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Qualitative and Quantitative Analysis of Phytochemical Constituents of *Alternanthera brasiliana* (L.) Kuntze and *Cassia alata* (L.) using Different Organic Solvents

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Abstract

The therapeutic qualities of *Alternanthera brasiliana* and Cassia *alata* are due to the phytochemicals contained in these plants. This research was conducted to study the phytochemical analysis of leaf extracts of these plants using methanol, hexane, and chloroform solvents. The leaves were collected, cleaned, and dried for eight hours at 60°C in a cabinet dryer. Using the solvent extraction method, the extract was made. Qualitative and quantitative analysis was done to determine the occurrence of flavonoids, terpenoids, tannins, phenols, amino acids, alkaloids, phlobatannins, glycosides, and saponins. The extraction yields in methanol, hexane, and chloroform were 15.77%, 3.01%, and 3.16% for *A. brasiliana* and 26.91%, 19.5%, and 18.32% for *C. alata*. Alkaloids, terpenoids, tannins, phenols, glycosides, flavonoids, carbohydrates, phlobatannins, and saponins were all found in *A. brasiliana*. Alkaloids, tannins, phenols, glycosides, flavonoids, carbohydrates, and saponins were found in *C. alata*. Total phenolic, flavonoid, and tannin content in methanol extract for *A. brasiliana* was found to be 127.76 mg GAE/g, 136.48 mg GAE/g, and 58.88 mg GAE/g. The total phenolic, flavonoid and tannin content found were 41.76 mg GAE/g, 103.21 mg GAE/g, and 58.67 mg GAE in hexane extract. Similarly, 102.4 mg GAE/g, 112.49 mg GAE/g, and 41.76 mg GAE/g in the chloroform extract. Total phenolic, flavonoid, and tannin content of *C. alata* in the methanol extract were 36.3 mg GAE/g, 22.43 mg GAE/g, and 10.89 mg GAE/g, in the hexane extract, 25.98 mg GAE/g, 16.68 mg GAE/g, and 3.25 mg GAE/g, in the chloroform extract, 28.52 mg GAE/g, 20.33 mg GAE/g, and 4.56 mg GAE/g. From extraction yields and qualitative and quantitative analysis, methanol was the best solvent.

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1. Introduction

Due to the presence of phytochemicals that have been used to treat various diseases since the dawn of time, medicinal plants are among the most significant species in the plant kingdom. Phytochemicals are substances found in plants. Phytochemicals are naturally occurring chemical substances that are biologically active and present in different parts of the plant, like roots, stems, leaves, flowers, and fruits (Saxena et al., 2013). Phytochemicals found in plants have essential applications in agriculture, human medicine, and veterinary medicine cosmetics, which are important in the discovery of new medication leads for the treatment and prevention of different diseases. Due to their actions on the cells and tissues in live animals' bodies, phytochemicals exhibit certain biological activities and are beneficial to human health (Lawati and Parajuli, 2021). Phytochemicals help plants develop, protect them by triggering defence mechanisms, and give them colour, flavour, and odour (Sharma et al., 2020). Phytochemicals, made of natural materials and their derivatives are more efficient than their synthetic counterparts and cause fewer side effects (Essiett and Bassey, 2013). To prevent and treat human diseases, plant derivatives have been employed. The antifungal, anticancer, antioxidant, antibacterial, anti-inflammatory, analgesic, and immune-stimulating

properties of phytochemicals contained in plants have been demonstrated (Lahare et al., 2020).

The opulence of phytochemicals in medicinal plants aids in the effective and side-effect-free treatment of disease (Sharma et al., 2020). As a result, the W.H.O. estimates that almost 80% of the world's population depends on the use of medicinal plants as their major form of healthcare (Ekor, M. 2014). Nepal uses ethnobotanical knowledge to supplement its reliance on natural remedies for primary healthcare (Adhikari et al., 2011). The vast varieties of medicinal plants found in Nepal not only treat illnesses but also offer nutritional value. Nearly all biological reactions involve phytochemicals and mineral components (Shad et al., 2014). The potential for creating new pharmaceuticals that will greatly help humanity is huge when it comes to medicinal plants. The bulk of pharmaceutical businesses heavily relies on the wild population for the raw materials needed to extract crucial phytochemicals for medicine (Karthika et al., 2016). There are many methods used to find novel physiologically active components (Sunmathi et al., 2016). Phytochemical screening is the most popular method of isolating medicinal principles. Studying, researching, extracting, and experimenting to identify various classes of phytoconstituents is a scientific process. It is a scientific method for examining, extracting, experimenting, and identifying diverse types of phytoconstituents present in various plant sections (Sharma et al., 2020). The existence of bioactive substances produced by plants, such as flavonoids, alkaloids, tannins, terpenoids, saponins, glycosides, phenolic compounds, etc., is confirmed by phytochemical screening (Shad et al., 2014).

The Brazilian native herb *Alternanthera brasiliana* (L.) Kuntz, popularly known by the names Brazilian joy weed and Penicillin, belongs to the Amaranthaceae family. It grows in poor and deforested soil. It has a persistent, horizontal, branchy, and curved to the multilateral stem. Its flowers are bisexual, monocyclic and actinomorphic (Duarte and Debur, 2004). The plant contains several bioactive chemicals with therapeutic potential, including vitamins, minerals, antioxidants, anticarcinogens, and a wide range of other substances. (Novak and Haslberger, 2000). A. *brasiliana* is used to treat burns and wounds. In Brazilian remedies, it is frequently used to treat inflammation, cough, and diarrhoea (Brochado et al., 2003).

Cassia alata L. (Roxb.), belongs to the Fabaceae family of plants, which grows in intertropical regions (Habtemariam, 2019). It is also referred to as Aththorai in Sri Lanka, candle bush in Malaysia, winged senna, and dadmardan in India (Habtemariam, 2019). *Cassia alata* is used to treat allergies, ringworm, scabies, and other skin conditions. Its leaves are used to treat a variety of illnesses, including diabetes, dermatologic, gastrointestinal, and anti-infectious conditions. They can also be used as a mouthwash, expectorants, purgatives, and astringents. Additionally, the leaves are particularly effective in treating ringworm, eczema, scabies, athlete's foot, herpes, bug bites, etc. (Fatmawati et al., 2020).

Nevertheless, compared to other higher plants, these plants are not taken into consideration as therapeutic herbs. In order to understand the medicinal contents of these plants, qualitative and quantitative research on these plants is required. It is crucial to conduct early phytochemical screening of plants sequentially to uncover and create novel therapeutic compounds with higher potency (Yadav et al., 2014). There are various main types of phytochemicals found in various plant species. Two such important plant species are *Alternanthera brasiliana* and *Cassia alata*; however, little is known about their phytochemical makeup. Any remarkable work regarding these plants from the Hattisar area has not been done yet. Thus, this work contributes to our understanding of the phytochemical makeup of *A. brasiliana* and *C. alata* of Hattisar, Dharan by generating relevant data. Thus, the current study was conducted with an objective to determine the biologically active compounds present in these two plants and the optimal solvent for their extraction that may be used to treat a variety of diseases.

2. Materials and Method

2.1 Study area

Alternanthera brasiliana and *Cassia alata* leaves were collected in the vicinity of Hattisar, Dharan, Nepal. It is situated 428 metres above mean sea level at latitude 26° 47' 39.79" N and longitude 87° 16' 54.218" E. The research was conducted in a lab at the Central Campus of Technology in Province No. 1 of the Sunsari region of Nepal's Dharan. The location of the study region is shown in Figure 1.

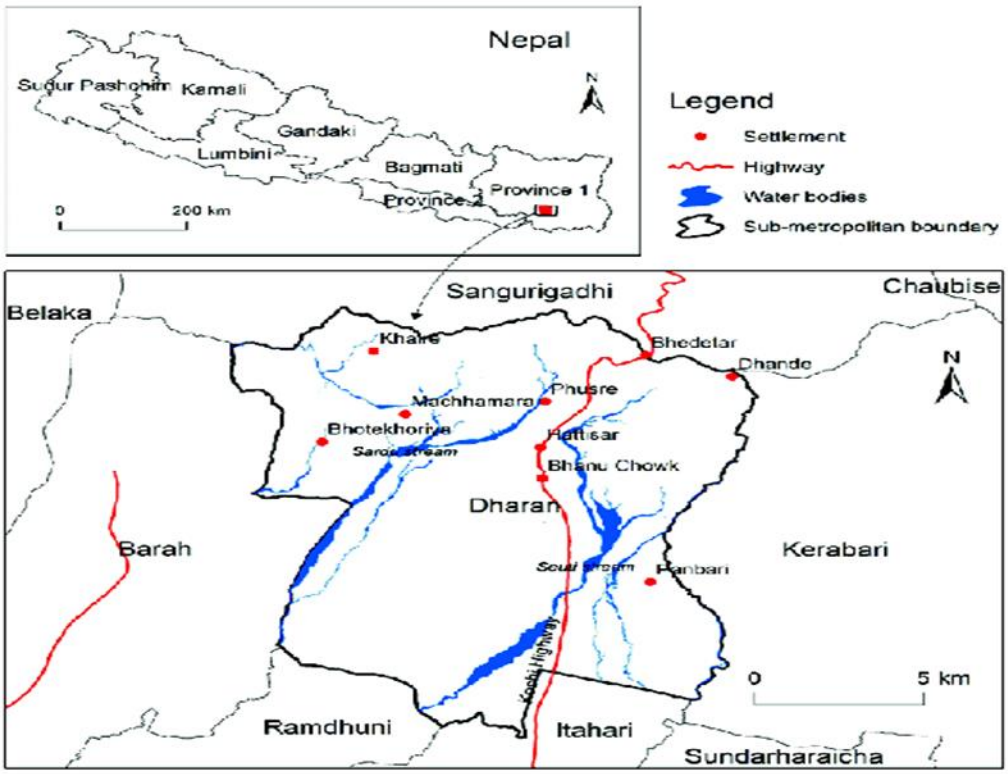

Figure 1. Location of the Study Area

2.2 Chemicals

Chemicals and reagents used for the study were Methanol, Glacial aceti acid, Chloroform, Sodium chloride, Molisch's reagent, Wagner's reagent, Ferric chloride, Conc. Sulphuric acid, Acetic anhydride, Fehling's solution, Hexane, Folin ciocalteu, Ninhydrin solution, Sodium nitrite, and Sodium carbonate obtained from Merk, India.

2.3. *Collection of plant samples*

The samples of *Alternanthera brasiliana* and *Cassia alata* leaves were collected by hand from healthy and vigorously growing plants and then immediately transported to the laboratory of CCT, Dharan, for drying within two hours from the study site for further investigation. to 70℃ till 12% moisture was obtained and in the third stage malt was dried at 71-90℃ till the moisture dropped to 4-5% (Hornsey 2013).

2.4. *Extraction*

The collected leaves were dried in a cabinet dryer at 60○C for 8 hours and were ground in an electric grinder to make a fine powder. Using the Soxhlet equipment and a solvent extraction procedure, 250 ml of methanol, hexane, and chloroform were used for the preparation of the extract from 10 g of the plant material. The extract was stored at 4°C until the qualitative and quantitative analyses were performed.

2.5. *Extraction yield of samples*

To calculate the extraction yield, the plant extract was collected in a flask with a round bottom from the rotatory evaporator, which was then spun while the system was partially emptied. To get the solvent to boil, the pressure was decreased. The concentrated chemical was taken out of the flask, withdrawn from the solvent, and weighed.

The extraction yield (%) was calculated according to Truong et al., (2019).

$$\text{Extraction Yield (\%)} = \frac{\text{Weight of the Extract after evaporating Solvent}}{\text{Dry Weight of Sample}} \times 100$$

2.6. *Qualitative Analysis of Sample Extracts*

Alkaloids, amino acids, terpenoids, saponins, phenols, tannins, glycosides, flavonoids, carbohydrates, and saponins were among the chemical components whose presence was tested using the methods described by Joshi et al. (2011).

2.7. *Screening of Alkaloids*

Wagner's test: In a test tube with 1 ml of extract, 1 ml of Wagner's reagent was added. Alkaloids were present, as evidenced by the reddish-brown precipitate that formed (Mir et al., 2013)..

2.8. Screening of Terpenoids

Salkowski test: 2 ml of chloroform was added to 1 ml of extract in a test tube, and then a thin coating of concentrated sulfuric acid was carefully placed down the tube's side. The presence of terpenoids was confirmed by the development of a reddish-brown colour at the interface (Mir et al., 2013).

2.9. Screening of *Tannins* and Phenolic compounds

Ferric chloride test: To perform this test, 1 ml of water was mixed with 0.5 ml of extract, and 2 drops of ferric chloride solution were added. The green-black colour showed the presence of tannins (Joshi et al., 2011).

Ferric Chloride Test: 0.5 ml of the extract was combined with 1 ml of water, and then 1-2 drops of ferric chloride solution were added. Tannin was evident from the green-black hue (Joshi et al., 2011).

2.10. *Screening of Amino acids*

Ninhydrin Test: 1 ml of ninhydrin solution was added to 2 ml of extract. In a hot water bath, the mixture was boiled. A blue-to-purple tint appeared which indicated the presence of amino acids (Mir et al., 2013).

2.11. *Screening of Glycosides*

Keller-Killiani test: A test tube containing 1 ml of glacial acetic acid and 1 ml of concentrated sulfuric acid was added. Next, 1 ml of the extract was carefully added. The presence of glycoside was shown by the formation of a brown ring at the contact. The appearance of a violet ring beneath the ring may also occur (Mir et al., 2013).

2.12. Screening of Flavonoids

Shinoda test: 1.5 ml of 50% methanol solution was added to 4 ml of extract solution. Metal magnesium was added after the solution had been warmed. Five to six drops of strong HCl were also added to this solution. The appearance of a red colour indicated the presence of flavonoids (Joshi et al., 2011).

2.13. Screening of Carbohydrates

Fehlings Test: This test involved mixing and boiling two reagents for 1 minute. For this, 1 ml of Fehling's A (copper sulphate in distilled water) and 1 ml of Fehling's B (potassium tartrate and sodium hydroxide in distilled water) reagents were taken and then mixed with an equal volume of test solution. In a bath of boiling water, the solution was heated. A brick red precipitate was produced, which was a sign of the presence of carbohydrates.

2.14. Phlobatannins testing

1% aqueous HCl was heated with 1 ml of plant extract. Phlobatannins were present because a red precipitate was formed (Mir et al., 2013).

2.15. Screening of Saponins

Foam test: For 5 to 10 minutes, 1 ml of extract and 20 ml of distilled water were rapidly shaken. There were saponins present because a one-centimetre layer of foam was formed (Mir et al., 2013).

2.16. Quantitative analysis

2.16.1.. Determination of total phenolic content (TPC)

Using a small modification, the spectrophotometric method was used to quantify the amount of phenolics in the plant extract (Tambe and Bhambar, 2014). To determine the total phenol content, the Folin-Ciocalteu test method was employed. A test tube was filled with a combination that contains 0.5 ml of extract and 4.5 ml of distilled water. After giving the mixture a good shake, 0.5 ml of the Folin-Ciocalteu phenol reagent was added. Five minutes later, the mixture was treated with 5 ml of a 7% sodium carbonate solution. The following concentrations of gallic acid were created as standard solutions: 20, 40, 40, 60, 80, and 100 g/ml. After the mixture had been incubated for 30 minutes at room temperature, an ultraviolet (UV) or visible spectrophotometer was used to measure the absorbance of the test and standard solutions in comparison to the blank reagent at 550 nm. The total phenol content was calculated as mg of GAE per gram of extract (Tambe and Bhamber, 2014).

2.16.2. Determination of total flavonoid content (TFC)

The Aluminium Chloride ($AlCl_3$) technique was used to calculate the total flavonoid content (mg/ml). 0.3 ml of 5% sodium nitrite, 0.5 ml of distilled water, and 0.5 ml of plant extract were put into a test tube. The assay mixture was incubated at room temperature for 5 minutes. 0.3 ml of 10% aluminium chloride was applied right away following incubation. The mixture was mixed with 2 ml of sodium hydroxide, and the absorbance was gauged at 510 nm. Quercetin served as a benchmark (Sankhalkar and Vernekar, 2016).

2.16.3. Determination of total tannin content (TTC)

The Folin-Ciocalteu technique was used to calculate total tannin contents. In a test tube with 7.5 ml of distilled water and 0.5 ml of the Folin-Ciocalteu phenol reagent, 0.1 ml of the sample extract was added. After that, 1 ml of a 35% Na2CO3 solution was added, and 10 ml of distilled water was used to dilute it. The mixture was stored at room temperature and then thoroughly shaken. Gallic acid standard reference solutions (20, 40, 60, 80, and 100 g/ml) were made. Using a UV/Visible spectrophotometer, absorbance for test and reference solutions was measured at 725 nm in comparison to the blank. Using mg of GAE/g of extract, the tannin concentration was calculated (Khaleel, 2018).

3. *Data analysis*

The obtained data were processed and analysed using Microsoft Excel.

4. Results and Discussion

4.1. Extraction Yield of Alternanthera brasiliana and Cassia alata

The maximum yield was found in the methanolic extract, i.e., 15.77% and 26.91% in *A. brasiliana* and *C. alata*, respectively. In a similar way, 3.16% and 19.5% were found in chloroform extracts from both plants. The minimum yield was found in hexane extract, i.e., 3.01% and 18.32% (Table 2). Thus, the result showed that methanol is the best solvent for the phytochemical analysis of *A. brasiliana* and *C. alata*.

Solvents used	***A. brasiliana***	***C. alata***
Methanol (%)	15.77	26.91
Hexane (%)	3.01	19.5
Chloroform (%)	3.16	18.32

4.2. Qualitative analysis of phytochemicals for Alternanthera brasiliana

The qualitative analysis was performed by preparing the plant extract in methanol, hexane, and chloroform extracts for the conformation of alkaloids, terpenoids, tannins and phenols, amino acids, glycosides, flavonoids, carbohydrates, phlobatannins, and saponins. The outcomes are displayed in Table 2

.

Table 3: Qualitative evaluation of phytochemicals for *Alternanthera brasiliana*

S.N	**Phytochemicals**	**Methanol**	**Hexane**	**Chloroform**
1.	Alkaloid	+	+	+
2.	Terpenoid	+	_	_
3.	Tannin and phenol	+	_	_
4.	Amino acids	_	_	_
5.	Glycosides	+	+	_
6.	Flavonoid	+	_	_
7.	Carbohydrates	_	_	+
8.	Phlobatannins	+	_	_
9.	Saponins	+	_	_

*The "+" sign represents the occurrence of phytochemicals, and the "-" sign represents the lack of phytochemicals.

Chandran et al. (2013) reported the presence of alkaloids, phenols, flavonoids, tannins, saponins, and Carbohydrates in the leaves of *A. brasiliana*, which is similar to our study. In our study, we found there is the presence of alkaloids, terpenoids, tannins, phenols, glycosides, flavonoids, carbohydrates, phlobatannins, and saponins. But amino acid was absent in all three solvents.

4.3. Qualitative evaluation of phytochemicals for Cassia alata

The qualitative analysis of phytochemicals in *C. alata* was performed in methanol, hexane, and chloroform, and the results obtained are shown in Table 4.

S.N	Phytochemicals	Methanol	Hexane	Chloroform
1	Alkaloid	+	+	+
2	Terpenoid	_	_	_
3	Tannin and phenol	+	_	_
4	Amino acids	_	_	_
5	Glycosides	+	_	+
6	Flavonoid	+	_	_
7	Carbohydrates	_	_	+
8	Phlobatannins	_	_	_
9	Saponins	+	_	_

* The "+" sign represents the occurrence of phytochemicals, and the "-" sign represents the lack of phytochemical

Lahare et al. (2020) reported the presence of saponins, tannins, terpenoids, flavonoids, and alkaloids in the leaf extracts of *C. alata*. In our study, alkaloids, tannins and phenols, glycosides, flavonoids, carbohydrates, and saponins are present. But terpenoids, amino acids, and phobatannins were absent in all three solvents.

4.4. Quantitative evaluation of phytochemicals in Alternenthera brasiliana

The TPC, flavonoid, and tannin content of the methanolic extract of *A. brasiliana* were 127.76 mg GAE/g, 136.48 mg Quercetin/g and 58.88 mg GAE/g respectively. Similarly, 41.76 mg GAE/g, 103.21 mg Quercetin/g and 58.67 mg GAE/g in hexane extract, and 102.4 mg GAE/g, 112.49 mg Quercetin/g and 41.76 mg GAE/g were found in a chloroform extract of *A. brasiliana* (Table 5).

Table 5. Quantitative evaluation of phytochemicals in *A. brasiliana*

Phytochemicals	Methanol extracts	Hexane extracts	Chloroform extracts
Total Phenol Content (mg GAE/g dry matter)	127.76	41.76	102.4

Total flavonoid content (mg quercetin/g dry matter)	136.48	103.21	112.49
Tannin content (mg GAE/g dry matter)	58.88	58.67	41.76

4.5. Quantitative analysis of phytochemicals in Cassia alata

The TPC, flavonoid, and tannin content of the methanolic extract of *C. alata* were 36.3 mg GAE/g, 22.43 mg Quercetin/g and 10.89 mg GAE/g respectively. Similarly, 25.98 mg GAE/g, 16.68 mg Quercetin/g and 3.25 mg GAE/g in hexane extract, and 28.52mg GAE/g, 20.33 mg Quercetin/g and 4.56 mg GAE/g was found in chloroform extract of *Cassia alata* (Table 6).

Table 6. Quantitative analysis of phytochemicals in *Cassia alata*

Phytochemicals	**Methanol extracts**	**Hexane extracts**	**Chloroform extracts**
Total phenol content (mg GAE/g dry matter)	36.3	25.98	28.52
Total flavonoid content (mg quercetin/g dry matter)	22.43	16.68	20.33
Total tannin content (mg GAE/g dry matter)	10.89	3.25	4.56

Pamulaparthi et al. (2016) discovered that the methanolic extract of *Cassia alata* has a TPC and flavonoid concentration of 41.6 mg GAE/g, and 31.9 mg Quercetin/g, 32.4 mg GAE/g, and 20.1 mg quercetin/g of the chloroform extract of *Cassia alata*'s TPC and flavonoid content, respectively. This outcome was discovered to be nearly similar to our investigation. Due to their high solubility in the extraction solvent, the methanolic extract displayed comparatively large levels of TPC, flavonoids, and tannins when compared to other extracts (Biney et al., 2021).

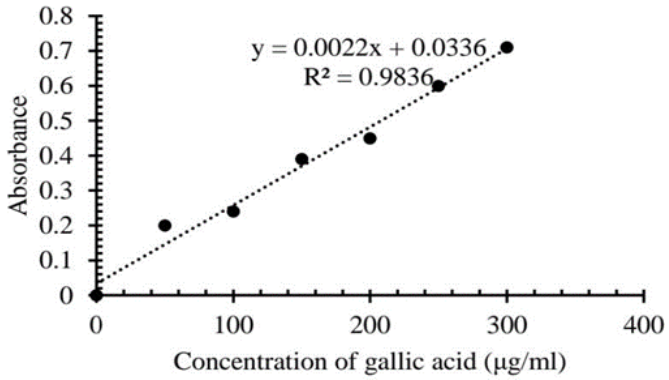

Figure 2. Standard curve of gallic acid at 550 nm

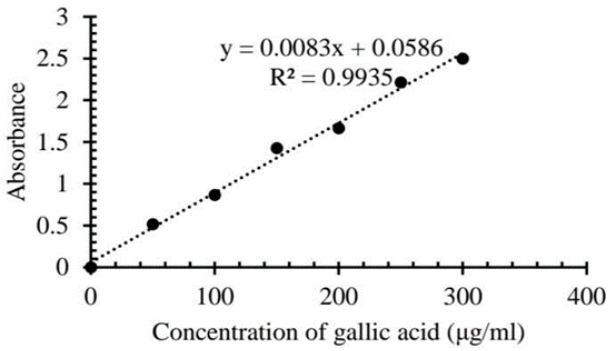

Figure 3. Standard curve of gallic acid at 725 nm

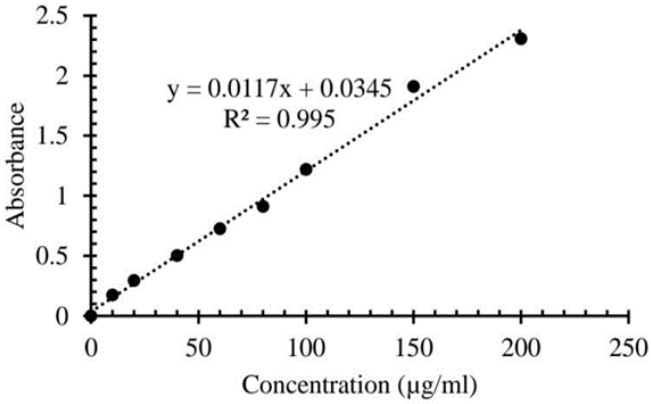

Figure 4. Standard curve of gallic acid at 510 nm

5. Conclusions

Alternanthera brasiliana and *Cassia alata* extract

from methanol, hexane, and chloroform were screened for phytochemicals, alkaloids, terpenoids, tannins and phenols, glycosides, flavonoids, carbohydrates, phlobatannins, and saponins. According to the study, *Alternanthera brasiliana* and *Cassia alata* are excellent sources of phytochemicals that are physiologically active and are the basis for their therapeutic properties, which can be utilised to treat a variety of disorders. Additionally, the aforementioned result demonstrated that, out of the three solvents, methanol was determined to be the best solvent for phytochemical screening because it produced the highest yield and had the highest concentration of detected phytochemicals. *A. brasiliana* and *C. alata* are utilised for a variety of therapeutic purposes by the locals in many nations, including Nepal, as a result of the existence of these metabolites.

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Competing interests

The authors declare no competing interests.

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