ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14937061

Unlocking the Therapeutic Potential of *Cassia fistula* Leaves: A Phytoconstituents-Based Investigation

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Publication Date: 2025/02/28

Abstract: *Cassia fistula*L is a shady tree found in different part of the World. Different part of the plant has been used to treat different diseases in traditional medicine. Certain chemical elements and compounds in plant leaves are mostly required for proper metabolic function, growth in Human and numerous health related benefits. In this study, the vitamin, mineral, proximate, phenolics contents of *Cassia fistula* leaves were assessed. The presence of proximate, minerals, vitamins and phenols were determined using standard methods, Atomic Absorption Spectroscopy (AAS) and High-Pressure Liquid Chromatography (HPLC) respectively. According to the standard method used in proximate analysis, the *Cassia fistula* leaves extracts was found to contain the following; carbohydrate (45 %), protein (28 %), moisture (13 %), lipids (6 %), fibre (4 %), and ash (4 %). The mineral element determined in the sample revealed Magnesium, Calcium, and Potassium having the highest concentrations of 177, 171, and 149 ppm, respectively. Iron, sodium, and zinc have moderate concentrations of 9.1, 8.2, and 7.1 ppm, respectively, and manganese, selenium, and copper have the lowest concentrations of 0.76, 0.73, and 0.011 ppm, respectively. HPLC results showed different vitamins in the leaves extracts such as vitamin C 19.5946 mg/100 g, while vitamin B_3 and vitamin E levels were moderate at 3.43 and 2.3 mg/100 g, respectively. Also, the HPLC phenolic analysis of *Cassia fistula* leaves showed prominent level of Kaempferol, quercetin, quercitrin, isoquercitrin, rutin etc. and trace amount of salicylic acid, p-hydroxybenzoic acid, gentisic acid, naringin acid etc. The outcome of the study indicated that the composition obtained from *Cassia fistula* leaves extract established the significance of these leaves in human health and therapy. This work reveals important bioactive compounds inherent in *Cassia fistula* leaves which serves as insightful information to push for further studies of *Cassia fistula* leaves in disease therapy.

***Keywords**: Phytoconstituents, Proximate, Minerals, Cassia Fistula, High Pressure Liquid Chromatography.*

How to Cite: Oyewole T. A.; Orji E.E., Ishaku E. (2025). Unlocking the Therapeutic Potential of *Cassia fistula* Leaves: A Phytoconstituents-Based Investigation. *International Journal of Innovative Science and Research Technology*, 10(2), 825-830. https://doi.org/10.5281/zenodo.14937061.

I. INTRODUCTION

Since ancient times, ethno-botanical statements about the effectiveness of some plants have been supported, and researchers are now creating medications, dietary supplements, and functional meals, among other things, from plants to manage or treat diseases (Duraipandiyan and Ignacimuthu, 2007; Bhavna *et al*., 2007). *Cassia fistula* is an ornamental Fabacea Family tree plant known as Golden shower and is commonly distributed in Africa, Asia and other part of the World (Bhalodia *et al*., 2012; Oyewole, 2022). Different parts of this plant such as the root, stem, leaves and many others have been identified in traditional medicine to treat different diseases (Ploeger and Shugar, 2017). According to traditional medicine claims made in several parts of the world, the leaves of *Cassia fistula* have been used traditionally in the treatment of different diseases and many studies have revealed these claims (Oyewole, 2022). It has been demonstrated that these tree plant parts contain some bioactive compounds (Oyewole, 2022). It is of note that Phytochemicals are compounds synthesized by plants, which provide health related benefits to human (Hasler and Blumberg, 1999) and these micronutrients and some compounds found in some of these therapeutically effective medicinal plants, have shown to trigger certain physiological activities in human that fosters health benefits (Akinmoladun*et al*., 2007).

One of these groups of compounds is called phenols. Phenols are one of such numerous secondary metabolites that exist in nature among plants as phenolic acids, flavonoids and tannins (Rakesh *et al*., 2019), which have been proven to confer therapeutic impacts in some plants efficacy to manage variety of diseases. In addition, flavonoids have been proven to possess diverse health beneficial activities such as anti-inflammatory, antimicrobial activity, antioxidant, anti-allergic activity and many other related health benefits (Tapas *et al*., 2008). Also, evidences have shown its anti-oxidant

ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14937061

actions against oxidative activities on biological macromolecules. Even though, Tannins are synthesized in plants due to exposure to stress, infection and excessive ultraviolet radiation it helps to manage damage on them (Roupe *et al.,* 2013).

Vitamins have been documented to foster health related benefits among Human, and some of these medicinal leaves have shown high level of these related benefits because of the vitamins in some of them. Also, majority of vitamins are obtained through meals to prevent malnutrition-related disorders and some fat and water-soluble vitamins are provided as supplements in doses base on daily recommended values to correct malnutrition diseases (Garcı´a, 2009). Since some of these therapeutic plants have shown medicinal properties due to their rich vitamin level, it is then paramount we unravel the vitamin profile of *Cassia fistula* leaves which may help to elucidate the potential of this plant as an alternate medicinal source of vitamins to Human and nutritional source to some animals or important component of their feeds. Additionally, as minerals have reportedly been linked to the prevention of several diseases in humans, the majority of medicinal plants include free and/or bound mineral elements that alter biochemical reactions when bio-available in biological systems (Hannah and Krishnakumari, 2015). Several minerals from various plant foods and parts are necessary for proper health functioning. Since mineral deficiency has been a significant health challenge in different parts of the world, increasing dietary mineral intake is a great therapy in overcoming these challenges in humans and even in other animals (Oyewole *et al*., 2022). Since certain reports have shown that the level of crude fibre, moisture and ash could signify good digestibility and palatability of some plant parts this, coupled with the vitamin and mineral component of this plant will help give enough information of *Cassia fistula* and its significance in disease prevention and management (Hannah and Krishnakumari, 2015). This current study identified the proximate analysis, vitamin, mineral and phenolic profiles of *Cassia fistula* leaves. Additionally, it's critical to determine the vitamin, mineral and proximate analyses of these plant leaves to understand their therapeutic/nutritional potential in human or animals.

Fig 1: The *Cassia Fistula* Leaves in its Natural Habitat in Ado-Ekiti Metropolis

II. MATERIALS AND METHODS

A. Sample Collection and Authentication

*Cassiafistula*leaves was collected from The Federal Polytechnic, Ado-Ekiti. The leaves were washed with water and air dried for seven days. It was grinded to powder and stored in a polythene bag. Botanical identification and validation were done at Herbarium unit of Ekiti State University.

B. Determination of Proximate Analysis

The powdered samples were subjected to proximate analysis for the determination of the nutritional composition; moisture, ash, crude protein, crude fat (ether extract), crude fibre and nitrogen-free extract (carbohydrate) as described by (AOAC, 2005).

C. Determination of Mineral Contents

The mineral contents of the samples were determined by atomic absorption spectrometry, flame photometry and spectrophotometry according to the methods of AOAC (2003).

D. Analysis of Water and Fat-Soluble Vitamins Simultaneously

In order to allow the samples to acclimate to the laboratory environment, they were removed from the compartment that was kept at a temperature lower than 4^{o}C and placed on a bench.

E. Extraction of Water-Soluble Vitamins:

The sample was grinded with the aid of the laboratory mortar and pestle. The accurately weighed 0.100g of ground powder was put into 100 ml volumentric flasks and 80 ml of water was added. After 15 minutes of ultrasonic extraction, the water added to the volumentric flask mark.

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F. Extraction of Fat-Soluble Vitamins:

The accurately weighed 0.125g of grounded sample was added into 10 ml volumentric flasks and 8 ml of CH_30H-CH_2CH_2 (1: 1, v/v) was added to the flask. After 15 minutes of ultrasonic extraction, CH_30H-CH_2Cl_2 (1:1, v/v) was added to the volumentric flask mark. The prepared sample solution was stored in the dark; and diluted if necessary. Prior to injection, the solutions were filtered through a 0.2 µm filter (Miliex-GN).

G. Optimized Chromatography Conditions for Vitamin analysis

Under the following improved chromatographic settings with valve switching, double injection, envelope-injection, and wavelength switching, water- and fat-soluble vitamins were separated concurrently. The HPLC Column consist of Acclaim PA, 3µm, 120A, 3.0 x 150mm for fat soluble and Acclaim C18, 3µm, 120A, 3.0 x 150mm for water soluble at a Column Temperature of 25ºC. While the mobile Phase for the Water-soluble Vitamin Determination is made up of: a 25 mm phosphate buffer (3.4g KH2PO4 dissolved in 100 ml water; then pH should be adjusted to 3.6 with H_3PO_4), CH3CN Mobile Phase A (7:3, v/v) using an injection volume of 10 µl with all determinations on dry weight basis.

The mobile phase for the determination of the Fat-soluble Vitamin consists of: CH3OH-CH3CN (8:2, v/v), Methyl tert-butyl ether (MTBE) using an injection volume of 10 µl with all determinations on dry weight basis. The samples were out of the less than 4ºC compartment in the Laboratory and placed on the bench acclimatizing to the laboratory conditions.

H. Phenol HPLC System

The aqueous extract of *Cassia fistula* leaves was performed using Agilent 1200 series HPLC system consisting of an Agilent 1260 detector (DAD), delivery system, an auto-sampler. The chromatographic analysis was carried out on Chromspher 5, C18 column with hamilton microliter syringe with dimension 5µm × 3mm × 250mm and Helium gas at a constant flow rate of 0.7 ml/min and pressure of 180×10^5 Pa was used as a carrier gas. Isocratic elution mode was prepared with 2% (v/v) Acetic acid in water – methanol mixture 82:18 v/v with UV detector set at 320 nm. The flow rate and injection volume were set at 100 µL and 0.7 ml/min respectively. *Cassia fistula* leaves extracts were centrifuged at 10,000 rpm for 10 min, this was repeated. The standards of different concentrations were prepared for the injection into the HPLC system for calibration and correlation coefficient establishment. The supernatants of the *Cassia fistula* leave extracts were collected and filtrated through a 0.22 µm membrane filter, and 20 µL aliquots each from the filtrate were then injected into the HPLC system following the same procedure as standard mixtures.

I. Statistical Analysis

All experiments were performed in triplicate and the results or values are expressed as mean; standard deviation denoted by error bars.

III. RESULTS

The qualitative analysis of *Cassia fistula* leaves aqueous extracts was carried out base on standard protocols nine phytochemicals show positive result as shown in (Table 1).

Table 1: Phytochemical Analysis of *Cassia Fistula* Leaves

Secondary metabolites	Test	Inference
Alkaloid	Mayer's test	+ve
Tannin	Ferric chloride test	+ve
Glycoside	Keller's test	+ve
Saponin	Frothing test	+ve
Flavonoid	Ferric chloride test	+++ve
Steroid	Salkowski test	+ve
Terpenoid	Salkowski test	+ve
Phenol	Ferric chloride test	+++ve
Anthraquinone	Carbon tetrachloride test	+ve

Legend: + = Absent, + = Trace, ++ = Moderate, +++ = Abundant

Table 2: Quantitative composition of *Cassia fistula* leaves

Secondary metabolites	Values (%)
Alkaloid	1.0361
Terpenoid	0.2340
Tannin	1.500
Flavonoid	8.7400
Glycoside	0.200
Phenol	3.1741
Trypsin inhibitor	0.4131

The HPLC phenolic analysis of *Cassia fistula* leaves revealed prominent level of Kaempferol, quercetin, quercitrin, isoquercitrin, rutin, epicatechin, catechin among others (Table 5.0).

ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14937061

Table 3: The Phenolic Contents of *Cassia fistula* Leaves using High Pressure

Phenolic Compounds	Amount in (mg/100g)
Salicylic Acid	0.4765
Gentisic Acid	0.09150
Protocatechuic Acid	6.44038
O-coumaric Acid	0.3421
Vanillic Acid	0.06407
p-hydroxybenzoic Acid	1.4297
Cinnamic Acid	0.1644
Catechin	81.4703
Gallic Acid	25.9422
Caffeic Acid	172.5637
Ferulic Acid	0.2114
Syringic Acid	0.0439
Naringin	0.9050
Ellagic Acid	51.0941
Kaempferol	186.3807
Piperic Acid	0.0202
Sinapinic Acid	0.0625
Epicatechin	238.0434
Quercetin	144.6226
Chlorogenic Acid	29.8217
Quercitrin	130.0922
Isoquercitrin	150.0369
Hesperidin	0.06503
Rutin	118.2180

The proximate analysis of *Cassia fistula* leaves showed that the leaves (Figure 1) have 45% carbohydrate higher than 28% proteins, 13% moisture contents, 6% lipids and 4% ash.

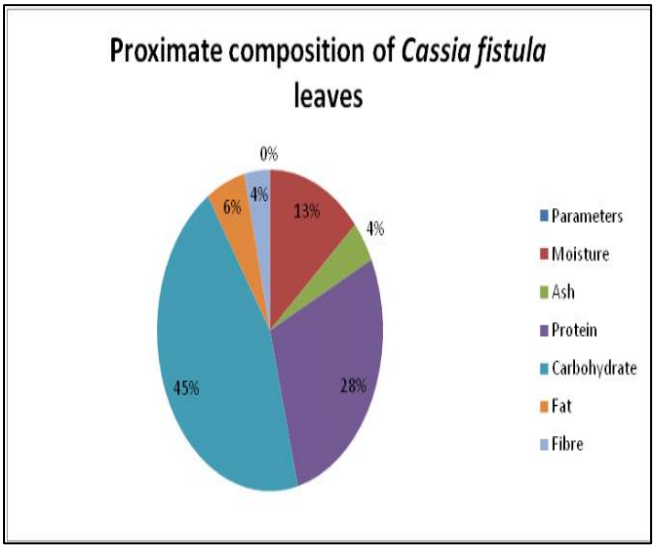

Fig 1: Proximate Analysis of *Cassia fistula* Leaves Extracts

A. Mineral Analysis of Cassia Fistula Leaves

Table 4 indicated the mineral components found in the leaf aqueous extracts of *Cassia fistula*, showing the order of the composition: potassium, magnesium, phosphorus, and calcium have higher values than other minerals like iron, sodium, zinc, manganese, among others.

Table 4: The Mineral Composition of *Cassia fistula* Leaves

SAMPLE (ppm)	LEAVES
Sodium (Na)	8.240
Calcium (ca)	171.130
Potassium (K)	418.170
Iron (Fe)	9.140
Manganese (Mn)	0.755
Zinc (Zn)	7.131
Phosphorus (P)	149.160
Magnesium (Mg)	177.080
Selenium (Se)	0.731
Copper (Cu)	0.011

B. Vitamin Profile of Cassia Fistula Leaves

Furthermore, the vitamin profile was analyzed using HPLC and it showed that vitamin c level was the highest (Table 5) at 19.59 mg/100g with lower amount of vitamin B3 at 3.434 mg/100g, then vitamin E at 2.302 mg/100 g and vitamin B2 at 0.8718. Additionally, vitamin D and vitamin B_{12}, with corresponding concentrations of 0.0345 and 0.0104 mg/100g were the lowest values.

Table 5: The Vitamin Quantities of *Cassia fistula* Leaves

Vitamins	Amount (mg/100g)
Fat Soluble Vitamins	
A	0.1552
D	0.0345
E	2.3022
K	0.7963
Water Soluble Vitamins	
B_1	0.5241
B_2	0.8718
B_3	3.4344
B_5	0.5000
B_6	0.6031
B_9	0.4770
B_{12}	0.0104
C	19.5946

IV. DISCUSSION

From result in (Table 3), Epicatechin level was 238.0434mg/100g and quercetin level was 144.6226mg/100g with Epicatechin the highest in terms of polyphenolic level in *Cassia fistula* leaves. Epicatechin from green tea and quercetin from the outer skin of onions with some other phenolic compounds have shown some therapeutic actions (Shin *et al*., 2013; Horton *et al*., 2013). And some evidences have shown the anti-inflammatory and antioxidant activity of epicatechin and quercetin against oxidative stress among some mice samples in radiation experiment revealing their radioprotective effect (Nicolas *et al*., 2017).

Caffeic acid level was 172.5637mg/100g while Chlorogenic acid level was 29.8217 mg/100g in *Cassia fistula* leaves. Reports have shown that some mice fed with polyphenolic compound such as chlorogenic acid and caffeic acid significantly have reduced body weight, visceral fat mass, plasma leptin and triglycerides and significant increased HDL-cholesterol/total cholesterol ratio compared

ISSN No:-2456-2165 https://doi.org/10.5281/zenodo.14937061

to high fat fed diet mice group (*Cho et al*.,2010). All these phenolic contents established the possible therapeutic potentials of *Cassia fistula* leaves.

In addition, the amount of rutin level in *Cassia fistula* leaves was 118.2180 mg/100g while that of gallic acid level was 25.9422 mg/100g. Some animal experiments have shown the antioxidant activity of gallic acid in reducing oxidative stress biomarkers (Punithavathi*et al*., 2011), therapeutic activity in reducing hyperglycemia and improving neurological function on nerve cells (Latha and Daisy, 2011; Moradi *et al*., 2020) etc. In addition, the activity of rutin in ameliorating $HgCl_2$-induced inflammation and oxidative damages in the tissues of experimental animals have been reported (Cuneyt *et al*., 2019).The level of Ellagic acid in *Cassia fistula* leaves was 51.0941 mg/100g. And some reports have shown the anti-tumorigenic and anticancer activity of Ellagic acid against different forms of cancer in an *in-vitro* and *in-vivo* studies of some normal cell lines (Claudia *et al*., 2018). All these unveil the therapeutic values of *Cassia fistula* leaves in disease management and many more.

The results of the proximate analysis indicate that the leaves of *Cassia fistula* are abundant in protein, fiber, carbohydrates, and other nutrients (Figure 1.0), indicating that they would likewise make ideal nutritional supplements, as has been proven in several mushrooms (Moore and Chi, 2005).

Magnesium, potassium, calcium, and phosphorus, some of which have been considered essential for maintaining equilibrium in organisms, have been found in significant concentrations in *Artemisia annua* and *Camellia sinensis* leaves. These minerals are also present in significant amounts in *Cassia fistula* leaves, providing a glimpse into the potential of these leaves. Additionally, various trace mineral components, including manganese, iron, copper, and zinc, have been claimed to be crucial in the fight against parasite infections (Alassane, 2013).

Also, almost all herbal plants offer health advantages that are closely tied to the nutrients they contain. Poor growth, development, and abnormalities have been linked to calcium deficiency in humans and animals (Hortz and Brown, 2004). According to reports, zinc has a positive effect on removing ulcers and promoting the healing of wounds. Its deficiency could be characterized by recurrent infections caused by a lack of or low immunity, poor growth, and even coronary diseases (Alassane, 2013). For the breakdown of lipids and cholesterol, an antioxidant cofactor like manganese is thought to be necessary (Chaturvedi *et al*., 2004).

Since vitamin C can donate an atom of hydrogen and create a relatively stable ascorbyl free radical, it is an effective antioxidant. Ascorbate has been demonstrated to be an efficient ROS scavenger against the superoxide radical anion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen (Rose, 1989; Weber *et al*., 1996). Vitamin E cannot be synthesized by poultry and its content decreases in stressful environments, according to research. Reports have shown that the alpha-tocopherol concentration of organs and products declines when dietary PUFA levels rise, which may be due to vitamin E's ability to intercalate with phospholipids and protect PUFAs from oxidation. And studies have demonstrated that when enough vitamin E was given, the unfavorable oxidative effects of n-3 PUFAs on rat erythrocytes could be prevented (Ando *et al*., 1998).These results have proven the significance of *Cassia fistula* leaves in traditional medicine and also as dietary products among some animal feeds considering its vitamins, minerals and proximate results.

V. CONCLUSION

Since ancient times, different plants have been prepared as herbs, medicine to treat several diseases. The phenolic compounds in Cassia fistula revealed some of the bioactive compounds playing major roles in management in these diverse diseases. This work gives information to the potential of *Cassia fistula* leaves for further novel studies in disease management and drug design.

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