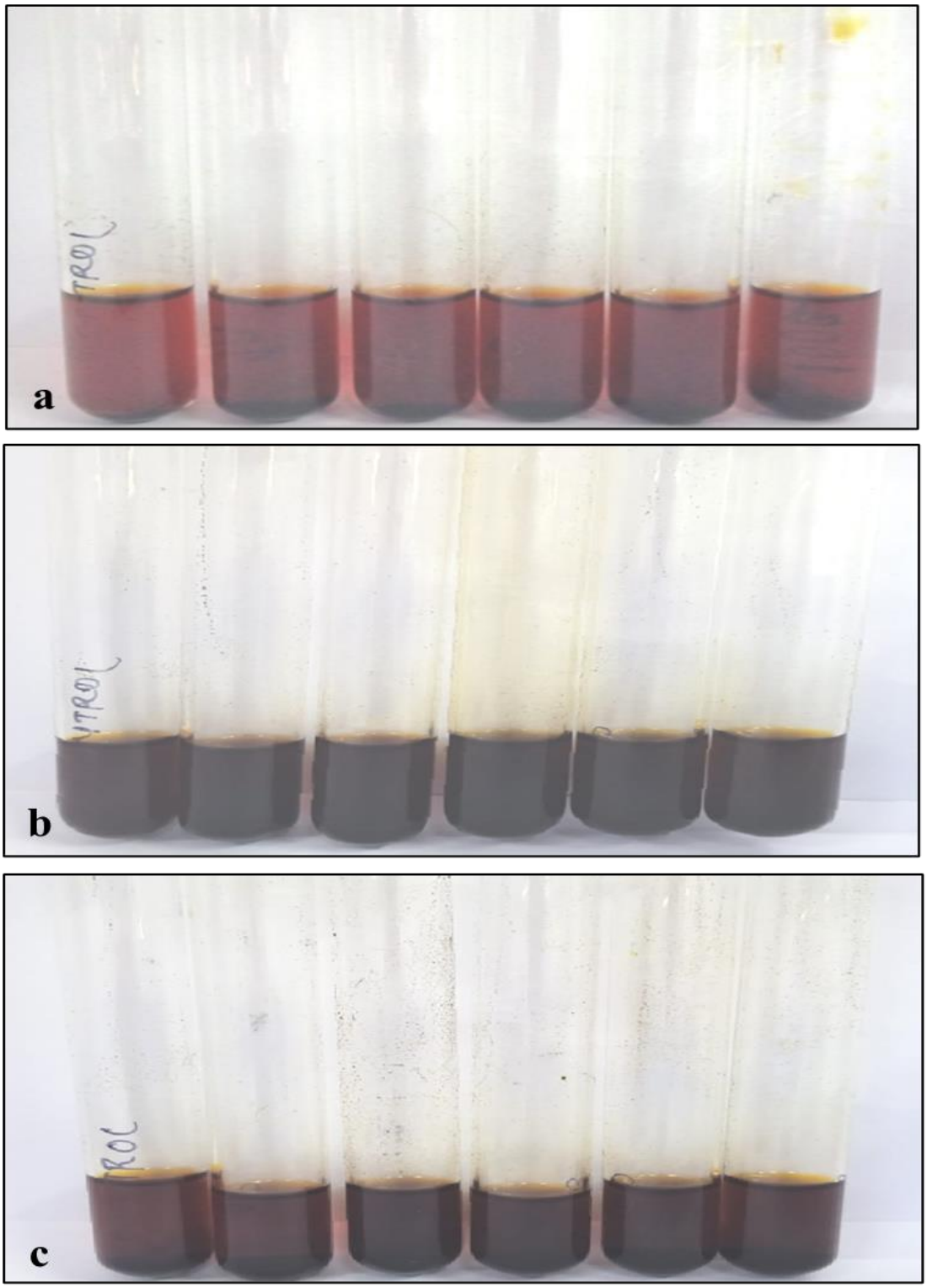


Plate 4.14. Determination of anti-diabetic activity. a. Acarbose b. Methanol extract of *Costus speciosus* c. Chloroform extract of *Costus speciosus*.

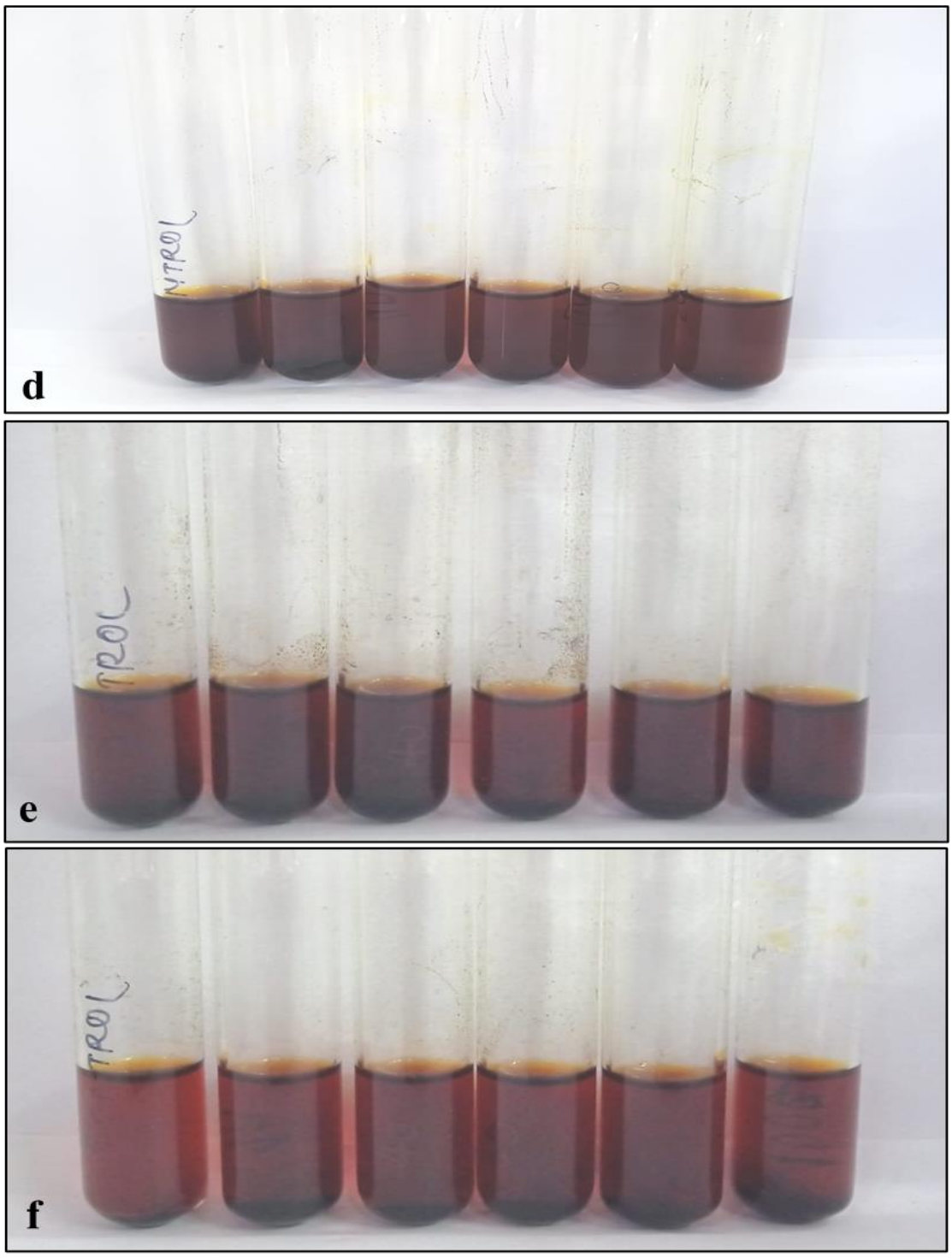


Plate 4.15. Determination of anti-diabetic activity. d. Water extract of *Costus speciosus* e. Methanol extract of *Tinospora cordifolia* f. Chloroform extract of *Tinospora cordifolia*.

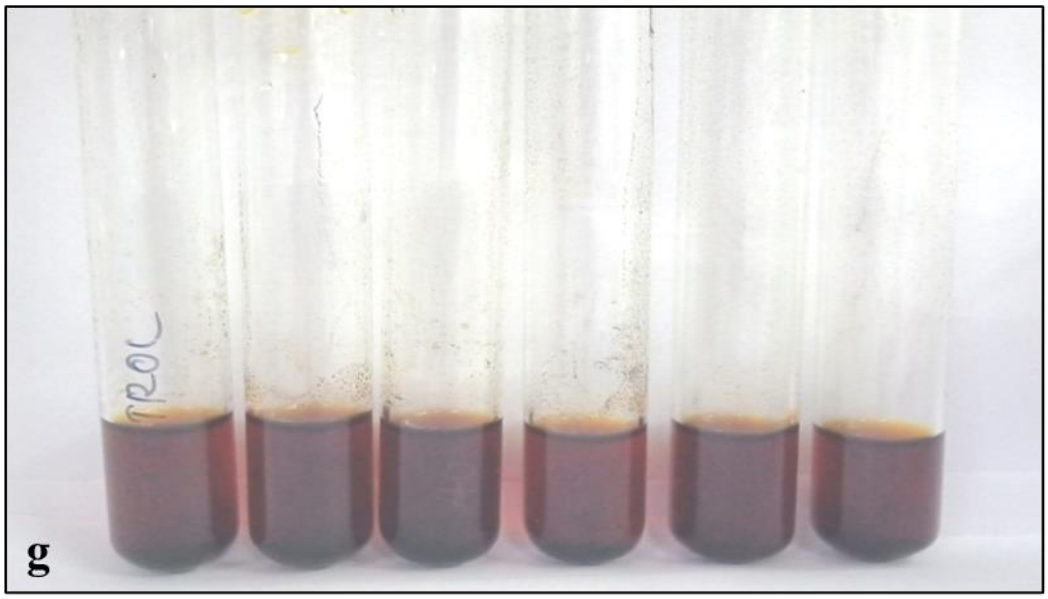


Plate 4.16. Determination of anti-diabetic activity. g. Water extract of *Tinospora cordifolia*.

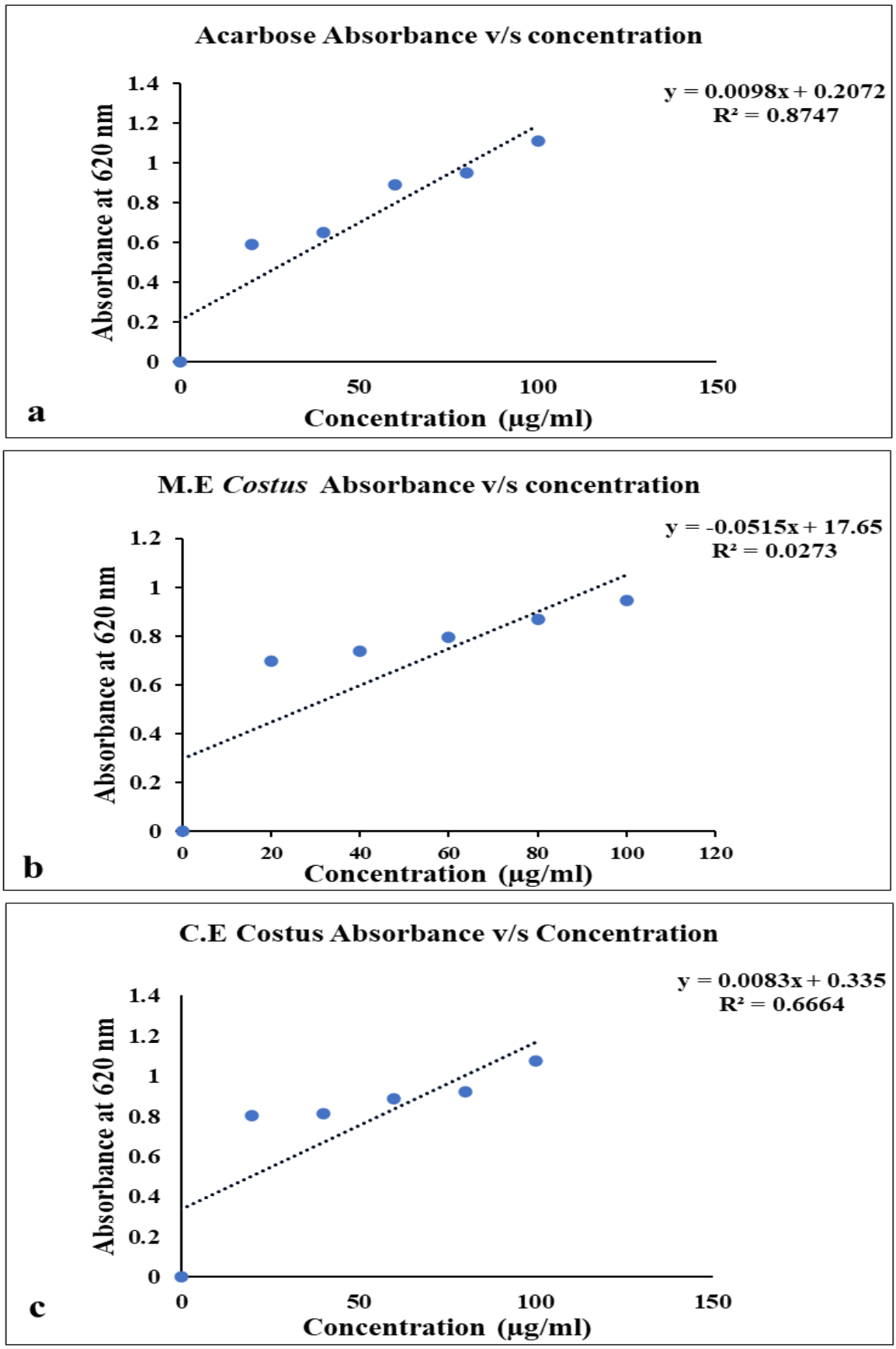
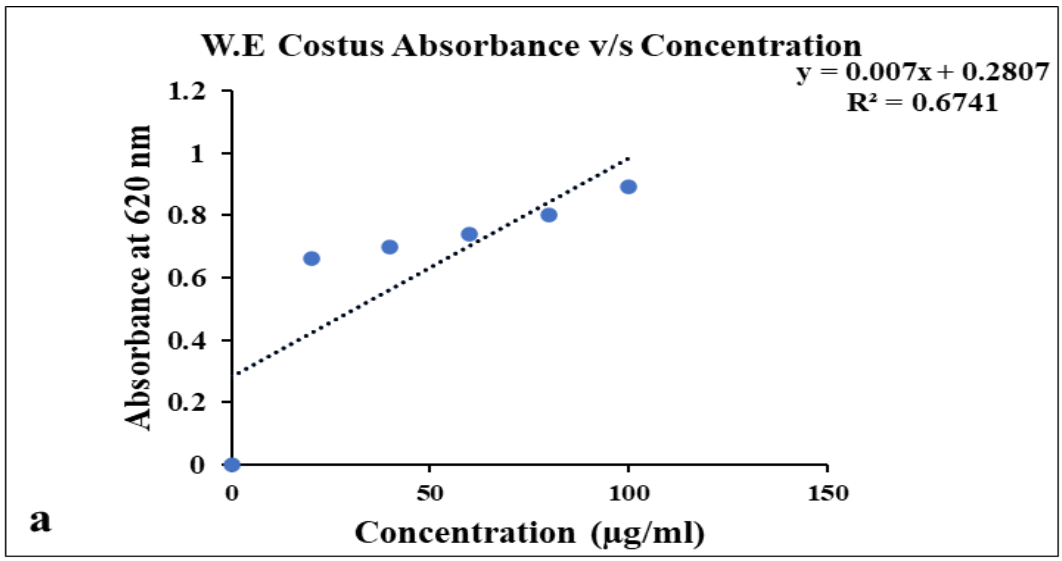
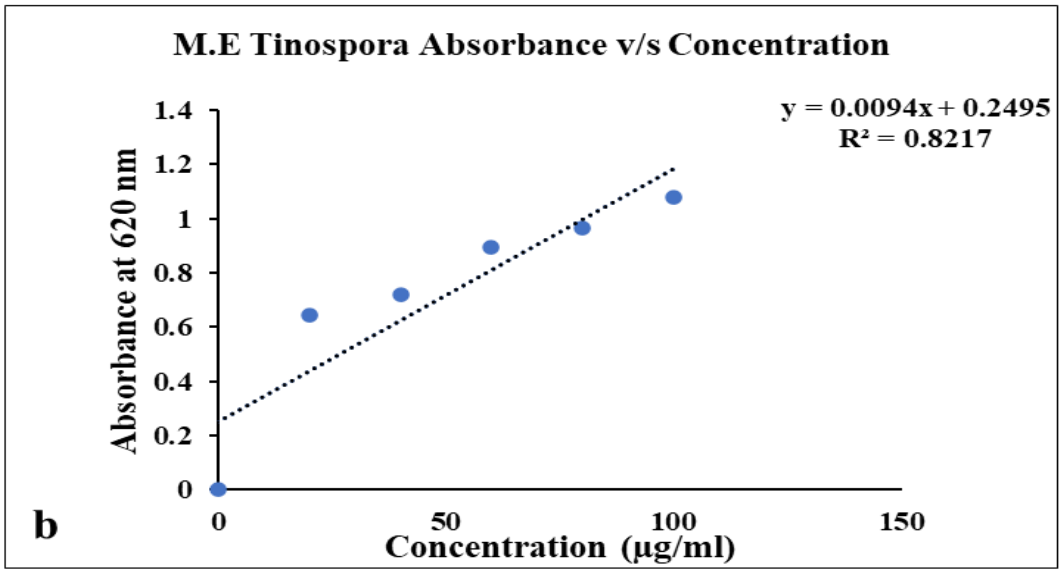


Figure 4.9. Graph of determination of anti-diabetic activity. a. Acarbose b. Methanol extract of *Costus* c. Chloroform extract of *Costus*.





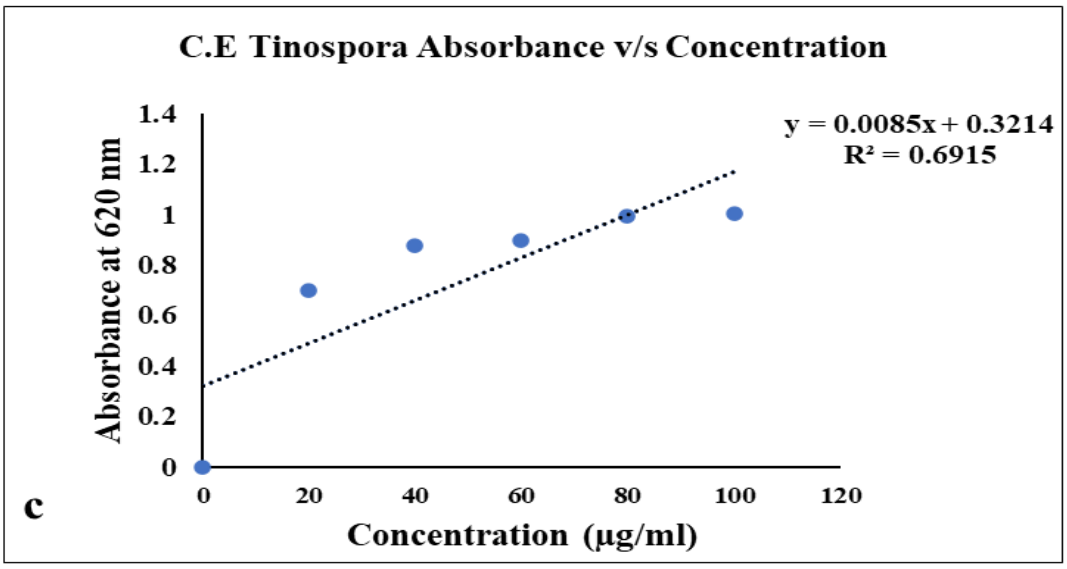


Figure 4.10. Graph of determination of anti-diabetic activity. a. Water extract of *Costus* b. Methanol extract of *Tinospora* c. Chloroform extract of *Tinospora*.

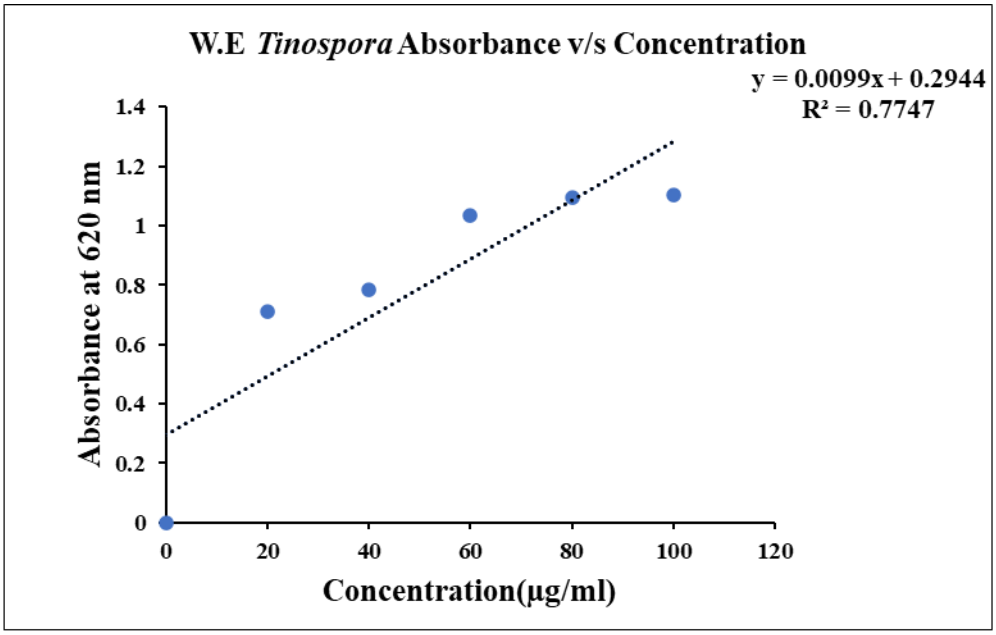


Figure 4.11. Graph representing determination of anti-diabetic activity of water extract of *Tinospora*.

The chloroform extract of *Tinospora cordifolia* also showed prominent anti-diabetic activity than *Costus speciosus*. *Tinospora cordifolia* at 20 µg/ml displayed an absorbance value of 0.698 and for 100 µg/ml as 1.005. The same extract of *Costus speciosus*, the absorbance was noted at 20 µg/ml as 0.802 and 1.076 at 100 µg/ml. The water extract of both the plants showed similar activity. In *Costus speciosus* the absorbance was 0.644 at least concentration and 0.108 at the highest concentration. In *Tinospora cordifolia* absorbance was noted as 0.713 and 1.102 at least and highest concentration respectively.

It was reported that the anti-diabetic activity of leaves of *Costus speciosus* with methanol extract was showing good activity which was carried out using normal and alloxan-induced rats (Siriwaedhene *et al*., 2023). *Costus speciosus* plant extract has a significant relative potency compared to commercial insulin in this preliminary *in-vitro* study, which can be used to treat type II diabetes. After carefully planned pre-clinical and clinical research, this plant source may prove to be a viable, sustainable, and natural anti-diabetic medication (Bindu *et al*., 2016). But the present study shows that chloroform and water extract of leaves of *Costus speciosus* were exhibiting good anti-diabetic activity.

Tinospora cordifolia leaves of ethanolic extract reported good anti-diabetic activity (Singh *et al*., 2013). Sonkamble and Kamble (2015) also reported that *Tinospora cordifolia* has good antidiabetic activity. Present study also showed *Tinospora cordifolia* with good anti-diabetic activity as compared to *Costus speciosus*.

4.6. Anti-inflammatory activity

Anti-inflammatory activity was determined by egg albumin protein denaturation method. Diclofenac sodium was used as the positive control to detect the activity, which showed good activity with an IC_{50} value of 54.38µg/ml as depicted in **(Tables 4.29**, **4.30**, **4.31**, **4.32**, **4.33**,**4.34**,

4.35 and **4.36)**. Methanol extract of *Tinospora cordifolia* showed best anti-inflammatory activity than *Costus speciosus* with 10 and 71.72µg/ml IC_{50} values respectively **(Plate 4.17**, **4.18** and **4.19)**. Chloroform extract of *Tinospora cordifolia* resulted in good activity with 14.53µg/ml IC_{50} value than the *Costus speciosus* with 69.56µg/ml IC_{50} value. In water extract, *Costus speciosus* revealed more activity with 48.70µg/ml IC_{50} value and *Tinospora cordifolia* possesses less activity with 85.62µg/ml as IC_{50} value **(Figure 4.12 4.13**and **4.14)**. Hence, from the results obtained we can conclude that *Tinospora cordifolia* possess good anti-inflammatory activity which was observed more in water extract than the positive control Diclofenac sodium followed by chloroform extract.

Whereas, the flower of *Costus speciosus* with methanol as the extracting solvent reported good anti-inflammatory activity proved using bovine serum albumin method (Fathirah *et al*., 2023). The flower of *Costus speciosus* contain good anti-inflammatory activity with methanol and ethyl acetate (Fathirah *et al*., 2023). Here, the present study shows that leaves with water extract of *Costus speciosus* showed good anti-inflammatory activity. The methanolic extract of *Tinospora cordifolia* reported better inhibition activity, which indicates that the plant possess good anti-inflammatory property (Chauhan *et al*., 2016). The same extract also showed high anti-inflammatory activity in present study.

Table no. 4.29. Anti-inflammatory activity of Diclofenac sodium by Egg Albumin Protein Denaturation.

Sr. No	Concentration (µg/mL)	Absorbance at 660 nm			Mean	% inhibition
		Absorbance 1	Absorbance 2	Absorbance 3		
1	control	0.435	0.257	0.755	0.482	0
2	20	0.445	0.438	0.445	0.442	8.29 ± 0.01
3	40	0.758	0.409	0.158	0.210	56.43 ±0.01
4	60	0.256	0.012	0.221	0.163	66.18 ±0.01
5	80	0.116	0.230	0.080	0.142	70.53 ±0.01
6	100	0.154	0.102	0.112	0.112	76.76 ±0.01

Table no. 4.30. Anti-inflammatory activity of methanol extract of *Costus speciosus* by Egg Albumin Protein Denaturation.

Sr. No	Concentration (µg/mL)	Absorbance at 660 nm			Mean	% inhibition
		Absorbance 1	Absorbance 2	Absorbance 3		
1	control	1.093	1.095	1.099	1.095	0
2	20	1.074	1.085	1.070	1.076	1.73 ± 0.01
3	40	0.633	0.628	0.630	0.630	42.46 ±0.01
4	60	0.503	0.546	0.543	0.544	50.31 ±0.01
5	80	0.463	0.460	0.460	0.461	57.89 ±0.02
6	100	0.450	0.443	0.441	0.444	59.45 ±0.01

Table no. 4.31. Anti-inflammatory activity of chloroform extract of *Costus speciosus* by Egg Albumin Protein Denaturation.

Sr. No	Concentration (µg/mL)	Absorbance at 660 nm			Mean	% inhibition
		Absorbance 1	Absorbance 2	Absorbance 3		
1	control	1.106	1.115	1.119	1.113	0
2	20	1.048	1.049	1.047	1.048	5.84 ± 0.10
3	40	0.631	0.630	0.630	0.631	43.30 ±0.11
4	60	0.574	0.568	0.592	0.578	48.06 ±0.14

5	80	0.492	0.489	0.480	0.487	56.24 ±0.07
6	100	0.384	0.385	0.384	0.384	65.49 ±0.11

Table no. 4.32. Anti-inflammatory activity of water extract of *Costus speciosus* by Egg Albumin Protein Denaturation.

Sr. No	**Concentration (µg/mL)**	**Absorbance at 660 nm**			**Mean**	**% inhibition**
		Absorbance 1	**Absorbance 2**	**Absorbance 3**		
1	control	0.953	0.960	0.943	0.952	0
2	20	0.541	0.541	0.540	0.540	43.27 ±0.03
3	40	0.537	0.537	0.539	0.537	43.59 ±0.03
4	60	0.305	0.308	0.307	0.306	67.85 ±0.27
5	80	0.285	0.287	0.285	0.285	70.06 ±0.02
6	100	0.183	0.181	0.182	0.182	80.88 ±0.05

Table no. 4.33. Anti-inflammatory activity of methanol extract of *Tinospora cordifolia* by Egg Albumin Protein Denaturation.

Sr. No	**Concentration (µg/mL)**	**Absorbance at 660 nm**			**Mean**	**% inhibition**
		Absorbance 1	**Absorbance 2**	**Absorbance 3**		
1	control	1.012	1.014	0.983	1.003	0
2	20	0.103	0.940	0.101	0.099	90.12 ±0.01
3	40	0.064	0.062	0.060	0.062	93.8 ± 0.01
4	60	0,031	0.033	0.028	0.030	97 ± 0.10
5	80	0.028	0.029	0.029	0.028	97.20 ±0.04
6	100	0.021	0.021	0.020	0.020	98 ± 0.01

Table no. 4.34. Anti-inflammatory activity of chloroform extract of *Tinospora cordifolia* by Egg Albumin Protein Denaturation.

Sr. No	Concentration (µg/mL)	Absorbance at 660 nm			Mean	% inhibition
		Absorbance 1	Absorbance 2	Absorbance 3		
1	control	0.0953	0.0942	0.0909	0.0934	0
2	20	0.177	0.111	0.162	0.150	83.94±0.07
3	40	0.112	0.106	0.104	0.107	88.54±0.06
4	60	0.057	0.056	0.049	0.054	94.21 ±0.02
5	80	0.043	0.041	0.041	0.041	95.61 ± 0.01
6	100	0.039	0.037	0.037	0.037	96.03 ± 0.01

Table no. 4.35. Anti-inflammatory activity of water extract of *Tinospora cordifolia* by Egg Albumin Protein Denaturation.

Sr. No	Concentration (µg/mL)	Absorbance at 660 nm			Mean	% inhibition
		Absorbance 1	Absorbance 2	Absorbance 3		
1	control	1.038	1.130	1.203	1.125	0
2	20	0.827	0.851	0.880	0.852	24.26 ± 0.01
3	40	0.802	0.769	0.819	0.796	29.24 ± 0.01
4	60	0.755	0.743	0.720	0.739	34.31 ± 0.06
5	80	0.550	0.651	0.572	0.591	47.46 ± 0.01
6	100	0.493	0.496	0.490	0.493	56.17 ± 0.01

Table no.4.36. IC_{50} values of methanol, chloroform and water extract of *Costus speciosus* and *Tinospora cordifolia*for anti-inflammatory activity.

Sr. No	**Sample**	**Type of extract**	**IC_{50} Value**
1.	Diclofenac sodium		54.38
2.	*Costus speciosus*	Methanol extract	71.72
		Chloroform extract	69.56
		Water extract	48.70
3.	*Tinospora cordifolia*	Methanol extract	10
		Chloroform extract	14.53
		Water extract	85.62

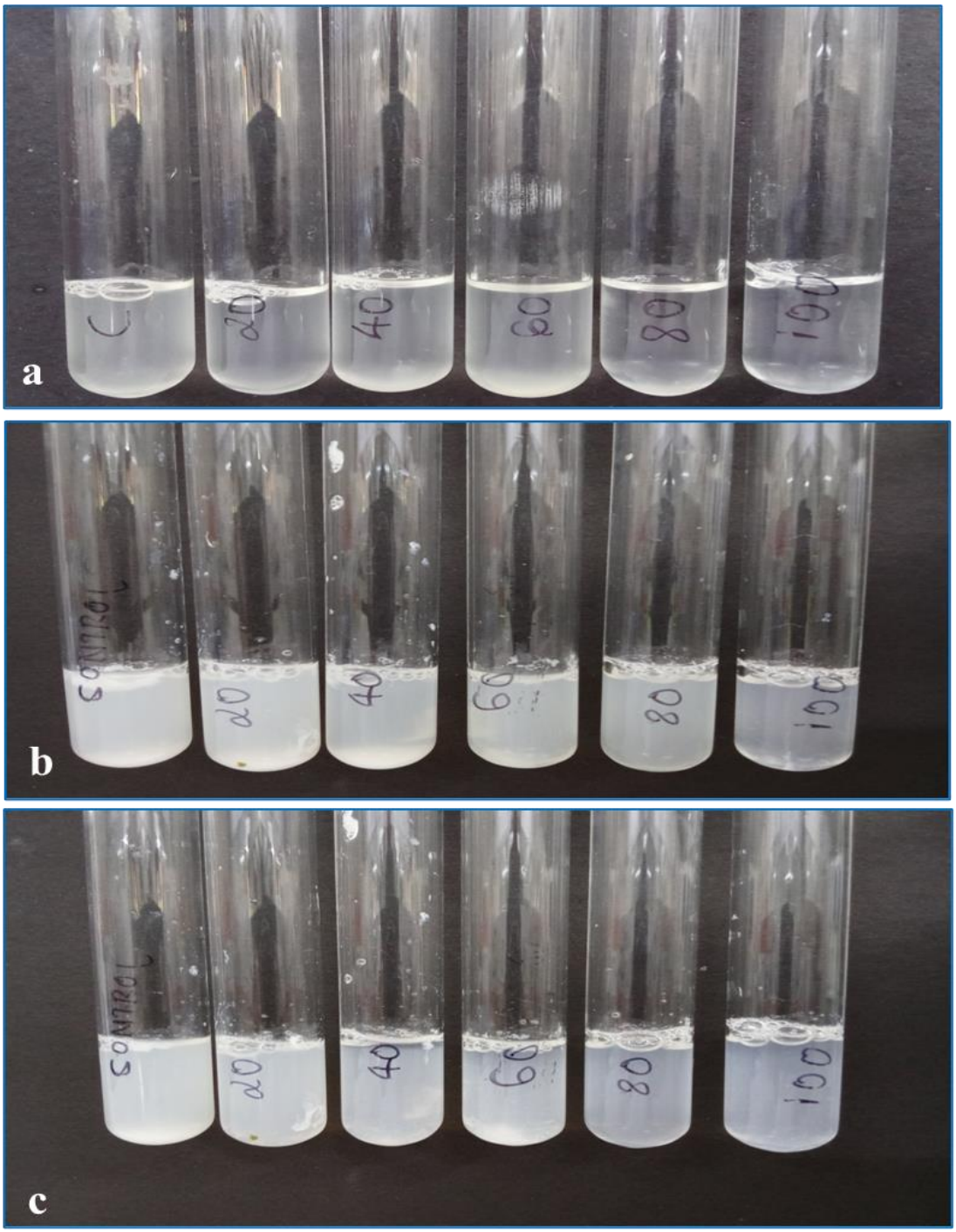


Plate 4.17. Determination of anti-inflammatory activity. a. Diclofenac sodium b. Methanol extract of *Costus speciosus* c. Chloroform extract of *Costus speciosus*.

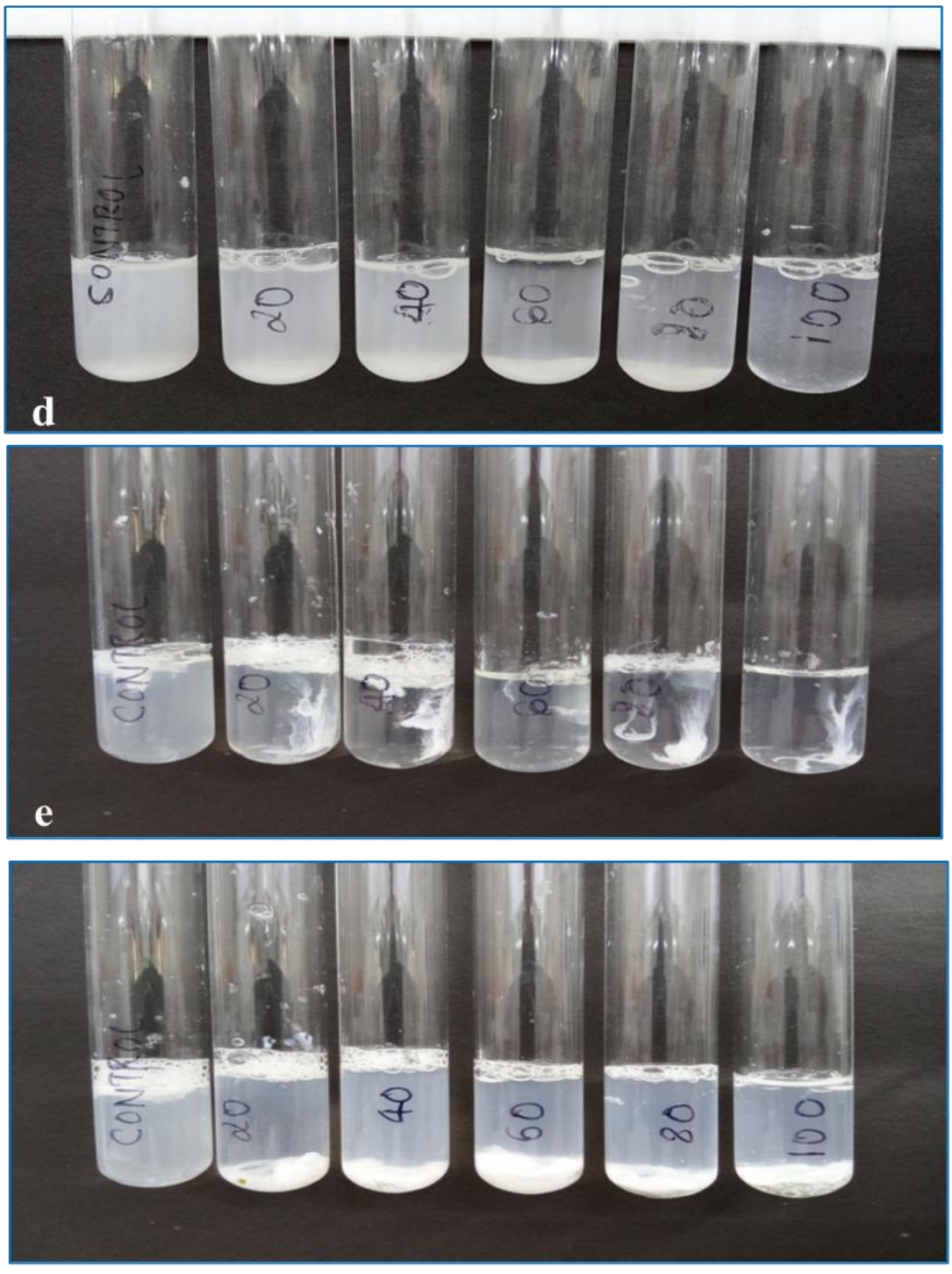


Plate 4.18. Determination of anti-inflammatory activity. d. Water extract of *Costus speciosus* e. Methanol extract of *Tinospora cordifolia* f. Chloroform extract of *Tinospora cordifolia*.

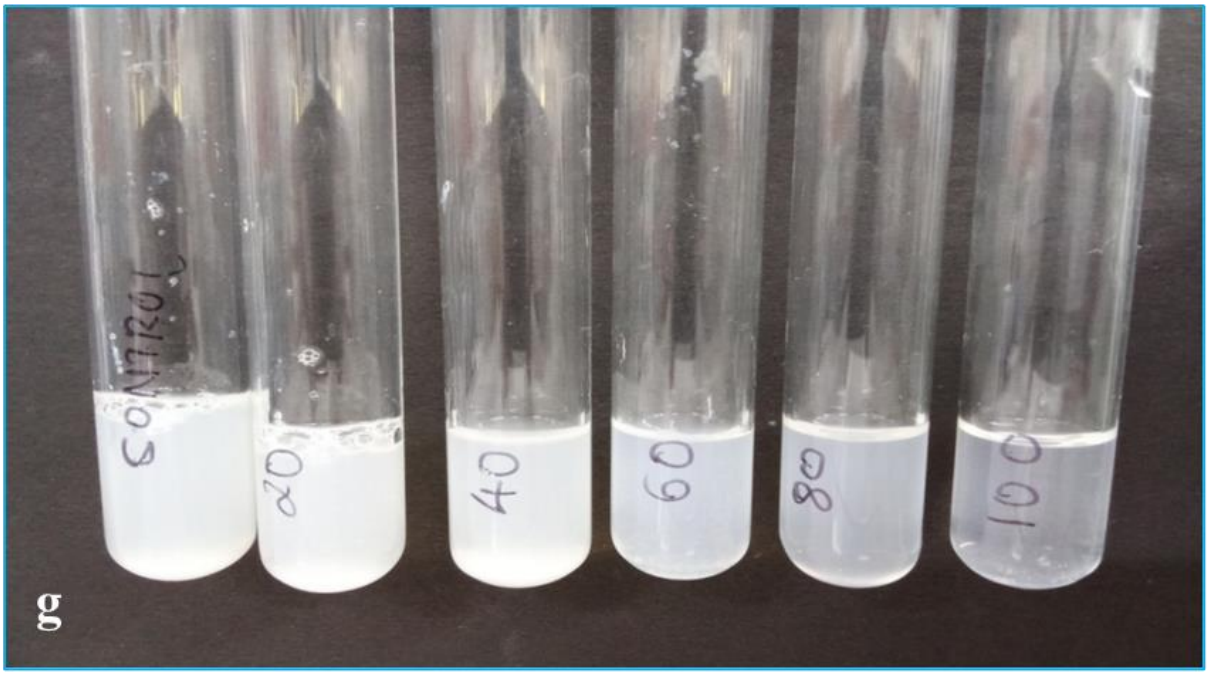


Plate 4.19: Determination of anti-inflammatory activity g. Water extract of *Tinospora cordifolia*.

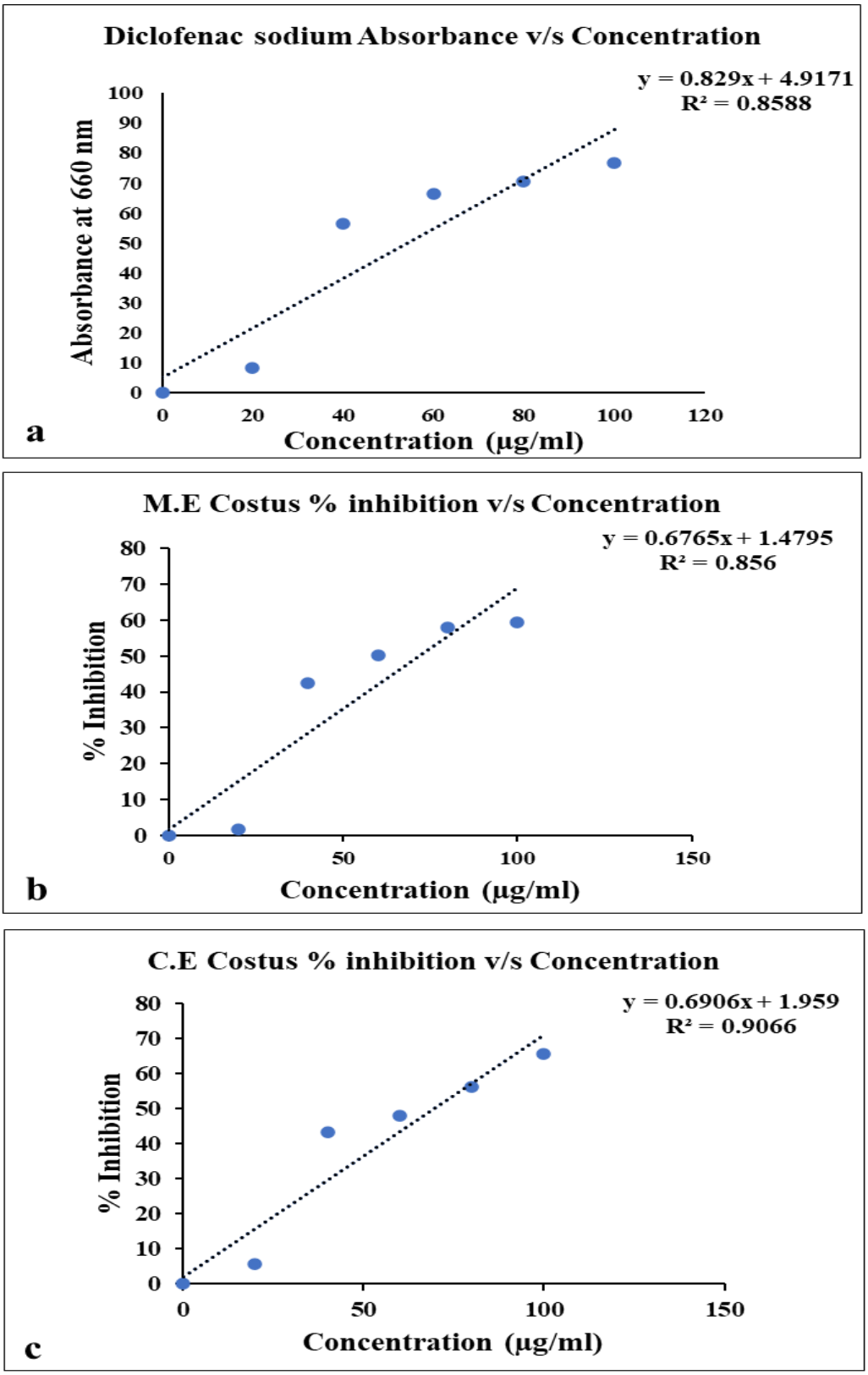


Figure 4.12. Graph representing determination of anti-inflammatory activity. a. Diclofenac sodium b. Methanol extract of *Costus* c. Chloroform extract of *Costus*.

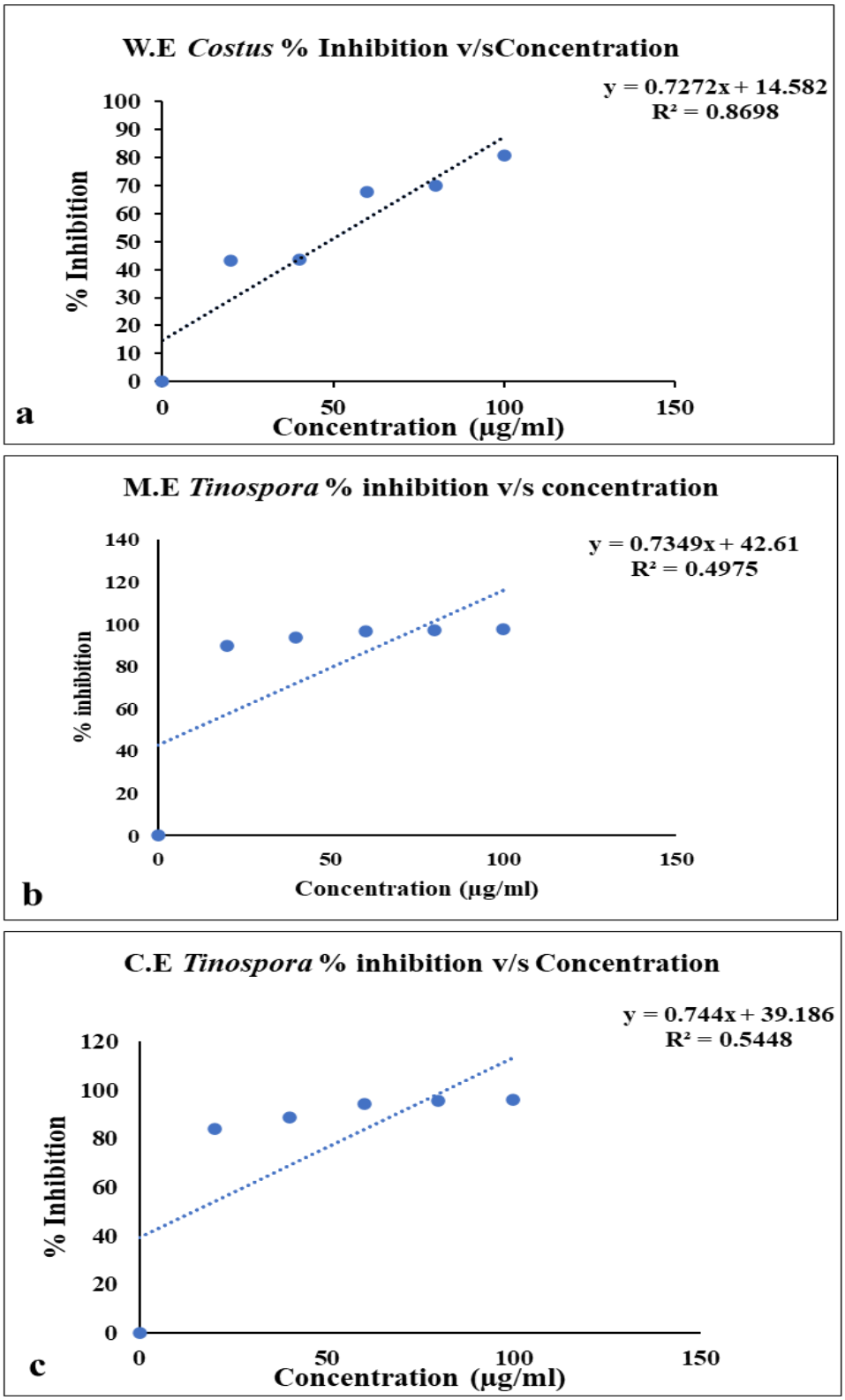
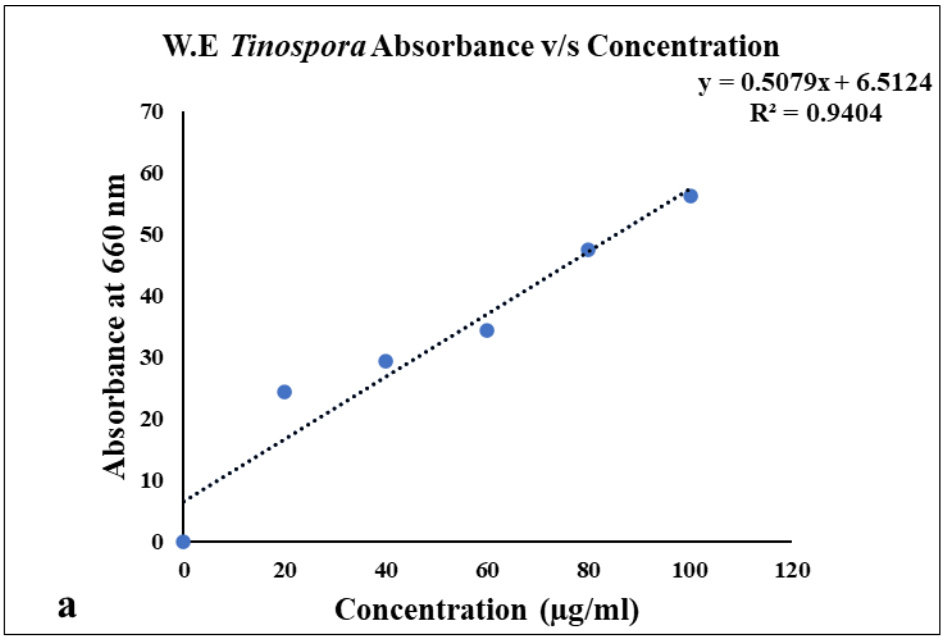


Figure 4.13. Graph representing determination of anti-inflammatory activity. a. Water extract of *Costus* b. Methanol Extract of *Tinospora* c. Chloroform extract of *Tinospora*.



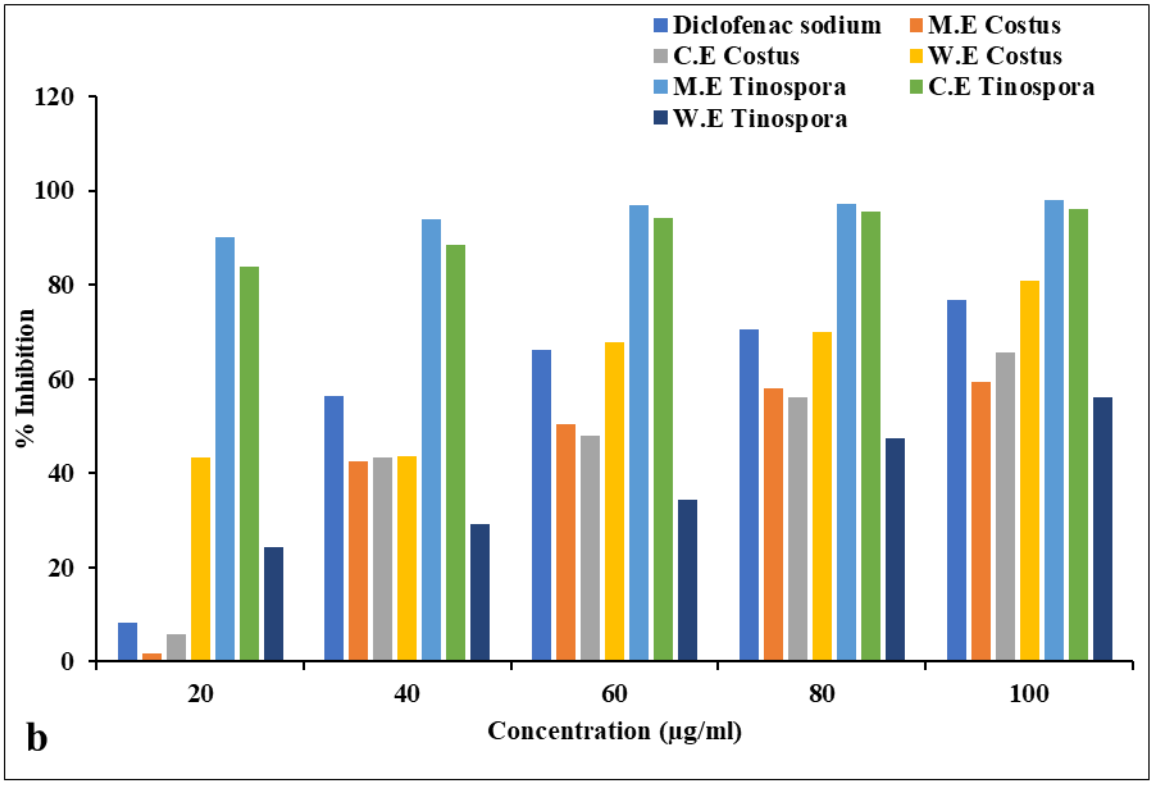


Figure 4.14. Graph representing determination of anti-inflammatory activity. a. water extract of *Tinospora* b. Comparative graph of diclofenac sodium and all extracts.

CHAPTER 5
CONCLUSIONS

5. CONCLUSIONS

Ethanobotanical study shows that *Costus speciosus* and *Tinospora cordifolia* are used chiefly by the local practitioners for treating diabetes mellitus. They mainly use leaves for the treatment and the method of taking medication is in the form of decoction. The extract was prepared by following maceration method using methanol, chloroform and water as solvents. The water extract of leaf of *Costus speciosus* and stem of *Tinospora cordifolia* showed good amount of extract yield as compared to the methanol and chloroform solvents. Among this, the water extract of *Tinospora* showed better yield. Chloroform extract showed less yield as compared to the water extract.

The qualitative phytochemical screening shows better results with water extracts followed by chloroform and methanol in both selected plants. Both the plants show similar phytochemical constituents. The quantitative phytochemical analysis resulted in higher amount of flavonoid content followed by alkaloid, phenols, and tannins. *Costus speciosus* showed presence of more flavonoid, alkaloid, and tannin content than *Tinospora cordifolia*. The total phenolic content was found to be similar in both the plants. Overall, the quantitative analysis suggests that leaves of *Costus speciosus* contain more amount of phytoconstituents than the stem of *Tinospora cordifolia*.

The antioxidant activity studied using DPPH free radical scavenging activity suggests that the chloroform extract of leaf of *Costus speciosus* showed good antioxidant activity as prominent colour change from dark purple to yellow was noticeable compared to all the other extracts. But the overall performance of *Tinospora cordifolia* shows that it has better antioxidant activity than *Costus speciosus*.

The water and chloroform extracts of stem of *Tinospora cordifolia* possesses good anti-diabetic activity than *Costus speciosus* leaf extract which shows little less activity when detected using starch-iodine alpha amylase inhibition method. The anti-inflammatory activity was performed using egg albumin protein denaturation method which suggests that the methanol extract of stem of *Tinospora cordifolia* showed best anti-inflammatory activity as it was showing clear inhibition of protein denaturation followed by the chloroform extract. *Costus speciosus* showed less anti-inflammatory activity. It can be concluded that *Tinospora cordifolia* showed better biological activities in comparison to *Costus speciosus* thus proving its medicinal supremacy over *Costus speciosus*.

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