

# Phytochemical and Anticancer Assessment of *Cassia siamea* Extracts

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## ABSTRACT

In the present study, the leaves of *Cassia siamea* were subjected to extraction, fractionation, and isolation procedures, followed by comprehensive phytochemical screening. Antioxidant activity was assessed using the DPPH radical scavenging assay, while cytotoxic potential was evaluated through the MTT assay on the MDA-MB-231 human breast cancer cell line. Phytochemical analysis revealed the presence of glycosides, flavonoids, phytosterols, and phenolic compounds in the ethyl acetate, aqueous, and ethanol extracts. Among all extracts tested, the ethanol and methanol extracts exhibited the highest antioxidant activities, whereas the chloroform extract demonstrated the lowest. Additionally, the methanolic extract showed notable cytotoxicity, reducing cell viability to 49% at a concentration of 10  $\mu$ M. These findings indicate that ethanol and methanol extracts of *C. siamea* leaves possess significant antioxidant and anticancer potential, warranting further investigation for the isolation and characterization of bioactive constituents.

**Keywords:** *C. siamea*, Cancer, Anti-oxidant, Extraction

## INTRODUCTION

Cancer is a disease which is life-threatening to the humans, where the growth of cells is uncontrollable which invades and damages surrounding tissues in the body. Nowadays its incidence and prevalence increasing day by day which leads to high number of cases diagnosed with the cancer every year. <sup>1</sup>In case of cancer body cells lost their capacity of inhibiting uncontrolled cell growth which leads to the formation of group of cell which is commonly known as tumour, nodule, lump, mass or a lesion which may be arises from any part of the body and generally not detected easily but some time diagnosed “accidentally” in case of any other medical checkups. <sup>2</sup>But when person knows about that it already leads to the formation of millions of cells in body where it can infect either single tissue or start infecting neighbour tissues and organ. If it remains at a particular position in particular tissue, it is called as “Benign tumour” and when it starts infecting neighbour tissues and spreading to the other part of the body known as “Malignant tumour” and overall process of spreading and infecting other tissues generally named as metastasis. As benign tumour is infecting at particular position and it does not appear in the body once it is removed but “malignant tumour” behaves totally opposite to benign tumour and appears after some time in body.<sup>3</sup> Cancer is due to its prevalence becomes top cause of death in the United States, which is near to 25% deaths in humans. As per the data of 2020, 10 million peoples were died because of that, which is about one in every six deaths.<sup>4</sup> Because of that it is regarded as a foe of modernization and the sophisticated

socio-cultural pattern dominated by western medicine and for that multidisciplinary scientific studies are going on to tackle this sickness, but there has yet to be a sure-fire, perfect remedy need to the world of medicine.<sup>5</sup>

Breast cancer is the most common type of cancer which is diagnosed in females and leads to death of individual. It affects 85% lining cells of glandular tissues and 15% of lobules of breast. It starts from “in situ” where malignancy of breast cancer is restricted to the lobule or duct of breast with no symptoms and having least probability of spreading to the surrounding tissues. Overtime, “in situ” breast cancer converts into “invasive” which started invading surrounding breast tissue, after that it starts regional metastasis then leads to distant metastasis which ultimately causes extensive metastasis this results in death of individual. It is not only affecting females it also diagnosed in males that is extremely uncommon and accounting less than 1% malignancies in males and less than 1% of all breast cancer globally.<sup>6</sup> Since breast cancer in male is uncommon, the majority of breast cancer literature, research, clinical trials as well as the discovery of therapeutic options focuses on female breast cancer.<sup>7</sup> Breast cancer, on the other hand, is the most prevalent cancer diagnosed in the majority of nations which is around 140/184, equal to the one fourth malignancies detected in the females. Among addition, it is the major cause of cancer related fatalities in females. Previously, it was thought that cancer of breast is link to western woman’s only, however, now it was infect half (52%) of the cases which is diagnosed newly from it results in death of 62% cases, caused in developing countries.<sup>1</sup>In accordance with WHO data of breast cancer of 2020, 2.3 million females were impacted with it which result in death of 6.8 lakh females and overall data of five year up to end of 2020 explains that 7.8 million females diagnosed.<sup>1</sup>

There are so many medicinal plants, which have active constituents for cancer treatment and management. Cassia is one of them and belongs to the family of Fabaceae with over 250-300 recognised species widely distributed in tropical and subtropical climates such *C. fistula*, *C. grandis*, *C. javanica* The Cassia genus has 580 different species of herbs, shrubs, and trees which is annually and that found all over the world.<sup>8</sup> Cassia species have been mentioned in ancient ayurvedic literatures, and a literature review revealed that it is used to treat a variety of skin problems.<sup>9</sup>This genus contains many phytoconstituents such as tannins, flavonoids, alkaloids, glycosides, and so on. Due to their structural variety of bioactive compounds cassia shows spectrum of biological and pharmacological activity which is due there aerial and underground parts and it is traditionally and medicinally used for various diseases.<sup>10</sup>

## Material and Methods

The leaves of *Cassia siamea* plant were collected from the Kurukshetra University campus which is located in Kurukshetra, India. Identification of *cassia siamea* was done in department of Botany, Kurukshetra University by Dr. B. D. Vashita and voucher specimen was preserved in the Kurukshetra University under the faculty of pharmaceutical sciences.

## Cold Extraction

Cold extraction is an isocratic type of extraction method that is suitable for thermolabile substances. The leaves of *C.siamaea* were crushed into a coarse powder and macerated with different solvents according to the polarity.<sup>11</sup> Various solvents were added to closed vessels for several days to yield the macerates. These macerates were collected and stored in a China dish for drying, and the extract yield was calculated.<sup>12</sup>

### Phytochemical screening

Preliminary phytochemical screening of each extract of *C. siamea* was done to identify the presence of phytoconstituents such as carbohydrates, alkaloids, glycosides, saponins, sterols, phenols, tannins, flavonoids, proteins and amino acids.<sup>13</sup>

### Tests for alkaloids

**Dragendorff's test:** To the filtrate, a few drops of Dragendorff's reagent (solution of potassium bismuth iodide) were added and observed for the development of orange brown precipitate.

**Hager's test:** To the filtrate, a few drops of Hager's reagent (saturated aqueous solution of picric acid) were added and observed for the formation of yellow precipitate.

**Wagner's test:** To the filtrate, a few drops of Wagner's reagent (solution of iodine in potassium iodide) were added separately to each filtrate and observed for the formation of reddish-brown precipitate.

**Mayer's test:** To the filtrate, a few drops of Mayer's reagent (potassium mercuric iodide solution) were added and observed for the formation of white or cream coloured precipitate.<sup>14</sup>

### Tests for steroids

**Salkowski reaction:** To 2 ml of filtrate, 2 ml of chloroform and 2 ml of conc. sulphuric acid were added through the sides of test tube and shaken well and observed. Chloroform layer appears red and acid layer shows green fluorescence, if steroids are present.

### Tests for flavonoids

**Sodium hydroxide test:** To the extract, increasing amount of sodium hydroxide solution was added and observed for the development of yellow colour for the presence of flavonoids.

**Lead acetate test:** To the extract, lead acetate solution was added and observed for the formation of yellow ppt. for the presence of flavonoids.

**Shinoda test:** Each extract was separately shaken with ethanol in different test tubes. Now, 0.5gm of magnesium turnings and a few drops of conc. HCl from the sides of test tube were added and observed for the development of pink colour for the presence of flavonoids (Red-flavanone, orange-flavanol).<sup>15</sup>

### Tests for tannins

**Ferric chloride test:** About 2 ml of 5% ferric chloride solution was added to the filtrate and observed for the formation of green or blue colour.

**Lead acetate test:** To extract filtrate, 3-4 drops of 10% w/v lead acetate solution was added and noticed for the formation of white precipitates.

### Tests for amino acids and proteins

**Ninhydrin test:** 2 drops of 2% ninhydrin reagent (in acetone) was added to 2ml of aqueous filtrate. The appearance of violet colour confirmed the presence of free amino acids.

**Xanthoproteic test:** When the aqueous extract is treated with conc. HNO<sub>3</sub>, a yellow colour is produced. It is a test for proteins which contain aromatic amino acids.<sup>16</sup>

**Millon's test:** To 2 ml of the extract filtrate, 5-6 drops of Millon's reagent were added and observed for the formation of red precipitate as an indication of the presence of proteins.

### Tests for carbohydrates

**Molisch's test:** To extract filtrate (2 to 3 ml), a few drops 20% w/v alpha-naphthol solution was added and observed to form a violet colour ring at the junction of two liquids after adding about 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub> from the sides of test tube.

**Benedict's test:** Equal volumes of filtrate and Benedict's reagent is mixed in a test tube, heated on a boiling water bath and observed for the development of yellow, green or reddish colour for the presence of reducing sugars. (Aliphatic aldehyde).

**Fehling's test:** 1 ml of each of Fehling's A and Fehling's B were mixed and added to equal volume of filtrate. Mixture was placed in water bath for 5 minutes and colour change from yellow to brick red ppt. showed reducing sugars (glucose, fructose, lactose).<sup>17</sup>

**Seliwanoffs test:** To 1 ml of aqueous extract add few drops of seliwanoffs reagent (resorcinol in HCl) and heat for 5 minutes. Appearance of cherry red colour confirms keto sugar presence.

### Tests for glycosides

#### Anthraquinone glycosides

**Borntrager's test:** A small amount of each extract was hydrolysed with dilute HCl for a few hours on a water bath. To the hydrolysate, about 1.0 ml of benzene/ether and 0.5 ml of dilute ammonia solution was added. The Appearance of reddish-brown colour or pink colour at the junction of the two layers confirmed the presence of glycoside. (O-glycoside present).

**Modified Borntrager's test:** To 5ml of extract add 5ml of 5%FeCl<sub>3</sub> & 5ml of dil. HCl. Heat for 5 minutes on a water bath. Cool and add benzene, shake well, separate the organic layer. Add an equal volume of dil. Ammonia in the organic layer. (C-type anthraquinone).

#### Cardiac glycosides

**Keller-Kiliani test:** A small portion of each extract was stirred with about 1 ml of glacial acetic acid and after cooling, a few drops of ferric chloride solution were added. The contents were then transferred to a test tube containing about 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. After standing, a reddish-brown layer acquiring bluish-green colour was observed as an indication for the presence of glycoside.

**Lieberman's test:** To 3ml of extract add 3ml of acetic acid. Heat for 5 minutes and cool then add few drops of sulphuric acid to it. Blue colour gives confirmation of cardiac glycoside.

**Baljet's test:** Sodium picrate was added to extracts and observed for colour development from yellow to orange to test the presence of cardiac glycosides.

**Legal's test:** To the extracts, pyridine and sodium nitroprusside were added and then made alkaline and observed for the development of green colour to test the presence of cardiac glycosides.

### Tests for resins & phenolic compounds

**Lead acetate test:** To extract filtrate, 2-3 drops of lead acetate solution was added and observed for the white precipitates. Indicate the presence of phenolic compounds.

**FeCl<sub>3</sub> test:** 3 ml of 5 % ferric chloride solution was added to the filtrate and noticed for the formation of green or blue colour. (Blackish blue colour gives presence of gallic Tannis).

**HCl test:** 1gm of drug is dissolved in a few ml of acetone further 3ml of dil. HCl is added to it. Heat for 30 mins in a water bath. Observance of pink colour shows the presence of resin.

### Tests for saponins

**Foam test:** About 1 ml of each extract was separately diluted to 20 ml with distilled water and further shaken in a graduated cylinder for 15 minutes. Formation of about 1 cm thick layer of foams confirmed the presence of saponin.<sup>18</sup>

### In vitro Antioxidant assay

The free radical scavenging activity of the *C. siamea* plant extracts was analysed using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and ascorbic acid was taken as standard. The sample solution was prepared with 10mg of extract in 100ml of methanol and the standard solution was prepared by taking 10mg of ascorbic acid in 100ml of methanol. A stock solution of plant extracts and DPPH were prepared in methanol, various concentrations (20 µl, 40 µl, 60 µl, 80 µl & 100 µl) of plant extracts were prepared from the stock solution and subsequently added to it. The reaction mixture was kept for 30 min at 37°C and the absorbance was measured at 517 nm. %RSA (radical scavenging activity) calculated as:<sup>19</sup>

$$\%RSA = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Where,  $A_{\text{control}}$  is the absorbance of the control which includes all reagents except the sample, and  $A_{\text{sample}}$  is the absorbance of the extracts/standard. (32-33)

### TLC Optimization and Isolation of the Compounds

The TLC was analysed and optimized using different solvents. Then multiple spots were seen in UV at 254 nm and 365 nm. Fluorescent & non fluorescent spots were observed with different  $R_f$  value on the TLC. Isolation of compounds was performed by preparative TLC. Preparation of silica was done by using distilled water and silica gel G and applied to a glass plate with thickness of 1.0-1.5 mm. Chromatoplate was subjected for activation at 130°C for 3 hours in an oven. 250 mg of the sample was applied to activate the chromatoplates. TLC chamber was saturated using solvent kept for few hours. Fractionation of aqueous extract was done by using solvent in increasing order of polarity and carry forward for TLC optimization.<sup>20</sup>

### Results and Discussion

The plant leaves of the *Cassia siamea* have been evaluated for their quality by performing extraction, phytochemical screening and In vitro studies. The plants found to contain phenols, saponins, phytosterols, flavonoids, carbohydrates, and cardiac glycosides. Extracts of *Cassia siamea* have been subjected for in vitro antioxidant activity by DPPH assay.

### Extraction

From cold extraction method coarse powder of the plant leaves were macerated with different solvents according to the polarity to obtain crude extract of leaves, the percentage yield of the extracts (chloroform, ethyl acetate, ethanol, methanol, water) were calculated as shown in **table 1**. The results show that percentage yield was obtained to be 6.24% in chloroform (VK-CS-CL-C-01), 6.92% in ethyl acetate (VK-CS-EA-C-02), 6.04% in ethanol (VK-CS-EtOH-03), 5.16% in methanol (VK-CS-MeOH-C-04) and 12.60% in aqueous (VK-CS-Aq-C-05).

**Table 1 Percentage yield of crude extracts of *Cassia siamea* by maceration (Cold extraction).**

S. No.	Extracts	Yield (%)
1	Chloroform (VK-CS-CL-C-01)	6.24

2	Ethyl acetate (VK-CS-EA-C-02)	6.92
3	Ethanol (VK-CS-EtOH-C-03)	6.04
4	Methanol (VK-CS-MeOH-C-04)	5.16
5	Aqueous (VK-CS-Aq-C-05)	12.60



**Figure 1 Various extracts of *C. siamea* plant leaves.**

### Fractionation

Fractionation of aqueous extract of *C. siamea* plant leaves were done by using separating funnel with different solvents according to the polarity. The percentage yield of the fraction (chloroform, ethyl acetate, petroleum ether) was calculated as shown in **table 2**. The results show that percentage yield was obtained to be 1.04 % in chloroform (VK-CS-Aq-C-F-CL-02), 0.83 % in ethyl acetate (VK-CS-Aq-C-F-EA-03) and 0.56 % in petroleum ether (VK-CS-Aq-C-F-PE-01).

**Table 2: Percentage yield of fractions obtained from aqueous extract of *Cassia siamea*.**

S. No.	Fractions	Yield (%)
1	Pet. Ether (VK-CS-Aq-C-F-PE-01)	1.04
2	Chloroform (VK-CS-Aq-C-F-CL-02)	0.83
3	Ethyl acetate (VK-CS-Aq-C-F-EA-03)	0.56

### Phytochemical screening

For assuring the presence of active constituents, different extracts of *C. siamea* obtained from cold extraction were projected to phytochemical screening. The results of phytochemical test performed are shown in **table 3**. From the cold extraction (table 3) positive Molisch test showed presence of carbohydrate and presence of reducing sugar was confirmed by Fehling, benedict test. Presence of glycoside is seen by Bontrager test in methanol extract (VKCS-MeOH-C-04). With positive killer killani test it showed specific cardiac glycoside existence as active constituent. Cold extraction showed positive Salkowski and Shinoda test in all the extracts confirming existence of phytosterols & flavonoids. Saponins were also present which is confirmed by foam test in ethanolic extract (VK-CS-EtOH-C-03).

**Table 3: Phytochemical test of Cold extract of *Cassia siamea***

Sr. No.	PHYTOCHEMICAL TEST	PE	CL	EA	EtOH	MeOH	Aqueous
A.	CARBOHYDRATE TEST						
1	MOLISH TEST	+	+	+	+	+	+

2	FEHLING TEST	-	-	-	+	+	-
3	SCLIWANOFF TEST	-	-	-	+	+	-
4	BENEDICT TEST	-	-	-	-	+	-
<b>B.</b>	<b>PROTEIN TEST</b>						
1	BIURET TEST	-	-	-	-	-	
2	XANTHOPROTEIN TEST	-	-	-	-	-	
3	NINHYDRINE TEST	-	-	-	-	-	
<b>C.</b>	<b>GLYCOSIDE TEST</b>						
1	BORNTRAGER TEST	-	-	-	-	+	+
2	MOD. BORNTRAGER TEST	-	-	-	-	-	-
3	BALJET TEST	-	-	-	-	-	
4	KILLER KILLANI TEST	+	+	+	+	+	+
5	LIBERMAN TEST	-	-	-	-	-	-
6	FOAM TEST	-	-	-	+	-	+
7	SODIUM PICRATE TEST	-	-	-	-	-	
<b>D.</b>	<b>ALKALOID TEST</b>						
1	HAGER TEST	-	-	-	-	-	-
2	WAGNER TEST	-	-	-	-	-	+
3	DRAGENDROFF TEST	-	-	-	-	-	-
4	MAYER TEST	-	-	-	-	-	-
<b>E.</b>	<b>PHYTOSTEROL TEST</b>						
1	SALKOWASKI TEST	+	+	+	+	+	-
<b>F.</b>	<b>FLAVANOID TEST</b>						
1	FERRIC CHLORIDE	-	-	-	-	-	+
2	LEAD ACETATE	-	-	-	-	-	+
3	SHINODA TEST	+	+	+	+	+	-
4	ALKALINE REAGENT(NaOH)	-	-	-	-	-	+
<b>G.</b>	<b>PHENOLIC TEST</b>						
1	5% FERRIC CHLORIDE	-	-	-	-	-	+
2	LEAD ACETATE TEST	-	-	-	-	+	-
3	DIL.HNO <sub>3</sub> TEST	-	-	-	-	-	
<b>H.</b>	<b>FIXED OIL &amp; FAT TEST</b>						
1	SPOT TEST	-	-	-	-	+	
2	SAPONIFICATION TEST	-	-	-	-	-	

## TLC optimization

### TLC optimization of chloroform fraction of aqueous extract

The TLC was analysed and optimized using various solvents. TLC optimization of chloroform fraction (VK-CS-Aq-C-F-CL-02) of the *C. siamea* aqueous extract of leaves starts with single solvents Then optimization of fraction using mixture of two solvents in different ratios. Chloroform fraction of aqueous

extract of *C. siamea* leaves optimized in 16 % methanol: toluene and 2% methanol: DCM as mobile phase, multiple spots were seen at UV 254 nm and clear most intense spot observed from TLC analysis of chloroform fraction

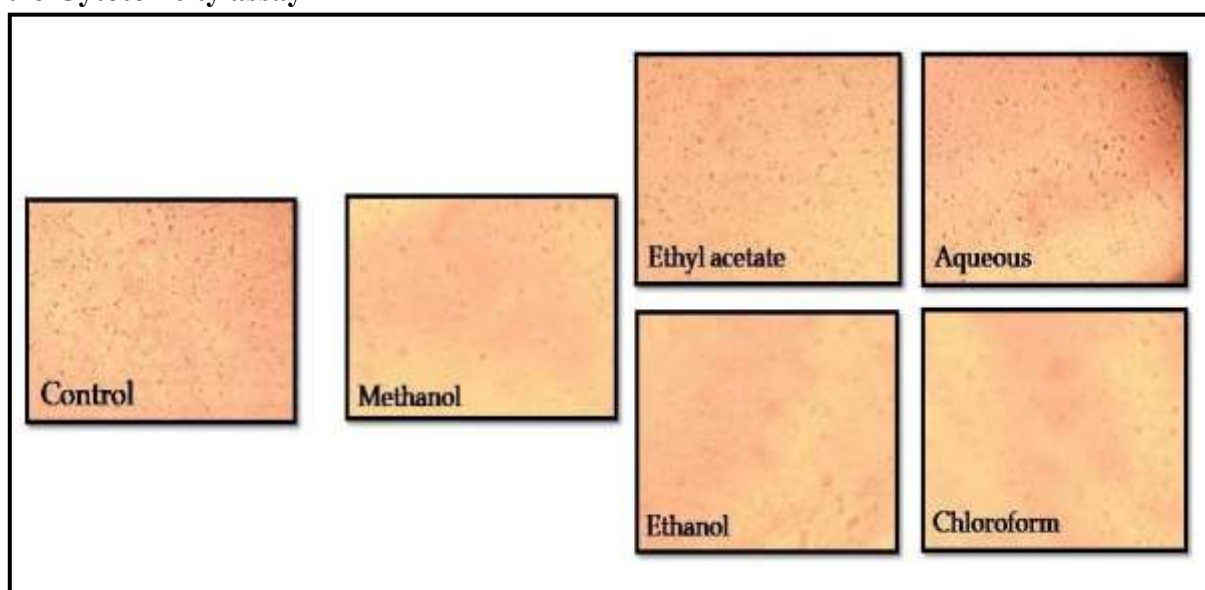
## TLC optimization of ethanol extract

The TLC was analysed using 20% ethyl acetate: petroleum ether as a mobile phase, which shows UV active multiple spots on TLC at 254 nm and fluorescence active various spots at 365 nm. TLC optimization was done by using 20 % ethyl acetate: petroleum ether as a mobile phase with 1 drop of triethyl amine in 1 ml of mobile phase, which shows that few of the compounds in it basic in nature, so to remove tailing triethyl amine was added to it. In first two TLC images showing multiple spots at 254 nm and 365 nm but after adding triethyl amine to the same concentration of mobile phase tailing between compounds reduces.

**Table 4 *In vitro* antioxidant activity of various extracts comparing with standard drug.**

Conc. (in µg/ml)	Abs. of control	% RSA					
		CHCl <sub>3</sub>	EA	Ethanol	Methanol	Aqueous	Standard
20	0.2666	0.1875	18.5296	33.2331	24.2342	2.4381	98.4996
40	0.2666	5.1012	19.8799	44.5611	40.7307	12.2655	99.4373
60	0.2666	11.7779	21.1177	54.6136	46.7307	15.3638	98.7246
80	0.2666	14.0285	22.5431	62.4531	58.3557	40.7351	99.5873
100	0.2666	16.3915	23.4808	63.0532	59.9677	57.9144	99.3998

## In-vitro Cytotoxicity assay



**Figure 5. Pictorial representation of MD-MB-231 cells treated with different extracts of *C. siamea* plant extract.**

## CONCLUSION

In this study, extraction, fractionation, isolation, phytochemical studies, *in vivo* antioxidant assay using DPPH radical scavenging & MTT assay on MD-MB-231 cell line was performed by various extracts of *C. siamea* leaves.

The results from the phytochemical studies concludes that the ethyl acetate, aqueous & ethanol extract shows the presence of glycosides, flavonoids, Phytosterols & phenols in *C. siamea*. The antioxidant property was observed maximum in ethanol and methanol extract whereas, least activity observed in chloroform extract. Additionally, methanolic extract shows maximum inhibition at 10 $\mu$ M with 49% cell viability. Hence, ethanolic and methanolic extracts will be useful for isolation of compounds with evaluation of antioxidant potential.

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