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Comparative Study of the Phytochemical, Proximate Composition, and Antioxidant Activity of Defatted and Non-Defatted Extracts of *Cassia fistula* Linn

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Abstract

Cassia fistula Linn has been used in the management of human diseases such as; asthma, leprosy, heart diseases and fever globally. This study evaluated the phytochemical, physicochemical, proximate composition and antioxidants activity of defatted and non-defatted ethanol extracts of *Cassia fistula* leaves and seed. Fresh leaves and seeds of *Cassia fistula* were collected from Augustine University Ilara-Epe, Lagos State. The materials were air-dried at room temperature and pulverized using electric blender. Homogenized leaves and seeds samples were divided into two portions. First portion (120 g) of leaves and seeds samples were separately defatted with 550 mL of n-hexane and then extracted with 1100 mL of ethanol. To the second portions, 1100 mL of ethanol was added to 120 g of leaves and seeds samples and kept for 72 hours. Filtrates of each extract were concentrated using rotary evaporator at 40 ºC. The results showed that the non–defatted seed had the highest percentage extract yield of 2. 6%. The physicochemical parameters showed that non-defatted seed extract had the highest pH (6.94 ± 0.005) while defatted leaves extract had a significant increase in ash content (6.22 ± 0.28) compared to others. The proximate analysis showed that the non-defatted seed extract had a significant increase ($P < 0.05$) in lipid content. Protein content and carbohydrate increased significantly ($P < 0.05$) in defatted seed extract when compared to the defatted leaves extract, non-defatted leaves extract and non-defatted seed extracts. Defatted leaves extract had a significant increase (3.20 ± 0.005) in crude fibre. The non-defatted leaves of the *Cassia fistula* exhibited the highest ferric reducing power while the defatted seeds sample showed the highest DPPH and H_2O_2 scavenging activities. This study revealed that defatting of *Cassia fistula* increased ash, crude protein, and carbohydrates contents with enhanced antioxidant activities.

Keywords: *Cassia fistula*, seeds, leaves, defatted, Comparative analysis, proximate composition, Antioxidant

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Introduction

Plants rich in phytochemicals have been used since ancient times for the treatment of various diseases. *Cassia. Fistula* is one of the trees that had been traditionally used for therapeutic purposes. Its barks, leaves, and fruits (pods) had been used for the treatment of many diseases. *Cassia fistula* pod contains a high amount of epiafzelechin, epicatechin, procyanidin, and saponins and can have protective role in metabolic disorders (Mwangi *et al.,* 2021*). Cassia fistula* belongs to the Leguminosae family of the plantae kingdom, and is an ornamental tree with its beautiful yellow flowers also known as 'Golden shower' due to its appearance. *Cassia fistula* is a semi-wild plant (Pawar *et al.*, 2017) that grows throughout many countries, mainly on the roadside (Rana *et al.,* 2017). The plant grows up to 20–30 m tall and the leaves are attached in the form of pinnate. The root, bark, and flowers are brownish-red, brown, and bright yellow in colour respectively (Pawar *et al.*, 2017; Rana *et al.,* 2017). The wood has high shelf-life and is very strong. The flowers have a glowing yellow colour and are 40 cm long at maturity, and this is the reason why *Cassia fistula* is called "Golden flower" (Singh *et al*., 2023)

Various parts of *Cassia fistula* have been reported to possess numerous medicinal activities such as anti-cancer, antioxidant potentials, laxative, and analgesic properties (Ali, 2014; Kumar *et al.*, 2017; Mwangi *et al.*, 2021). The stem bark and fruit of the plant displayed purgative effects, treats fever, heart related diseases, and nausea (Ali, 2014). Other studies on the medicinal activity of this plant have been documented (Wijaya *et al.,* 2000; Siddhuraju *et al.*, 2002; Bhalodia *et al.*, 2012). The plant possesses bioactive properties like antibacterial, antimicrobial, antifungal, anti-inflammatory, antitumor, anticancer, antioxidant, anti-yeast, and many skin-related bioactivities (Patel *et al*., 2016). The phytochemicals which were reported present in the *Cassia fistula* plant are carbohydrates, proteins, amino acids, flavonoids, tannins, anthraquinone and its derivatives, glycosides, terpenoids, sugars, alkaloids, saponins, and rhein, mucilage (Maqsood *et al.*, 2020; Sharma *et al*., 2020; Patel *et al*., 2016). From a commercial or economic perspectives, natural gum from *Cassia fistula* seeds is applied in the preservation of food products in various food industries. It has also been suggested that this property of *Cassia fistula* may be due to its antioxidant properties (Saha *et al.*, 2017). Due to the high cost and insufficient supply of synthetic drugs in rural parts of Nigeria, traditional herbal medicine is becoming more widely used for the treatment of various ailments and diseases (GRIN, 2019). This study investigated the phytochemical, proximate composition, and antioxidant activity of defatted and non-defatted ethanol leaves and seeds extracts of *Cassia fistula* were investigated.

Materials and Methods

Reagents and chemicals

The reagents used in this study were ferric chloride ($FeCl_3$), tetraoxosulphate (VI) acid (H_2SO_4), aqueous sodium hydroxide (NaOH), hydrochloric acid (HCl), Dragendoff's reagent, Molisch's reagent, acetic anhydride, ammonia, glacial acetic acid and sodium bicarbonate. The solvents used include n-hexane, ethanol, chloroform and petroleum ether.

Preparation of Cassia fistula extracts

Fresh leaves and seeds of *Cassia fistula* were collected on the campus of Augustine University Ilara-Epe, Lagos State. The collections were identified by Dr. Mudasiru O.M with the voucher number (FHA 4259) from the Department of Pure and Applied Botany, College of Biosciences Federal University of Agriculture, Abeokuta Ogun State Nigeria. The samples were then air-dried at room temperature and pulverized using electric blender prior to extraction. Homogenized leaves and seeds of *Cassia fistula* were each divided into two portions. A 120 g of the first portion of leaves and seeds respectively were defatted with 550 mL of n-hexane and then extracted with 1100 mL of ethanol. To the second portions, 1100 mL of ethanol was added to 120 g of leaves or seeds samples respectively and kept for 72 hours with frequent agitation (Adebusuyi and Dosumu, 2016). Filtration of the extracts was carried out using Whatman filter paper of the size 110 mm. The filtrates of each extract were concentrated using rotary evaporator at 40 ^oC. The crude air-dried extracts were weighed, percentage yield calculated, and stored in the refrigerator till further chemical analysis. The plant samples were code named: NDLE (Non-defatted leaves extract), DLE (Defatted leaves extract), DSE (Defatted seeds extract), and NDSE (Non-defatted seeds extract).

Phytochemical screening

Phytochemical screenings were carried out on defatted and non-defatted leaves and seeds extracts respectively using standard procedures to identify various metabolites present in the extracts (Varadharajan *et al.*, 2012; Adebusuyi and Dosumu, 2016).

Physicochemical and proximate analysis

Defatted and non-defatted leaves and seeds samples were analyzed for their moisture content, pH, loss on drying, and ash content (Ajayi *et al.*, 2011; Varadharajan *et al.*, 2012). Proximate analysis of the plant samples was carried out using AOAC, 1995 and Kamal *et al*. (2018) methods

Antioxidant assay of extracts

Scavenging activity of hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) scavenging activity of the plant extracts were determined using method proposed by Nabavi *et al*., (2008).

Ferric reducing power

The ferric reducing property of the extracts was determined using procedures described by Luqman *et al.*, (2012).

DPPH radical scavenging activity

The free radical scavenging activity of the extracts at different concentrations (0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL) was tested against 1,1-diphenyl-2-picrylhyrazyl (DPPH) (Dudonne *et al.*, 2012; Senguttuvan *et al.*, 2014).

Statistical analysis

Results were expressed as mean ± SD with n = 3. Dose-response relationship between the extracts was carried out using regression analysis. Linear regression analysis was performed to determine the correlation coefficient. Statistical significance was determined using one way ANOVA with $P < 0.05$, 56 was considered to be significant at 95% confidence level. Statistical analysis was carried out using SPSS version 20.

Results

Percentage yield of Cassia fistula extracts

The yield of the extracts revealed that the non-defatted seeds extract had the highest yield of 2.6% while defatted seeds extract had the lowest value (1.91%). The percentage yield of non-defatted leaves extract and defatted leaves extracts are 2.07 and 2.02% respectively.

Phytochemical constituents of defatted and non-defatted leaves and seeds extract of Cassia fistula

The phytochemical analysis of the extracts was carried out and the results obtained show that alkaloids, flavonoids, quinones, glycosides, carbohydrates, cardiac glycosides, terpenoids, triterpenoids and steroids are present in all the extracts. Tannins, anthocyanins and betacyanins are present in the leaf's extracts but absent in the seeds extracts while saponins, coumarins and acids were present in the seeds extract but absent in the leaves extract as presented in table 1.

Physicochemical properties of the extracts

Three physicochemical parameters of the extracts were carried out and the results revealed that the highest pH was recorded in the non-defatted seeds extract (6.94 ± 0.005) while the lowest pH was recorded in the defatted leaves extract (6.00 ± 0.01). The defatted leaves had the highest ash content (6.22 ± 0.28) while the non-defatted seeds had the lowest value (3.5 ± 0.5). The highest loss on drying at 105 ^{o}C was recorded in the non-defatted leaves (12.25 ± 0.25) while non-defatted and defatted seeds had the lowest (4.25 ± 0.25) (Table 2).

Results of proximate analysis

The results showed that defatted seeds had the highest protein and carbohydrate contents (35.6 ± 0.1 and 30.875 ± 0.01 respectively). The non-defatted leaves, defatted leaves, non-defatted seed, and defatted leaves had the highest moisture content (12.25 ± 0.25), crude fibre (3.20 ± 0.05), lipid content (31.375 ± 0.18) and ash content (6.22 ± 0.28), respectively (Table 3).

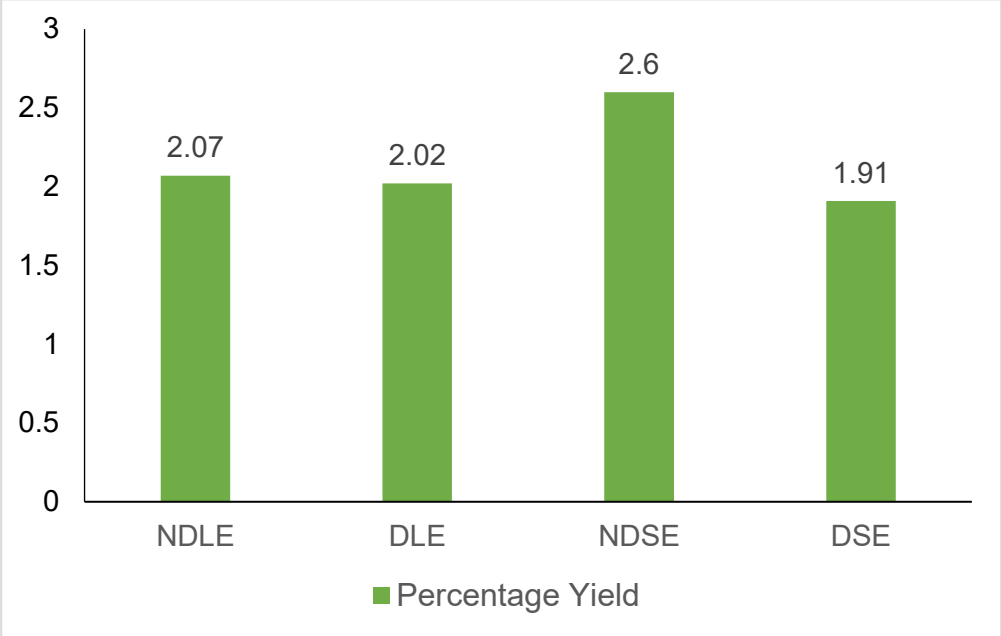

Figure 1: Percentage yield of ethanol extract of *Cassia fistula.* NDLE - non-defatted leaves extract, DLE - defatted leaves extract, DSE - defatted seeds extract, NDSE - non-defatted seeds extract

Table 1: Phytochemical composition of defatted and non-defatted leaves and seed samples extracts of *Cassia fistula*

Parameters	Non-defatted leaves	Defatted leaves	Non-defatted seeds	Defatted seeds
Alkaloids	+	+	+	+
Tannins	+	+	-	-
Flavonoids	+	+	+	+
Quinones	+	+	+	+
Anthocyanin and Betacyanin	+	+	-	-
Glycosides	+	+	+	+
Carbohydrates	+	+	+	+
Saponins	-	-	+	+
Cardiac glycosides	+	+	+	+
Terpenoids	+	+	+	+
Triterpenoids	+	+	+	+
Coumarins	-	-	+	+
Acids	-	-	+	+
Steroids	+	+	+	+

+ = Detected - = Not detected

Table 2: Physicochemical analysis of defatted and non-defatted leaves and seed samples of *Cassia fistula* Linn

Parameters	Non-defatted leaves	Defatted leaves	Non-defatted seeds	Defatted seeds
pH	6.40 ± 0.005	6.00 ± 0.01	6.94 ± 0.005	6.89 ± 0.01
Ash content	5.25 ± 0.25	6.22 ± 0.28	3.5 ± 0.5	4.75 ± 0.25
Loss on drying at 105 ^{o}C	12.25 ± 0.25	10 ± 0.5	4.25 ± 0.25	4.25 ± 0.25

Table 3: Proximate analysis of defatted and non-defatted leaves and seed of *Cassia fistula*

Proximate composition	Non-defatted leaves	Defatted leaves	Non-defatted seeds	Defatted seeds
Crude protein	26.05 ± 0.05	28.25 ± 0.25	31.4 ± 0.1	35.6 ± 0.1
Moisture content	12.25 ± 0.25	10.00 ± 0.5	4.25 ± 0.25	4.25 ± 0.25
Lipid content	27.825 ± 0.18	22.4 ± 0.15	31.375 ± 0.18	22.00 ± 0.5
Crude fibre	2.80 ± 0.08	3.20 ± 0.05	2.65 ± 0.03	2.525 ± 0.05
Ash content	5.25 ± 0.25	6.22 ± 0.28	3.5 ± 0.5	4.75 ± 0.25
Carbohydrate	25.825 ± 0.25	29.93 ± 0.02	26.825 ± 0.25	30.875 ± 0.01

*P < 0.005 shows significant difference

Antioxidant activity of Cassia fistula extracts

Ferric reducing power (FRAP) activity

Both the defatted and non-defatted leaves and seeds extracts exhibited some electron donation ability. The reducing power of the extracts increased with increase in concentration as (Figure 2). The order of decreasing power was DLE > NDLE > DSE > NDSE. Ascorbic acid had greater reducing power than all the extracts.

DPPH radical scavenging activity

DPPH radical scavenging activity of the extracts was found to be in the order: DSE > DLE > NDSE > NDLE. The DPPH scavenging activity increased with an increase in concentration of the ethanol extracts of *Cassia fistula* (Figure 3)

Hydrogen peroxide (H_2O_2) scavenging activity

Hydrogen peroxide (H_2O_2) scavenging study revealed that the extracts had dose-dependent capacity to scavenge OH^- free radicals (Figure 4). Hydrogen peroxide radical scavenging activity *of the* extracts was found to be in the order: DSE > DLE > NDSE > NDLE. All extracts showed OH^- free radicals' activity that was less than that of ascorbic acid which was used as the reference component

Comparative IC_{50} study of the defatted and non-defatted ethanol leaves and seeds extracts of Cassia fistula

The IC_{50} results obtained for the extracts (Table 4) showed that ascorbic acid had the lowest IC_{50} values of 0.13, 0.15, and 0.096 mg/mL for DPPH, H_2O_2, and FRAP respectively. The lowest IC_{50} value obtained for the plant extracts was 0.14 mg/mL for the defatted seeds extract (DSE) while the extract with the highest IC_{50} value was the non-defatted leaves extract with a value of 1.53 mg/mL.

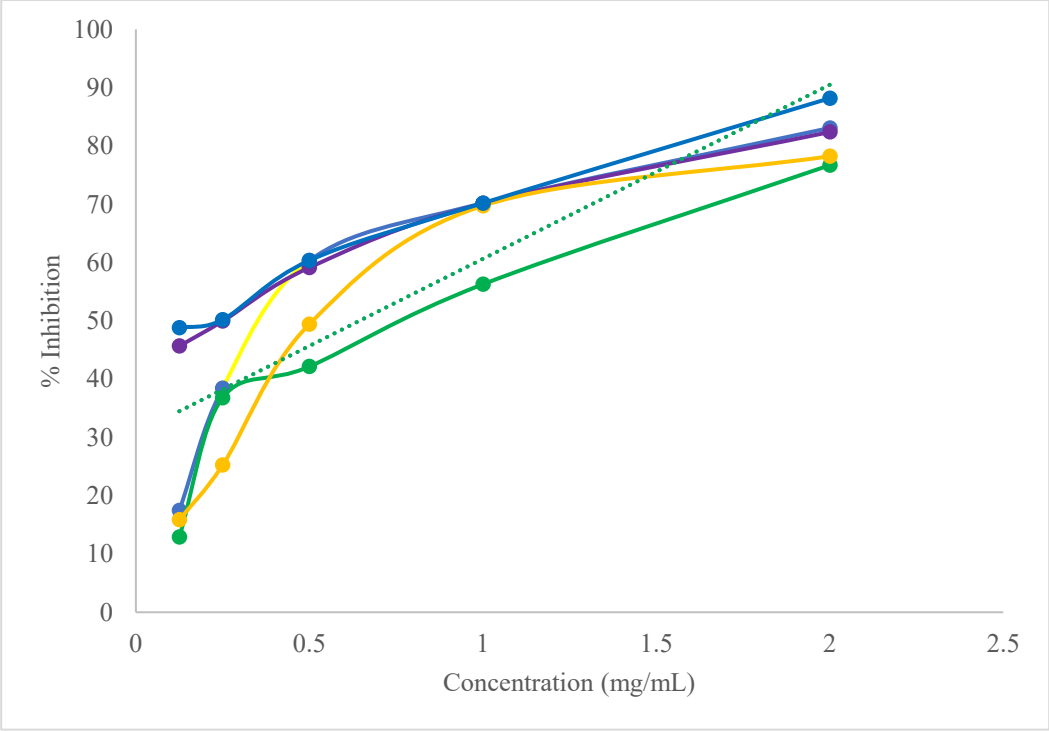

Figure 2: The ferric reducing power of ethanol non-defatted leaves extract (NDLE - Yellow), defatted leaves extract (DLP - Purple), non-defatted seeds extract (NDSE - Green), defatted seeds extract (DSE - Orange), and ascorbic acid (ABS - Blue). Values are expressed as mean ± standard deviation.

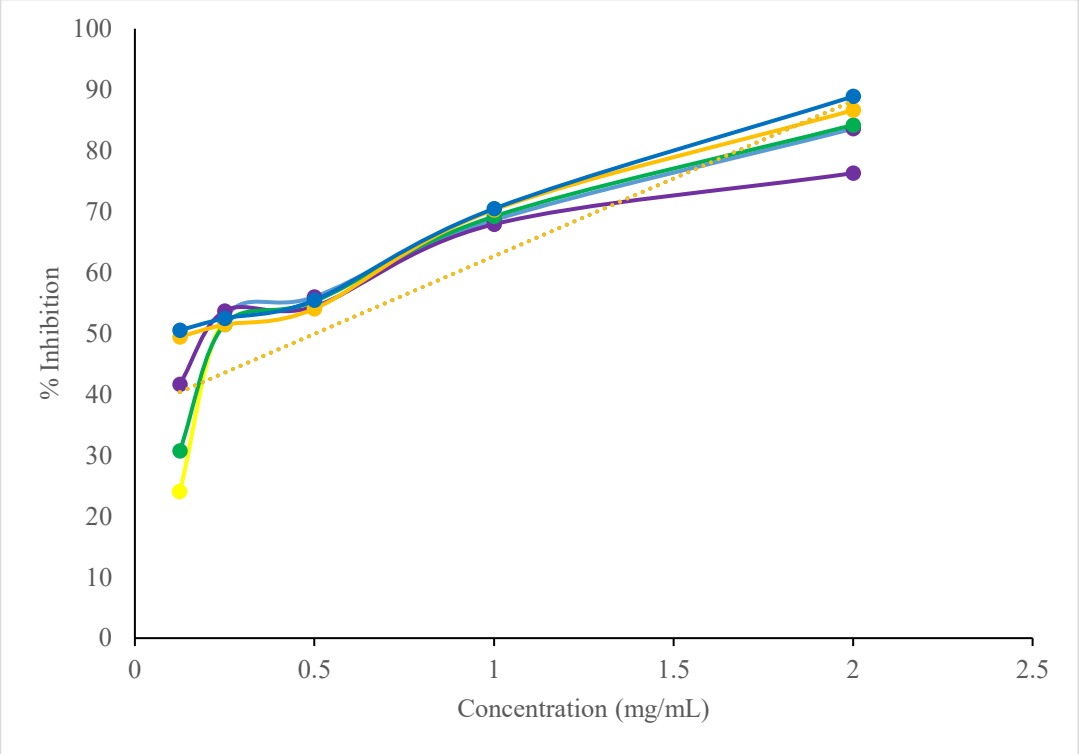

Figure 3: The DPPH scavenging activity of ethanol non-defatted leaves extract (NDLE - Yellow), defatted leaves extract (DLP - Purple), non-defatted seeds extract (NDSE - Green), defatted seeds extract (DSE - Orange), and ascorbic acid (ABS - Blue). Values are expressed as mean ± standard deviation (n = 3).

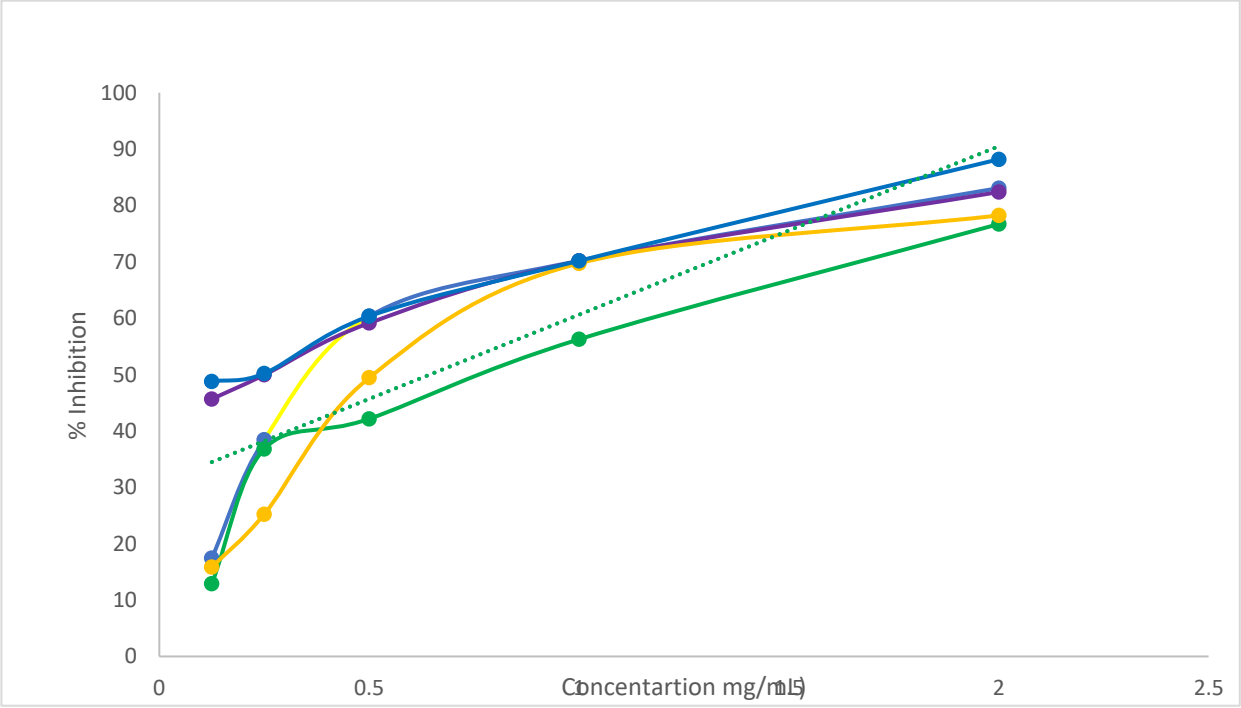

Figure 4: The hydrogen peroxide scavenging activity of ethanol non-defatted leaves extract (NDLE - Yellow), defatted leaves extract (DEL - Purple), non-defatted seeds extract (NDSE - Green), defatted seeds extract (DSE - Orange), and ascorbic acid (ABS - Blue). Values are expressed as mean ± standard deviation (n = 3).

Table 4: IC_{50} value of *Cassia fistula* ethanol defatted and non – defatted leaves and seed extracts

S/No	Extracts	DPPH	H_2O_2 IC_{50} (mg/mL)	FRAP
1	NDLE	0.5	1.53	0.64
2	DLE	0.24	1.17	0.95
3	NDSE	0.44	1.17	0.85
4	DSE	0.17	0.74	0.14
5	ABS	0.13	0.15	0.096

Non-defatted leaves extract (NDPE), defatted leaves extract (DEP), non-defatted seeds extract (NDSS), defatted seeds extract (DSS), and ascorbic acid (ABS).

Discussion

According to this study, *Cassia fistula* may help identify new bioactive natural compounds that will support the creation of medicinal products (Jangid *et al*., 2025).
The yield of the extracts as shown revealed that the non-defatted seeds extracts had the highest yield while defatted seeds extract gave the lowest value. A similar study Panda *et al*. (2011), found that the ethanolic extract of *Cassia fistula* leaves yielded higher amounts. Other study Aabideen *et al.* (2021) using hydroethanol extraction also recorded higher yield compared to pure ethanol. The low yield recorded in this study may be attributed to the solvent used in extraction.

Flavonoids, phenols, saponins, alkaloids, and other phytochemicals were reported abundant in *Cassia fistula* leaves (Sherwani *et al.,* 2025; Nirmala *et al*., 2024). The phytochemical results obtained in this study is similar to that obtained in a study by Kulkarni *et al*. (2015) on aqueous methanol and petroleum ether extracts of *Cassia fistula (*Bargah, 2017). A similar study by Gaurav *et al*. (2017) on leaves and seeds extracts of *Cassia fistula* using 70% ethanol revealed that carbohydrates, glycosides, steroids, and flavonoids are present in the two extracts while phenolics are present in the leaves extract but absent in seeds extract. The difference in results obtained may be due to difference in solvent used for extraction, defatting of samples and extraction methods used.

Earlier study on the physicochemical properties of *Cassia fistula* non-processed leaves samples revealed that the pH, ash content and loss on drying were 5.6, 4.35 and 13.58 for respectively (Agrawal *et al.*, 2014). The results obtained for both defatted and non-defatted leaves in this study are higher than what was reported by Agrawal *et al*., 2014 for pH (5.6) and ash content (4.35) while the loss on drying (13.58) was lower than what was reported by Agrawal *et al*. (2014). High ash primarily indicates an abundance of elements like calcium, potassium, magnesium, and iron, which are crucial for nutritional labeling in foods and feeds (principle of animal nutrition).
Defatting decreased the lipid and moisture content of the leaves but increased other proximate composition parameters. This result is in accordance with the study of Sneh (2015). A similar trend was observed in the seed except for the crude fibre content which was less in the defatted seeds compared to the non-defatted seeds. The seeds s contained higher contents of crude protein, lipid, and carbohydrate than their corresponding leaves in line with previous (Kdam, 2022).

Defatted and non-defatted seeds and leaves both exhibited some electron donation in ethanol extract. The reducing power of the extracts increased with increase in concentration This study agrees with results obtained by Irshad *et al.* (2012) which revealed that *Cassia fistula* fruit pulp and seed extracts possessed good ferric reducing power, which supports the antioxidant activity of the plant. Patel and Vyas, (2016) stated that methanol and ethanol extracts of *Cassia fistula* leaves had a good antioxidant activities e as compared with other parts of the *Cassia fistula* plant (i.e., stem, bark, flowers, fruits, pulp). The results in this study and the report by Irshad *et al*. (2012) both indicate that ethanol extracts of *Cassia fistula* have good antioxidant property (Goldson *et al.,* 2016; Saha *et al*., 2017). IC_{50} values show that ascorbic acid which was used as reference standard had the lowest IC_{50} values of 0.13, 0.15, and 0.096 mg/mL for DPPH, H_2O_2, and FRAP respectively. The lowest IC_{50} value among the plant extracts was obtained from defatted seeds extract (DSE) while the extract with the highest IC_{50} value is the non-defatted leaves extract (NDLE) with value of 1.53 mg/mL. The lowest and highest IC_{50} values were recorded with DSE with a value of 0.17. Under FRAP method, the highest IC_{50} value was recorded for DLE with a value of 0.95.

This study revealed that ethanol leaf extract of the plant displayed less antioxidant activity in DPPH (IC_{50} = 61.3 ± 1.9 µg/mL) compared to the hydroethanol (Aabideen *et al.*, 2021).

Conclusion

These study research have revealed that the leaves and seeds of *Cassia fistula* contain phytochemicals with good antioxidant activity The results indicate that defatting increases d their phytochemical, physicochemical, proximate profiles and antioxidant activities.

Prospects for future research

Comparative study of the toxicological effects and antimicrobial activities of the plant extracts should be carried out to ascertain how defatting can affect both activities. Antimicrobial activity of the leaves and seeds extracts should be carried out to ascertain pharmacological effects on humans.

Declaration of interests

All authors declare no conflict of interest associated with this article.

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