

Allelopathic effects of *Cassia fistula* L on germination and seedlings growth of *Raphanus sativus* L

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ABSTRACT

Aqueous and organic solvent extracts of stem bark, leaves and pods of *Cassia fistula* L. and their residues were tested for their phytotoxic effects on seed germination and seedling growth of *Raphanus sativus* L. Bioassay with different solvents extracts revealed that inhibition by ethyl acetate extract of stem bark, leaves and pods was more than other extracts. Epiafzelechin and epicatechin (tannins or the building blocks of the proanthocyanidins) were present in the ethyl acetate extract of pods, kaempferol was obtained from the stem bark, while 1,8-dihydroxy-3-methyl anthraquinone (chrysophenol) and 1,8-dihydroxy-3-methyl-6-methoxy acetophenone (physcion) were found in the leaves of *Cassia fistula* L. The chemical studies revealed that epiafzelechin and epicatechin (tannins) most inhibited the plumule and radicle growth of *Raphanus sativus*.

Keywords: Allelochemicals, aqueous extract, bioassay, *Cassia fistula* L., ethyl acetate, extracts, germination, organic solvents, *Raphanus sativus* L., seedlings growth

INTRODUCTION

Allelopathy is the term used to describe the direct or indirect effects of one plant (or microbe) on another by the release of chemical substances into the environment (21,43). Plant metabolites or their byproducts that are released into the environment are known as allelochemicals. They might impact the plants at several stages of their life cycles (seed germination, seedling development, flowering and fruiting, vegetation formation and succession, and species regeneration) (7,12,29,54). Allelochemicals also interfere with various physiological processes viz. photosynthesis, respiration, water balance, and hormone balance, which affects the germination and growth of nearby plants (50). Seed germination directly influences the plant growth and survival (16,26,55). The seed germination test is most widely used biological detection methods in allelopathy research (43). The natural solvent of plants is water, which has the potential to leach away the chemicals from the plants.

Radish (*Raphanus sativus* L.) is a vegetable from Brassicaceae family, used mainly in salads, cooked and pickled. It is an excellent source of calcium, phosphorus, and ascorbic acid in both its roots and leaves (46). As the test plant for this study, radish was selected due to their high sensitivity to allelochemicals at low concentrations. (53). *Cassia fistula* L. (family Fabaceae) is a medium-sized deciduous tree (Figure 1), distributed throughout India up to an altitude of 1120 m. It is commonly planted in the vicinity of crops and affects crops through its allelopathic biochemical interactions. Its wood is more durable than sal (*Shorea robusta*), hence used for making house articles, and agricultural equipment and is an excellent fuel wood (1). The aqueous extract derived from the fruit, bark, and leaves of

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Cassia fistula exhibited varying degrees of toxicity when it was tested on *Pennisetum americanum*, *Lactuca sativa*, and *Setaria italica* (35).



Figure 1. *Cassia fistula* L. tree with seed pods

Cassia species are traditionally utilized primarily to treat jaundice, skin issues, respiratory illnesses, and gastrointestinal disorders (27). Besides it has antibacterial, antifungal and antioxidant properties (13,31). Aqueous extracts of leaves and seeds of *Cassia tora* affected the germination of mustard seeds, however, the root extract had little effect at all concentrations (47). Leaf material, leaf litter, and leave leachate of *Cassia siamea* inhibited the germination of maize, cassava, and *Malvastrum tricuspidatum* (15,18). Roots stem bark, leaves and flowers extracts of *Cassia sophera* L. and its isolated compound i.e., scopoletin, quercetin, triterpene inhibited the germination of wheat, rice, rape seed, black gram, and lettuce (9). *Cassia tora* L. seed coat extract inhibited seedling growth of sesame (6). The root extract of *Cassia auriculata* inhibited the root elongation of pearl millet (41). The allelopathic effect of *Cassia occidentalis* inhibits the growth and germination of seeds of *Parthenium hysterophorus* L., a harmful weed (28). Proanthocyanidins with flavan-3-ol (epiafzelechin and epicatechin) units with an abnormal 2S-configuration, as well as common flavan-3-ols and proanthocyanidins, were identified from the pods of *Cassia fistula* L. and the bark of *C. javanica* L. (Leguminosae) (25). Kaempferol, dihydroxy kaempferol, 1,8-dihydroxy-3-methyl anthraquinone, epiafzelechin, and epicatechin were isolated from the sapwood of *Cassia fistula* (38). A previous study revealed that proanthocyanidins from *Prunus armeniaca* inhibited the growth and germination of *Triticum aestivum* the most (42).

Isolating each component of plant extracts or active components individually and purifying them into monomer compounds using physical and chemical techniques is the process of separating phytochemicals. The current standard for isolation techniques is the continued use of traditional techniques such as solvent extraction, precipitation, crystallization, fractional distillation, etc. However, using various chromatographic separation techniques also greatly helps in the separation of phytochemicals (2,8,20). The separation of chemicals in a sample according to characteristics like polarity, size, and chemical functionalization is the aim of chromatography (48). The primary objective of spectral analysis is to obtain as much structural information as possible through measuring and analyzing various spectra (49).

This study aimed to investigate the allelopathic effects of *Cassia fistula* L. aerial parts and the resulting morphological changes in *Raphanus sativus* L., the field crop that was

affected. Assay of the aerial parts (Stem bark, leaves and pods) of *Cassia fistula* in natural form or the concentrated extracts (solvent extracts) on the germination and seedlings growth of *Raphanus sativus* L. in which changes were observed. Isolation and characterization of the allelochemicals and observation of the effect of allelochemicals on the germination and seedlings growth of *Raphanus sativus* L.

MATERIALS AND METHODS

The field experiment was undertaken from October to December 2022 at Nakronda, Doiwala, Dehradun, India [latitude: 30.24°N, longitude: 78.10°E, altitude: 485 m, moderate climate, annual rainfall: 2187 mm, summer temperature (Summer): 43 °C and winter: -0.5 °C].

Cassia fistula trees in the vicinity of crop fields were selected for detailed study. The observations were recorded 3 m away from the matured *Cassia fistula* L. tree to eliminate the direct shading effect on the *Raphanus sativus* L. crop. Test plant population and plant height were recorded from varying distances viz. 3-4, 4-5, 5-6 and 6-7m from base of *Cassia fistula* L. tree. The fifth quadrat, which was 7 meters from the tree, was regarded as normal, with interference being regarded as zero (36). To determine the percentage reduction in germination, seedlings in each quadrat were counted after 5 weeks after sowing and compared to the population of a normal quadrat, where germination was assumed to be 100 %. After the crop reached maturity, the average of 20 healthy plants per quadrat was used to collect data on height and yield, which were then compared to the average of the same number of healthy plants from the normal quadrat, where height and yield were considered to be 100 %. The average of height, yield, and germination was then calculated (Figure 2).

Extract Preparation

Brown ripened pods and matured leaves from the top and stem bark from the bottom of *Cassia fistula* L., were collected from Nakronda, Doiwala, Dehradun, India. The plant parts were shade-dried and ground to make a fine powder. Powdered materials were extracted in ethanol for 6 h. The extracted residues were subjected to Thin Layer Chromatography (TLC). The TLC of ethanolic extract revealed compounds of diverse polarities. For the bioassay, 0.5 kg dried and powdered plant material (aerial parts viz. pods, leaves, and stem bark) was extracted with ethanol. Following reduced pressure evaporation, the residue was extracted in a Soxhlet apparatus with light petroleum, chloroform, ethyl acetate, and ethanol. All extracts were vacuum-dried under reduced pressure. For the bioassay of allelochemicals and their characterization, 1.0 kg each of dried and powdered plant material (leaves, pods and bark) was extracted with ethanol. The residue was then extracted in a Soxhlet apparatus with light petroleum, chloroform, ethyl acetate, and ethanol under reduced pressure evaporation.

The aqueous extract was prepared by soaking 40 g of each plant part (stem bark, leaves, and pods) in 1 liter double distilled water at room temperature for 48 h (3,24) and tested for bacterial growth, which was not observed in the extract (52). The extract was filtered under vacuum through Whatman filter paper no. 1.

Bioassay

The bioassay of different organic solvent extracts and the aqueous extract (400 ppm concentration) was done on *Raphanus sativus* L. as the test crop. The 'Japanese white' (Asiatic or tropical) variety of radish was selected for the study. Fifty mg of water and each organic solvent residue were dissolved separately in 50 ml of the solvent in which they were extracted and 2 ml of this solution was poured onto Whatman filter paper No. 1. in Petri dishes of 9 cm diameter. In controls, only 2 ml of the respective solvents were used. After evaporation of the solvent, 5 ml double distilled water was added and 10 seeds of the test crop were set in each petri dish and covered with another filter paper. Each extract and control treatment were replicated four times. All the Petri dishes were kept in an incubator at 25 ± 2 °C for 72 h. The percentage inhibition of root, shoot length, and germination was calculated by considering the control as zero. The results were statistically analyzed for the F-test.

Chemical analysis of *Cassia fistula* L.

Bioassay with organic extracts showed that ethyl acetate extracts of the studied plant parts were more inhibitory to seedling growth than extracts of other plant parts. Therefore, ethyl acetate extract was used to separate allelochemicals. Column chromatography over silica gel (60-120 mesh) of the ethyl acetate extract of pods, stem bark, and leaves in Chloroform: Methanol.

Bioassay with isolated compounds

For bioassay of isolated compounds, 5 mg of the compound was dissolved in 50 ml of methanol (0.01% concentration). Two ml of this solution was used to moisten the Whatman filter paper no. 1 in petri dishes and other operations were made similarly as in the case of plant parts extracts. The results were statistically analyzed for the F-test.

Statistical Analysis : The results were statically analysed using ANOVA.

RESULTS AND DISCUSSION

Field study: Germination and root and shoot development of *Raphanus sativus* L. crop growing under the canopy of *Cassia fistula* L. trees were inhibited [Table 1, Figure 2 (a-c)] which may have been caused due to several potential growth inhibitory organic compounds released from plants into their immediate environment by leaching and volatilization from plant foliage via exudation from clough-off of plant roots and the decomposition of dead plant residues. Many of these natural products enter into the soil medium (36).

The aqueous extract of stem bark, pods, and leaves of the mature plant of *Cassia fistula* L. significantly inhibited the seed germination, shoot, and root lengths of *Raphanus sativus* L. A very early allelochemical activation might have caused this type of response in seeds to extracts. During the swelling stage, these chemicals might produce anatomical deformations in the seed coat and spare components, contributing to delayed germination (34). Seed germination is inhibited by allelochemicals because they suppress the mitotic activity of developing cells (43). Light petroleum, chloroform, ethyl acetate, and methanol extracts of stem bark, leaves, and pods of *Cassia fistula* were used to observe allelopathic effects, among them ethyl acetate extract showed maximum inhibition due to the presence of allelochemicals in higher concentration than other solvent extracts [Table 2,

Figure 3 (a-c)]. Therefore, the ethyl acetate extract of stem bark, pods, and leaves was taken for further chemical analysis.

Table 1. Effects of *Cassia fistula* on *Raphanus sativus* at varying distances from tree base

S. No.	Distance from <i>Cassia fistula</i> tree (m)	% Reduction over normal plants (>7 m) mean values		
		Germination	Height	Yield
1.	3.5	44.5	40.0	34.5
2.	4.5	32.0	34.0	28.5
3.	5.5	26.5	20.0	19.6
4	6.5	15.2	11.5	9.0
Standard Error of the Mean (SEM) \pm		6.08	6.49	5.55
F Value		17.41852	6.928892	11.43011
p-Value		0.005855	0.058048	0.014842
Standard Error of the Mean (SEM) \pm		6.08	6.49	5.55
F Critical ($\alpha=0.05$)		5.987378	7.708647	5.987378

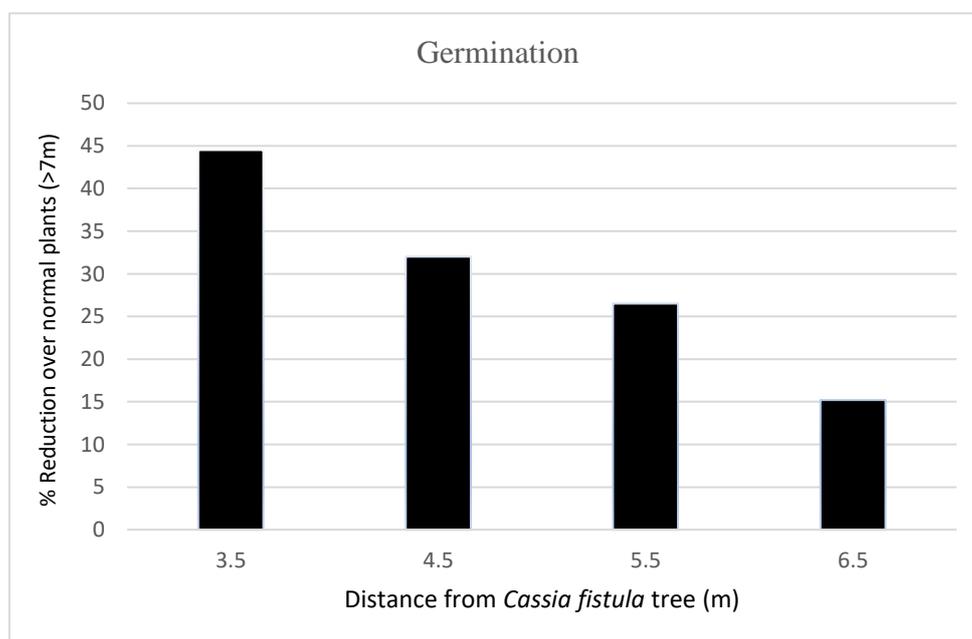


Figure 2 (a). Effects of *Cassia fistula* on germination of *Raphanus sativus* at varying distances from tree base

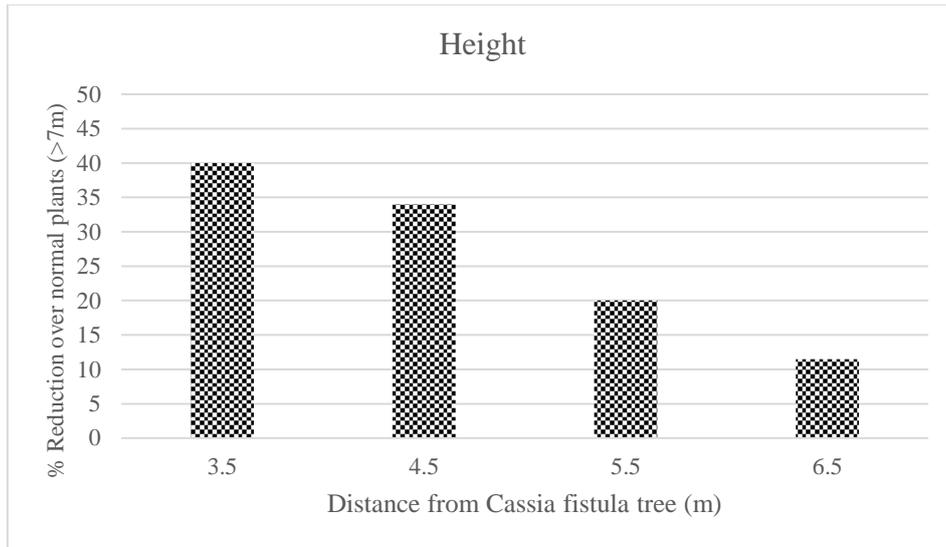


Figure 2 (b). Effects of *Cassia fistula* on height of *Raphanus sativus* at varying distances from tree base

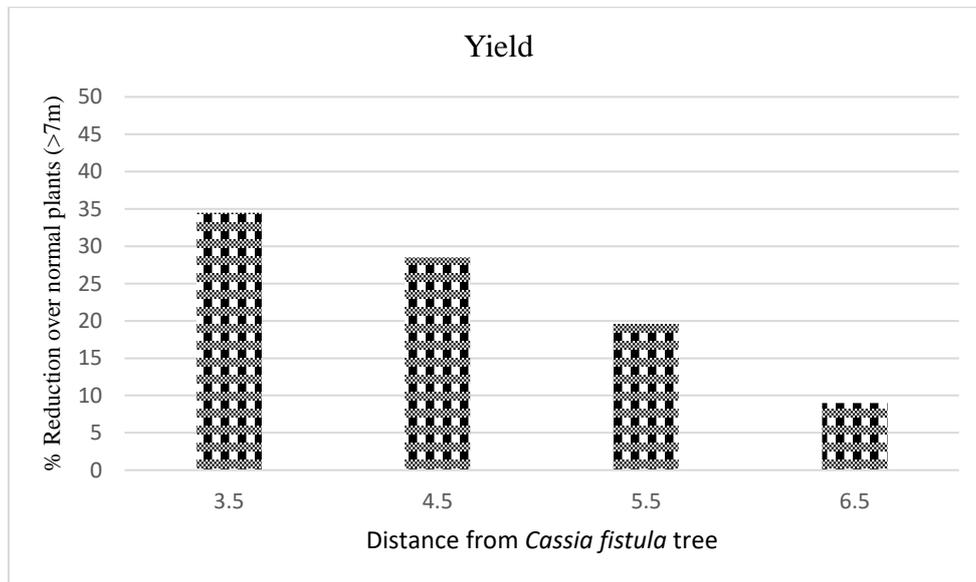
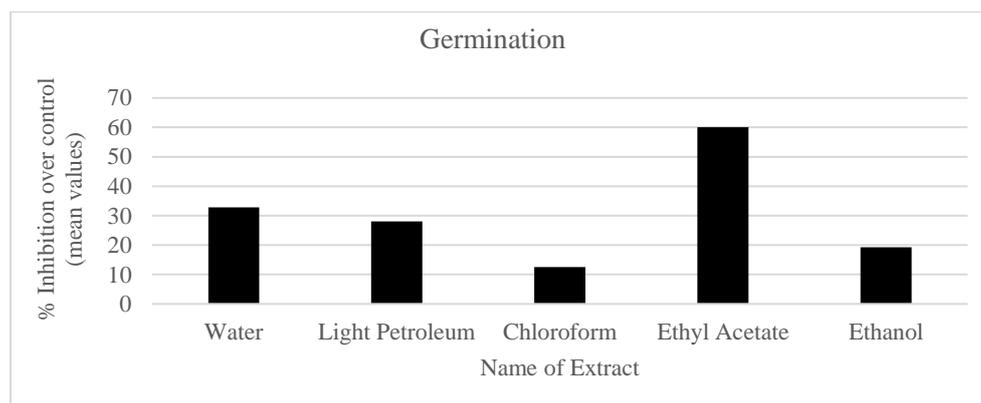
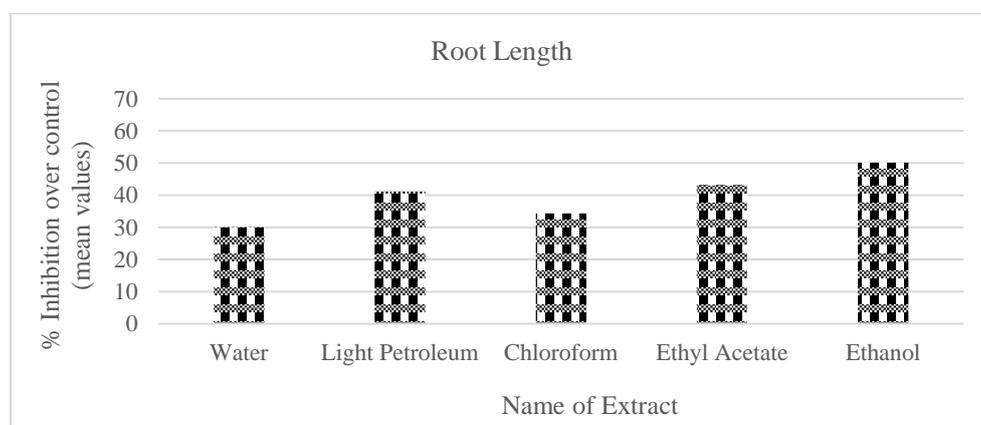


Figure 2 (c). Effects of *Cassia fistula* on yield of *Raphanus sativus* at varying distances from tree base

Table 2. Effects of aerial part extracts (400 ppm) of *Cassia fistula* on *Raphanus sativus* seedling bioassay

S. No.	Name of Extracts	% Reduction over normal plants (>7 m) mean values		
		Germination	Root Length	Shoot Length
1.	Water	32.78	30.09	41.87
2.	Light Petroleum	28.08	41.15	30.00
3.	Chloroform	12.58	34.30	40.10
4.	Ethyl Acetate	60.00	43.15	54.00
5.	Ethanol	19.22	50.08	54.94
	SEM \pm	8.15	3.48	4.66
F Value		12.1534	1.081374	2.111388
p-Value		0.008245	0.328795	0.184277
F Critical ($\alpha=0.05$)		5.317655	5.317655	5.317655

Figure 3 (a). Effects of aerial part extracts (400 ppm) of *Cassia fistula* on *Raphanus sativus* seedling (germination) bioassayFigure 3 (b). Effects of aerial part extracts (400 ppm) of *Cassia fistula* on *Raphanus sativus* seedling (root length) bioassay

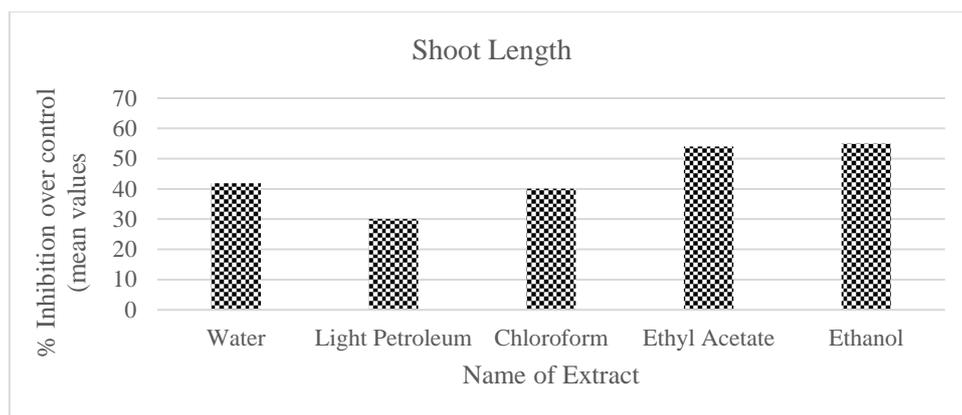


Figure 3 (c). Effects of aerial part extracts (400 ppm) of *Cassia fistula* on *Raphanus sativus* seedling (shoot length) bioassay

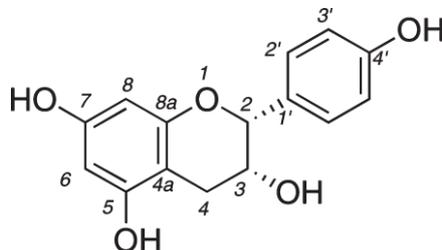
Chemical studies revealed that the ethyl acetate extract of *Cassia fistula* L. pods contained the following compounds.

Compound 1: Colourless solid, melting point: 225-227°C.

IR (ν max KBr cm^{-1}): 3416, 3317, 2936, 1614, 1513, 1469, 1355, 1284, 1219, 1167, 1140, 1094, 916, 864.

^1H NMR (300 MHz, CD_3COCD_3): δ 2.82 (m, - CH_2 -, 2H, H-4), 3.65 (d, 1H, H-2), 4.19-4.24 (m, 1H, H-3), 4.93 (br s, 1H, (CH) OH), 5.92 (d, $J = 2.27$ Hz, 1H, H-8), 6.0 (d, $J = 2.27$ Hz, 1H, H-6), 6.81 (d, $J = 8.69$ Hz, 2H, H-3', H-5'), 7.35 (d, $J = 8.31$ Hz, 2H, H-2', H-6'), 7.95 (br s, 1H, ArOH), 8.10 (br s, 1H, ArOH), 8.21 (br s, 1H, ArOH).

^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{C}=\text{O}$): δ 29.4 (- CH_2 -, C-4), 67.1 (CH, C-3), 79.8 (CH, C-2), 96.0 (CH, C-6), 96.4 (CH, C-8), 100.0 (C, C-4a), 115.7 (2C, C-3', C-5'), 129.5 (2C, C-2', C-6'), 131.8 (C, C1'), 157.4 (C, C-4'), 157.5 (C, C-7), 157.8 (2C, C-5, C-8a).



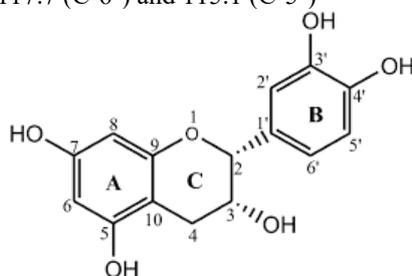
The IR spectra of compound 1 showed bands at 3416-3317 cm^{-1} (Ar-OH stretching), 1614 cm^{-1} (C-C multiplet bond stretching), and 864 cm^{-1} (C-H aryllic bending). The ^1H NMR spectra of the compound revealed eight methine protons at δ 3.65 (H-2), 4.19-4.24 (H-3), 5.92 (H-8), 6.0 (H-6), 6.81 (H-3' and H-5'), 7.35 (H-2' and H-6'), four hydroxyl group protons with chemical shift values recorded at δ 4.94, 7.95, 8.10, and 8.21, and a methylene proton reported at δ 2.82. The spectral peak of H-2 at δ 4.80 ppm appears as a singlet because

the coupling constant of H-2 and H-3 was insignificant. This verified the *cis* connection between H-2 and H-3. The compound's ^{13}C NMR analysis revealed that the cluster peaks at δ 131.8 ppm belong to C-1', while the peak at δ 115.7 ppm is attributed to C-3' and C-5'. The peaks δ 96.4, 96.0, and 129.5 ppm are attributed to C-8, C-6, C-6', and C-2'. At positions δ 157.8 (C-5, C-8a), 157.5 (C-7), 67.1 (C-3), and 157.4 (C-4'), there are four oxygenated carbon atoms. There are seven aromatic carbon (methine) peaks at δ 96.0 (C-6), 94.4 (C-8), 131.8 (C-1'), 129.5 (C-2' and C-6'), 115.7 (C-3' and C-5'), three aliphatic carbons at δ 79.8 (C-2), 67.1 (C-3), and 29.4 (C-4). Thus, the structure of Compound 1 was identified as epiafzelechin based on the above spectrum data and comparison with previously known spectral values (10, 22, 38) and verified by Co-TLC, and a mixed melting point test with a reference sample.

Compound 2: Brown amorphous solid, MP 175 - 177°C.

^1H -NMR spectrum (300 MHz, CD_3OD); δ 5.94 (1H, d, $J=2.2$ Hz, H-8), δ 5.92 (1H, d, $J=2.2$ Hz, H-6), δ 2.88 (1H, dd, $J=4.6, 16.7$ Hz, H-4b), δ 2.74 (1H, dd, $J=3.3, 16.7$ Hz, H-4a), δ 4.81 (1H, brs, H-2), δ 4.19 (1H, m, H-3), δ 6.94 (1H, d, $J=1.7$ Hz, H-2'), δ 6.80 (1H, dd, $J=1.8, 8.5$ Hz, H-6') and δ 6.79 (1H, d, 8.5 Hz, H-5').

^{13}C NMR (100 MHz, CD_3OD); δ C 156.3 (C-5), 156.9 (C-7), 156.8 (C-9), 98.9 (C-10), 131.3 (C-1'), 144.0 (C-4'), 144.9 (C-3'), 78.5 (C-2), 66.4 (C-3), 28.3 (C-4), 96.1 (C-6), 94.2 (C-8), 113.5 (C-2'), 117.7 (C-6') and 115.1 (C-5')



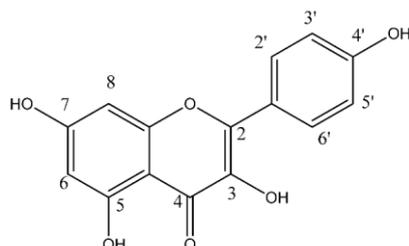
The ^1H NMR of compound 2 revealed the signals of protons observed at δ 5.94 (1H, d, $J=2.2$ Hz, H-8) and 5.92 (1H, d, $J=2.2$ Hz, H-6) suggested the presence of AX systems (tetrasubstituted benzene rings). A trisubstituted benzene ring was shown by the protons at δ 6.94 (1H, d, $J=1.7$ Hz, H-2'), 6.80 (1H, dd, $J=1.8, 8.5$ Hz, H-6'), and 6.79 (1H, d, $J=1.8, 8.5$ Hz, H-5'). The proton chemical shift values found at 4.81 (1H, brs, H-2) and 4.19 (1H, m, H-3), which correspond to an oxymethine and a carbinol proton, respectively, showed that an aliphatic ring existed. Two hydrogen atoms that can be attributed to C-4 and have chemical shift values of δ 2.74 (1H, dd, 2.9, 16.7 Hz, H-4a) and 2.88 (1H, dd, 4.6, 16.7 Hz, H-4b) define the 3-flavan-type flavonoid. The ^{13}C -NMR spectrum of Compound 2 showed that there were 15 carbon atoms present. Five oxygenated carbon atoms can be found at positions δ 156.3 (C-5), 156.3 (C-7), 156.4 (C-9), 144.0 (C-4'), and 144.9 (C-3'). Seven aromatic carbon peaks (methine) can be found at positions δ 96.1 (C-6), 94.2 (C-8), 98.9 (C-10), 131.3 (C-1'), 113.5 (C-2'), 115.1 (C-5'), and 117.7 (C-6'). The chemical shift value of carbon at δ 78.5 (C-2), and 66.4 (C-3) revealed three aliphatic carbons, whereas position δ 28.3 (C-4) for the methylene carbon. Catechin and epicatechin differ from one another due to the configuration of the C-2 and C-3 carbons. Catechin has chemical structures with (2R, 3S) or (2S, 3R) configurations, whereas epicatechin has (2S, 3S) or (2R, 3R) configurations.

It has been found that the vicinal protons H-2 in position 2 and H-3 in position 3 exhibit a weak coupling constant, suggesting that they are in the cis position about one another. Accordingly, the structure of Compound 2 was determined to be epicatechin using the data above (10, 25, 38, 40). The identification was verified by Co-TLC, and a mixed melting point test with a reference sample of epicatechin revealed that the two substances had the same R_f value and melting point.

The ethyl acetate extract of *Cassia fistula* L. stem bark yielded the following compound.

Compound 3: Yellow needles, MP 273-275°C.

¹³C NMR (100 MHz, CDCl₃): δ C 146.6 (C-2), 134.6 (C-3), 177.3 (C-4), 161.1 (C-5), 99.3 (C-6), 161.9 (C-7), 93.9 (C-8), 158.0 (C-9), 145.3 (C-10), 120.9 (C-1'), 130.8 (C-2' and C-6'), 116.3 (C-3' and C-5'), and 161.2 (C-4').



The ¹³C-NMR spectra of compound 3 revealed fifteen carbon signals in all. The DEPT experiment ascribed them to fourteen sp² carbons and a carbonyl signal at δ 177.3. The degree of unsaturation accounted for eight of the eleven total double-bond equivalents. The flavonol structure was consistent with the remaining three degrees of unsaturation. A comparison of NMR data of compound 3 with those of kaempferol (42), indicated that the structures of the two compounds are highly similar, which was confirmed by Co-TLC and mixed melting point with reference sample; hence, compound 3 was identified as kaempferol (38, 42).

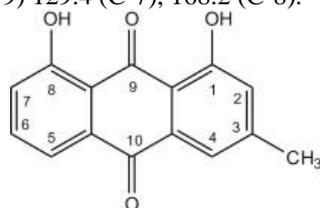
The ethyl acetate extract of *Cassia fistula* L. leaves afforded the following compounds

Compound 4: Yellow-coloured needles, melting point: 194-197°C.

IR (ν max KBr cm⁻¹): 3046, 2848, 1676, 1627, 1473, 1450, 1270, 1207, 1157, 1085, 1024, 902, 869, 839, 752.

¹H NMR (CDCl₃, 300 MHz): δ 2.7 (3H, s, CH₃), 7.1 (1H, brs, H-2), 7.3 (1H, brd, J=8.0 Hz, H-7), 7.6 (1H, brs, H-4), 8.0 (1H, brs, H-6), 7.8 (1H, brd, J=8.0 Hz, H-5), 12.0 (1H, s, -OH), 12.1 (1H, s, -OH).

¹³C NMR (CDCl₃, 100 MHz): δ C 22.3 (-CH₃), 108.7 (C-5), 109.6 (C-12), 114.1 (C-13), 121.0 (C-4), 124.5 (C-2), 133.3 (C-14), 135.6 (C-11), 149.0 (C-3), 162.0 (C-1), 166.5 (C-6), 182.0 (C-10), 190.2 (C-9), 129.4 (C-7), 168.2 (C-8).



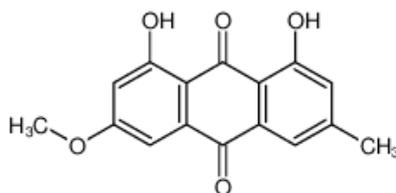
The ^1H -NMR spectrum of compound 4 showed two strongly downfield shifted signals at δ 12.1 and 12.0 owing to chelated hydroxyl protons involved in hydrogen bonding and ^{13}C -NMR spectrum with two downfield shifted carbonyl signals at δ 190.2 and 182.0. In addition, the ^1H -NMR spectrum revealed five aromatic and three methyl protons. Three mutually coupled aromatic protons with ABX spin system at δ 7.8, 8.0, and 7.3 were assigned to H-5, H-6, and H-7, respectively. Two meta-coupled aromatic protons with resonances at δ 7.1 and 7.6 that are attributed to H-2 and H-4, respectively. The di-substituted ring has an aromatic methyl group (δ 2.7) at position C-3. The ^{13}C NMR spectrum of compound 4 showed 15 carbon signals, including five aromatic methines at δ 124.5 (C-2), 121.0 (C-4), 108.7 (C-5), 166.5 (C-6) and 129.4 (C-7). Two carbonyl carbon signals were observed at δ 190.2 (C-9) and 182.0 (C-10) as well as a methyl group (δ 22.3). There are seven aromatic quaternary carbons at δ 135.6 (C-11), 109.6 (C-12), 114.1 (C-13), 133.3 (C-14), 162.0 (C-1), 149.0 (C-3) and 168.2 (C-8).

Therefore, based on the aforementioned spectrum data and comparison with previously known spectral values, the structure of Compound 4 was identified as 1,8-dihydroxy-3-methylanthraquinone (chrysophanol) (33,38,45) and comparison with an authentic sample.

Compound 5: yellow-colored needles, melting point: 205-207°C.

IR (ν max KBr cm^{-1}): 3403, 2920, 2831, 1626, 1480, 1367, 1321, 1272, 1225, 1161, 1031, 978, 904, 873, 712.

^1H NMR (CDCl_3 , 300 MHz): δ 2.4 (3H, s, CH_3), 3.9 (3H, s, $-\text{OCH}_3$), 6.6 (1H, d, $J=3.0$ Hz, H-7), 7.0 (1H, brs, H-2), 7.3 (1H, d, $J=3.0$ Hz, H-5), 7.6 (1H, d, $J=3.0$ Hz, H-4), 12.1 (1H, s, OH), 12.3 (1H, s, OH).



The IR spectra of compound 5 revealed characteristic absorption at 3403, 1272, and 1225 cm^{-1} (OH groups), 1626 cm^{-1} (conjugated $\text{C}=\text{O}$), and 1480 cm^{-1} (aromatic system). Two meta-coupled aromatic protons resonating at δ 7.0 and 7.6 assigned to H-2 and H-4, respectively, were seen in the ^1H -NMR spectrum of Compound 5. These protons were broadened by the methyl protons at C-3. With a coupling constant of 3.0 Hz, the narrow doublets at δ 6.6 and 7.3 may be assigned to H-7 and H-5, respectively. Methyl protons appeared as a singlet at δ 2.4, while methoxy protons resonated at δ 3.9 and 1,8-dihydroxy protons at δ 12.3 and 12.1 respectively.

Thus, based on the above spectral data and comparison to known spectral values, the structure of Compound 5 was assigned as 1,8-Dihydroxy-3-methyl-6-methoxyanthraquinone (physcion) (14, 30, 32) and comparison with an authentic sample. Compounds 4 and 5 exhibited a positive ferric reaction of phenol and the compound developed a red color on the chromatographic plate when sprayed with 10% methanolic KOH indicating the presence of anthraquinones (17).

Bioassay with isolated compounds revealed that the epiafzelechin and epicatechin (proanthocyanidins) have shown maximum inhibition in germination, plumule, and radicle growth of *Raphanus sativus* L. followed by kaempferol (flavonoid) and chrysophenol and physcion (anthraquinone) at the concentration of 40 ppm [(Table 3, Figure 4(a-c)]. Epicatechin and kaempferol have been studied for their impact on the differentiation of shoots and roots and the effectiveness of seed germination. It was discovered that these compounds are phytotoxic and are crucial for the dormancy, germination, and longevity of seeds (5, 11). Proanthocyanidins also known as condensed tannins, are a group of polymeric phenolic compounds consisting mainly of flavan-3-ol units such as afzelechin, epiafzelechin, catechin, epicatechin. Tannins were recorded as inhibitors of seed germination (43). The tannins possibly interfere with the growth of nitrifying bacteria (44) and inactivate plant β -glucosidases (51), which may cause inhibition in the growth and germination of the test crop. Proanthocyanidins have been reported as plant growth inhibitors (42). Some of these isolated compounds are reported to affect various metabolic processes of plants. For example, kaempferol is inhibitory to the synthesis of ATP acts as an energy transfer inhibitor, and also inhibits the phosphorylation mechanism (4). Anthraquinones too are recognized allelochemicals (23, 37). The ethyl acetate extract of leaves was dominated by anthraquinones (chrysophanol and physcion), which showed varying levels of allelopathic effects on plant seed germination and plant growth, suggesting a strong allelopathic activity. The considerable amount of phenolic and anthraquinones is most likely responsible for the allelopathic activity (39). The anthraquinone physcion has been described as allelochemical in *Polygonum sachalinense* (23). Several previous studies on the mechanisms by which anthraquinone (e.g., juglone) exerts its toxic effects on

Table 3. Effects of isolated compounds (40 ppm) from *Cassia fistula* on *Raphanus sativus* seedling growth

S. No.	Name of Isolated Compounds	% Reduction over normal plants (>7 m) mean values		
		Germination	Root Length	Shoot Length
1.	Epicatechin	65.40	56.30	54.08
2.	Epiafzelechin	60.50	59.50	52.50
3.	Kaempferol	45.00	42.50	38.90
4.	Chrysophanol	40.00	52.10	44.60
5.	Physcion	36.00	50.10	39.40
SEM \pm		5.77	2.90	3.18
F Value		63.59623	0.177197	0.279072
p-Value		0.00	0.684874	0.611646
F Critical ($\alpha=0.05$)		5.317655	5.317655	5.317655

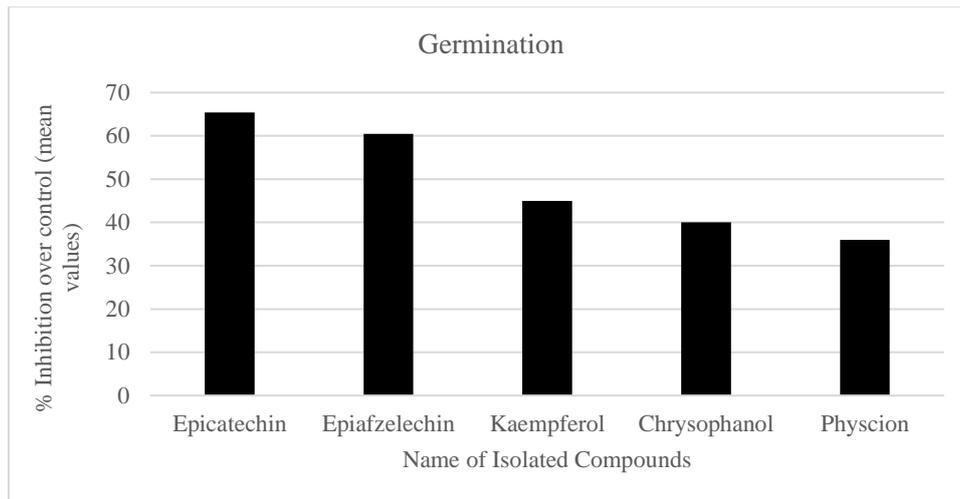


Figure 4 (a). Effects of isolated compounds (40 ppm) from *Cassia fistula* on *Raphanus sativus* seedling growth (germination).

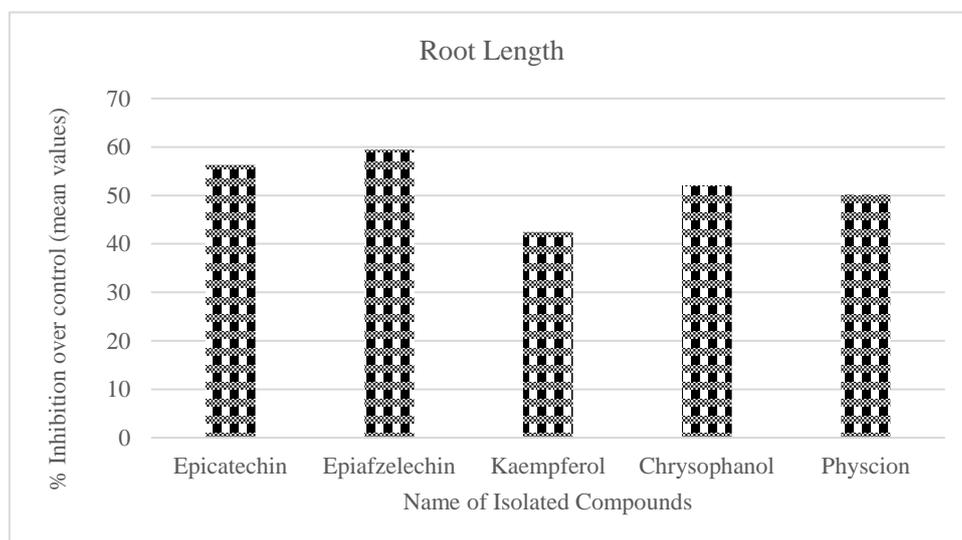


Figure 4 (b). Effects of isolated compounds (40 ppm) from *Cassia fistula* on *Raphanus sativus* seedling growth (root length).

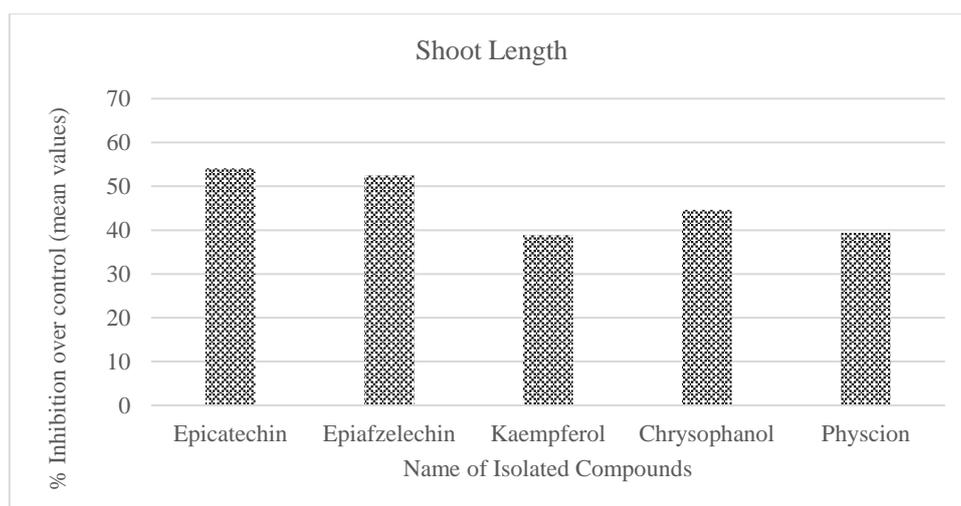


Figure 4 (c). Effects of isolated compounds (40 ppm) from *Cassia fistula* on *Raphanus sativus* seedling growth (shoot length).

susceptible plants indicated that it interferes with the electron transport systems in the mitochondria and the chloroplasts. Secondary result due to the action of juglone on H^+ -ATPase in root cells, causing loss of water absorption and thus stomatal closure with reduced photosynthesis (19). Therefore, the isolated compounds from *Cassia fistula* L. particularly proanthocyanidins, flavonoids, and anthraquinones can be regarded as inhibitors of seed germination and growth of *Raphanus sativus* L.

CONCLUSIONS

The *Raphanus sativa* seed germination and seedling growth were reduced at various levels, suggesting that the phytochemicals constituents (such as epicatechin, epiafzelechin, kaempferol, chrysophanol, and physcion) present in various parts of *Cassia fistula* L may have allelopathic effects. All treatments decreased the radish seed germination and seedling growth compared to control. Among the different extracts and isolated compounds of *Cassia fistula* L, the decreasing order of inhibition in seed germination of radish (ethyl acetate > water > light petroleum > ethanol > chloroform) and (epicatechin > epiafzelechin > kaempferol > chrysophanol > physcion). Due to its use in herbal remedies, farmers grow the *Cassia fistula* L. tree. To prevent the persistent accumulation of phytotoxins in tree vicinity, appropriate precautions should be taken.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct.

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