



Research Article

Ethno-medicinal Species of *Cassia* (*C. fistula*, *C. siamea*, *C. tora*): Documentation and Metabolites Estimation

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Abstract

Different species of *Cassia* found in Jharkhand are consumed as daily vegetables have high nutritional value. Most of them are underutilized and display varied ethnomedicinal values. The work was carried out with an aim to document three ethno medicinal properties of three different species of *Cassia*, *Cassia fistula*, *Cassia siamea* and *Cassia tora*. The documentation was done among 100 tribal people residing in the area near Morabadi, Ranchi. The knowledge revealed that the three plants were used in almost every tribal houses as a dietary supplement and various medicinal uses. According to the Munda Vaidya's the plant *Cassia* is of pitta nature and its different part have different uses. The root is used against tuberculosis glands, diabetes, as a tonic, the root and bark paste mixed in equal amount is used against snake bite. traditionally it is used as laxative, for the treatment of leprosy and various skin disorders. the present study aimed to screen and quantify primary and secondary metabolite by quantitative and qualitative method. The pigments (chlorophyll and carotenoid) were characterised by UV visible spectroscopy. Concentration of chlorophyll-a, chlorophyll-b and carotenoid was calculated by Arnon method (80% acetone extract). The spectrophotometric study of the different pigment of the three species of *Cassia*, *Cassia fistula* (chl-a 254.516, chl-b 305.741, total chl 533.7 carotenoid 10.6), *Cassia siamea* (chl-a 257.88, chl-b 393.16, total chl 630.2 carotenoid 9.395), *Cassia tora* (chl-a 266.36, chl-b 271.96, total chl 538.32 carotenoid 16.3). The result revealed that chl-a was highest in *Cassia tora* followed by *Cassia siamea* and *Cassia fistula*. Chl-b pigment followed *C siamea* > *C fistula* > *C tora* pattern, total chl *C siamea* > *C tora* > *C fistula* and the carotenoid amount *C tora* > *C fistula* > *C siamea*. In screening of secondary metabolites tannin, saponins alkaloids, terpenoids, resin Phyto-sterols were carried out on the aqueous extract showed +ve test for all except phyto-sterols and on the powdered specimen gave -ve result in aqueous, ethanol as well as acetone extract.

Keywords: *Cassia*; chl-a; chl-b; carotenoid; ethnomedicinal plants; underutilized; primary metabolite ; acetone extract.

Introduction

Number of medicinal systems is directly or indirectly dependent on plants nutraceuticals, food supplements, folk medicines, traditional systems of medicine, modern medicines, and pharmaceutical intermediates (Deori et al., 2007). Medicinal plants are used for the treatment of many

diseases. In nature number of medicinal plants are naturally grown and has relied on the vast variety of natural chemistries' found in plants for their biochemical and therapeutic properties (Seyyednejad and Motamedi, 2010). Medicinal plants are not having the pharmaceutical

approach, but there is a wider and diverse tendency to utilize herbal plant product to supplement the food, diet, and its main intense is to improving the quality of human as well as animal life and preventing the number of diseases (Ghosh and Sahu, 1986).

Jharkhand is land of nature transversed by plateaus and steeps with undulating valleys and is inhabited by about 23 ethnic groups of indigenous people (Keshari, 1983) with strange faith and practices which is associated with the local flora of the region. The rich and the vivid flora of the state acts as a substitute of nutrient supplement to the tribal. (Ghosh, 1971).

The simple home remedies practised especially by the Adivasi and the Adi Janjatis or the tribal communities all over India have been the Ayurveda system later developed by the Aryans. The simple system of and are used throughout the history of human beings either in the form of plant extracts or pure compounds against various diseases (Dabriyal and Narayana, 1988). Knowledge about these plants are passed from one generation to the other through folk tale (Ghosh (1971). The medicinal herbal plants popularly known as *saag* or *pothorbs* are beneficial for therapeutic uses as well as for curing of human diseases because of the presence of phytochemical constituents (Kumar and Kumari, 2010). *Saag* is the distorted form of Sanskrit word Saak meaning potherb. In Indian cuisine saag generally occupies a special place for both its taste as well as medicinal properties. Among the tribal of Jharkhand saags are as leafy laurels for the state is rich in varieties and different species of saag are essential part of the festivals and rituals (Singh et al., 2001). Some potherbs or saags are cultivated but most of them grow wild and are underutilized. Phytochemicals are naturally occurring in the medicinal plants, leaves, seeds and roots that have defence mechanism and safeguard from numerous diseases. (Oudhia, 2001). Phytochemicals are primary and secondary compounds (Porra et al., 1989). Sags or the pot herbs are rich in a wide variety of metabolites, which have anti-fungal, anti-bacterial. The primary metabolite like chlorophyll, amino acids, nucleotides (Markwell, 2002), simple carbohydrates or membrane lipids, play predict ale roles in photosynthesis, respiration, solute transport, translocation, nutrient assimilation and differentiation (Yen and Chung, 1999). Chlorophylls green colouring pigment is a primary metabolite that give the colour to the leaves. The spectral properties of chlorophylls are essential in harvesting light energy and in the transduction of absorbed light energy for photosynthesis. The variation of leaf colour, photosynthetic activity in most of the plants dependent on chlorophyll concentration. (Shibghatallah, 2013) Chlorophyll content determines the photosynthetic capacity of the plant per unit area of leaf, stress and nutritional deficiencies. (Vimala and Poonghuzhali, 2015) Secondary metabolites are synthesized by the plants as part of the defence system of

the plant (Indira et al., 2015). Higher plants contain Chl-a, Chl-b, accessory pigments and several additional forms of chlorophyll (Campeanu and Neata, 2012). The Chl-a and Chl-b are the best known among five main types of chlorophyll and are most commonly found in all autotrophic organisms except pigment containing bacteria. (Devlin & Witham 1997). Both Chl-a and Chl-b pigments are associated with light harvesting processes (Butnariu, 2016), which are solely responsible for photosynthesis in higher plants. Chlorophyll concentration in leaves is an indicator of plant health. The chlorophyll a:b ratio also indicates the developmental state of photosynthetic apparatus in plants. It has a determinative role in growth and development of higher plants (Wakefield and Bhattacharjee 2016). The chlorophyll content also indicates the photosynthetic capacity per uniarea of the leaf (Kozlowski et al. 1991) that determines the rate of photosynthesis in the plant (Dickman & Kozlowski 1968). Determination of chlorophyll content as an indirect method of estimating the productivity also provides a good understanding of the photosynthetic regime of plants (Costache et al.,2012). The chlorophyll content increases with leaf development and then decreases with the senescence phenomenon (Pereyra et al., 2014). The rate of photosynthesis is also higher in flowering and fruiting branches of sub-tropical fruit species in comparison to non-fruiting branches (Avery, 1977). However, the pigment is a factor that might also be responsible for the colour variation of leaf as well as the nutritional in different *Cassia* species. Chlorophyll concentration in leaves is an indicator of plant health (Iqbal et al., 2015).

Cassia tora is a small shrub which grows up in warm moist soil throughout the tropical parts of Asian and African countries, with a height of 30 to 90 cm. *Cassia tora* popularly known as chokd sag by the tribal is used as an important vegetable It grows as a wild shrub mostly in the tropical regions and is considered as a weed in most places. (Hemadri and Rao, 1984) his plant popularly known as 'Sickle pod' (Maity et al., 1998). The leaves and seeds are of use in cardiac disorders, dyspepsia, leprosy, ringworm, colic, constipation, flatulence, cough and bronchitis. Pods are used in dysentery as well as to treat eye diseases. Root is known to be bitter, tonic, stomachic and is antidote against snake bite (Hemadri and Rao, 1984). In Andhra Pradesh, the tribal people had been using the leaves of this plant grounded along with peppers and water into a paste, for the treatment of Jaundice. The leaves are alterative, aperient, antiperiodic and given to children suffering from intestinal disorders (Manojlovic et al., 2016). The leaves, roots, and even the whole *Cassia tora* is used as a natural pesticide in organic farms. The seeds yield yellow, blue and red coloured dyes used in dyeing and tanning therefore *Cassia tora* powder is most popularly used in the pet-food industry. It is mix with guar gum for use in mining and other industrial application .Alcoholic extract of *Cassia sp* were subjected to phytochemical screening to test for presence of

metabolites such as alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, This study would provide preliminary scientific evidence for different sp of *Cassia* as potent drug, because of *Cassia* leaves have more active principles like alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids. phyto-chemical study of is essential in order to evaluate active constituents responsible for its medicinal actions (Yen and Chung, (1999) studied the application of medicinal plants and to healing world as consumable. A special mention of *Cassia tora* is mentioned, significantly the presence of cinnamaldehyde from *Cassia tora* as effective in treatment of fungi as preventive measure (Wong et al., 1989). In tumor or cancer therapy chlorophyll or chlorophyll derivatives can be utilized as a photodynamic agent. (Brandis et al., 2006). It can be studied, modified and synthesized in chemistry and physics disciplines for different applications, that is electronic, photophysics, optoelectronic, electrochemistry (Patil et al., 2011). (*Cassia tora*. Linn) is well-known traditional medicinal plant, also called as ring worm plant which possesses kushtaghna, kandughna and dadrughna properties (Vimala and Poonghuzhali, 2015)

Cassia fistula is commonly known as golden showers, is a flowering plants sub family, of the legumes family, Fabaceae. (Haines, (1921-25) The species native to the Indian sub-continent is popularly known ornamental plant and it is also used as herbal medicines. It is the national trees national flowers of Thailand. In Ayurvedic medicine, the golden showers also known as, Aaragvadhā meaning, disease killer. The pulp is used in medicine is considered a purgative. Medication or any used without medical supervision is strongly advised against in Ayurveda texts (Francisco 2008). Through it has been used in herbalism millennia; little researched has been conducted in modern times. The seed is an in gradient mixed with other plants but some cases mass produced herbal laxative. (Khaleghi et al., 2012).

Cassia siamea the plants are of medicinal value and it contains compound named berakol (Haines, (1921-25). The leaves tender pods and seeds are edible, but the must be previously boiled and water discharge (Deori et al., 2007). Other use of include fodder plants in inter cropping systems wind breaks and shelter belts. As a hard wood it is used for ornaments, on instruments and decorative products (Kumar and Kumari, (2010)) It is used sometimes in Chinese furniture inter chargeable with wood from the *Cassia siamea* species. Chlorophyll fluorescence parameters and evaluation of chlorophyll content and relationships between chlorophyll a and b were determined in Dezfūl olive trees. José Francisco (2008) estimated the chlorophyll concentration in leaves of tropical wood species from Amazonian forest using portable chlorophyll meter. Kousar et al. (2007) extracted estimated and determined

chlorophyll and different pigment in black gram leaves using different methods, the main pigments are chlorophyll-a, -b and pheophytin (Eri, 2016). It was studied at different wave lengths. A simple and easy incubation method grinding and centrifugation procedures were described among few methods. The recovery of chlorophyll pigments by incubation method in which tender leaf tissue in 80% buffered acetone at 4°C give higher yield of pigments compared to other methods (Hiscox and Israelite, 1979).

documentation of ethnomedicinal properties, estimation of chlorophyll content and carotenoid in the mature leaf of three species of *Cassia* growing in Ranchi district, Jharkhand. And its Phyto-chemical study will certainly lead a path for identification of active constituents responsible for bringing out drug action and preliminary information on the quality of the drug.

Material and Methods

Collection of the Plant

Plant was collected from the Morhabadi Saritand from the terrestrial habitat. Plant leaves are collected from the Morabadi, Ranchi Jharkhand India behind the stadium. The plant was identified from the Ranchi University Department of Botany.

Documentation of Ethenomedicinal Properties

On the documentation basis an interview by various tribal community residing in the villages of Jharkhand near Ranchi District

Preparation of Plant Extract

Fresh extract for primary metabolites

Fresh plants were collected and washed properly to remove all the dust and soil particles with distilled water than rinsed it. After rinsing the plants were crushed in the mortar pestle and fresh extract was prepared.

Dry Extract for Secondary Metabolites

Fresh leaves were collected, washed thoroughly and properly with distilled water dried under shade, dust free condition for one to two week at room temperature. The leaves were then made powdered in a mechanical grinder. Powder was kept in an air tight jar for their further use.

Estimation of Chlorophyll (Arnon 1949)

1gram fresh leaves were crushed in the mortar pestal and then the extract was mixed with 25 ml 80% acetone solution (80% acetone and 20% distilled water) leave the solution for 24 hours in the refrigerator and then add a pinch of calcium carbonate in the solution and then centrifuge the solution at 5000 rpm for 5min repeat the solution till the pilletes become colorless. Transfer the extract into cuvet and take the O.D. in the spectrophotometer at 663 nm and 645 nm.

Calculation of Chlorophyll:

Chlorophyll A: $12.7 \times \text{O.D}(663) - 2.69 \times \text{O.D}(645) \times 40$

Chlorophyll B: $22.9 \times \text{O.D}(645) - 4.68 \times \text{O.D}(663) \times 40$

Total chlorophyll: $20.2 \times \text{O.D}(645) - 8.02 \times \text{O.D}(663) \times 40$

Estimation of carotenoids (Arnon)

1 gram fresh leaves were crushed in the mortar pestle and then the extract was mixed with 25 ml 80% acetone solution (80% acetone and 20% distilled water) leave the solution for 24 hours in the refrigerator and then add a pinch of calcium carbonate in the solution and then centrifuge the solution at 5000rpm for 5min repeat the solution till the pellets become colourless. Transfer the extract into cuvette and take the

O.D. in the spectrophotometer at 479 nm.

Calculation of Carotenoids: The relative efficient between prediction and measurement (μ) and standard error are as follows $\mu\text{g/ml}$

Carotenoids: $0.854 - 0.312 \times \text{O.D. (645)} + 0.039 \times \text{O.D}(663) - 0.005$

Screening of Secondary Metabolites

Screening of secondary metabolites was done by normal laboratory methods.

Test for alkaloids (Mayer's reagent)

3 ml filtrate was taken in a test tube and few drops of Mayer's reagent was added cream precipitate was observed, indicates the presence of alkaloids.

Test for Phyto steroid (Salkowaski test)

3ml filtrate was taken in a test tube 5 ml chloroform was added, chloroform layer was subjected to Salkowaski test. 1 ml of the Salkowaski solution was taken in a test tube and few drops of conc. H_2SO_4 was added solution color changed to cherry red indicates the presence of Phyto steroid.

Test for Resin

3 ml filtrate was taken in a test tube and 2-3 ml acetone was added to this solution. solution turned to Turbid, indicates the presence of resin.

Test for Saponin

5 ml of filtrate was taken in a test tube few drops of olive oil was added. Formation of emulsion indicates the presence of saponin.

Test for Tannin

5 ml of filtrate was taken in a test tube and few drops of 0.1% FeCl_3 was added. Brownish green or blue green color appeared, indicates the presence of Tannin.

Test for Phenol

5 ml filtrate was taken in a test tube and 2 ml of 0.1% FeCl_3 was added. Blue green color appeared, indicates the presence of Phenol.

Test for Terpenoids

5 ml of filtrate was taken in a test tube add 2 ml of chloroform add 3 ml of conc. H_2SO_4 to form a layer. Reddish brown colored is observed, indicates the presence of Terpenoids.

Results and Discussions

Documentation of the use of three species of *Cassia* suggests that there is immense potential of the three species sps of *Cassia* to be used as an ethnomedicinal plant and the cultivation of these plants can be promoted for commercial purpose. The study has revealed that the Chl-a ranges from 254.516 to 266.36 $\mu\text{g/ml}$ and Chl-b ranges from 271.96 to 393.16 $\mu\text{g/ml}$ (Table 2) and the total chlorophyll Chl (a+b) (table 3 Graph 5) ranges from 533.7 to 630.2 $\mu\text{g/ml}$ in three *Cassia* spp, *Cassia fistula* (Chl-a 254.516, Chl-b 305.741, total chl 533.7 carotenoid 10.6 Fig1(A &B)). *Cassia siamea* (Chl-a 257.88, Chl-b 393.16, total chl 630.2 carotenoid 9.395, Fig 2(A&B) *Cassia tora* (Chl-a 266.36, Chl-b 271.96, total chl 538.32 carotenoid 16.3 Fig 3(A &B). The result revealed that Chl-a was highest in *Cassia tora* followed by *Cassia siamea* and *Cassia fistula*. (Fig. 2) Chlb pigment followed *C. siamea* > *C. fistula* > *C. tora* pattern (Graph5), total chl *C. siamea* > *C. tora* > *C. fistula* and the carotenoid amount *C tora* > *C fistula* > *C Siamea*. (Graph 6) From the result it is also seen that *C. tora* has the highest concentration of Chl-a whereas *C. Fistula* was found to have least quantity of Chl-a. Highest amount of Chl-b in *C. siamea* and lowest in *C. tora*. among the three species (Table 1). The highest variation of chlorophyll *a* and *b* were observed in *C. tora*. The quantitative variation of chlorophyll content in *Cassia* spp. may be due to the health condition of the plants, habitat condition, leaf surface area and nutrients of the soil. Earlier Bojovic & Stojanovic (2005) reported that chlorophyll and carotenoid content in wheat cultivars depends on the presence of mineral elements in the substrate and plant physiological and environmental factors. According to Pandey & Sinha (1998) Chl-a and Chl-b occur together in the higher plants in the ratio of 2:1. The typical Chl-a/b for shade plants is about 1.6-2.2 and daily maximum sunlight exposed plants have the typical Chl-a/b content approximately 2.6-3.4 (Anderson 1986). In *Cassia* species the Chl-a/b was observed in between 0.73-2.08 which is much lower than Anderson's view. The reason of dissimilarities of Chl-a/b with other plants may be due to the presence of inactive light harvesting complexes in *Cassia* resulting effect in growth and development. Several literature and pieces of evidence suggest that the Chl-a:b ratio plays an important role to higher plants to adapt to new light regions to make optimal use of ambient light intensities and quantities (Arnon 1949). The ratio of Chl-a and *b* is stable in fully green leaves at nearly 3, but it can vary depending on the physiological status of the plant (Kouril et al., 1999). An average Chl-a:b ratio of .895 in *Cassia* plants. Bondada & Syvertsen (2003) reported that the nitrogen-deficient leaves contain less

chlorophyll per unit area, but a greater chlorophyll *a:b* ratio than N-fertilized leaves. The Chl-*a/b* in maximum sunlight exposure and shade plants reflect the adaptation of the chloroplast to prevailing intensity through regulation of the amount of photosystem I (PS I) relative to photosystem II (PS II) and the size and composition of the light harvesting complexes (LHCs) of each photosystem. Moreover, the leaf colors of a plant can be used to identify stress level due to its adaptation to environmental change (Shibghatallah 2013). Therefore, from the observation it can be said that the *C. Tora* and *C. siemea* were well adapted under local environmental condition. Kamble *et al.* (2015) opined that the leaf chlorophyll concentration plays a vital role in maintenance of photosynthetic mechanism as well as plant metabolism. Apart from these the seasonal variation and maturity of the leaf also affect the concentration of chlorophyll content in leaf and chlorophyll *a:b* ratio remains substantially lower in plants growing in high CO₂ condition (Cave *et al.*, 1981). Nevertheless, in the present investigation, a variability of chlorophyll content in the *Cassia* species observed but it has a scope to consider as tools for identification of *Cassia* species and varieties. From the (Table 1 & Fig. 2), an inference can be made that the Chl-*a*, Chl-*b* and total chlorophyll content in mg/g leaf tissue of different *Cassia* species are an individual character of each *Cassia* species studied. Remarkable variation of green colour of the leaf was observed as the same is dependent on the variability of chlorophyll content in the species. Two types of chlorophyll, *a* and *b* are present in green algae and terrestrial plants. Chlorophyll *a* possesses a green-blue colour, and chlorophyll *b* possesses a green-yellow color (Arnon, 1849). Moreover, the leaf colors of a plant can be used to identify stress level due to its adaptation to environmental change (Shibghatallah, 2013). Therefore, from the observation it can be said that the three species of *Cassia* were well adapted under local environmental condition and have immense potential as an ethnomedicine and nutritional supplement plant. Kamble *et al.* (2015) opined that the leaf chlorophyll concentration plays a vital role in maintenance of photosynthetic mechanism as well as plant metabolism. Apart from these the seasonal variation and maturity of the leaf also affect the concentration of chlorophyll content in leaf and chlorophyll *a:b* ratio remains substantially lower in plants growing in high CO₂ condition (Cave *et al.* 1981). Nevertheless, in the present investigation, a variability of chlorophyll content in the *Cassia* species observed but it has a scope to consider as tools for identification of *Cassia* species and varieties. From leaf tissue of different *Cassia* species are an individual character of each species. Remarkable variation of green colour of the leaf was observed as the same is dependent on the variability of chlorophyll content in the species. The alteration of Chlorophyll content may due to the factors like water, soil, temperature the (Table 1 & Fig. 2 & 3), an

inference can be made that the Chl-*a*, Chl-*b* and total chlorophyll content in mg/g etc. which indirectly affect the leaf area, morphology, thickness and chloroplast distribution (Campeanu and Neata, 2012).

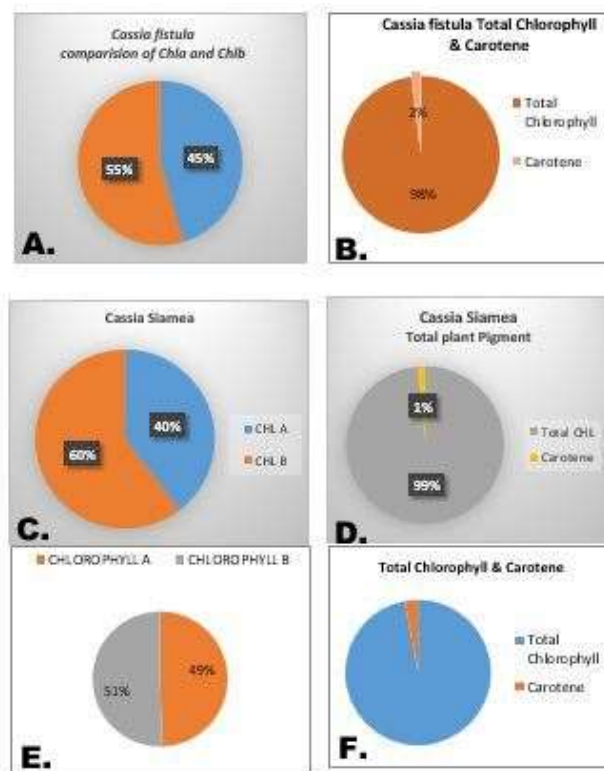


Fig. 1: Comparative study of various pigments. *Cassia fistula* (A & B); *C. siamea* (C & D); *C. tora* (E & F)

Analysis of the plant extracts revealed the presence of alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides, carboxylic acid, Resin and anthraquinone. (Table 2).

Phyto-chemical study helps to identify active constituents which are responsible for bringing out drug action. It also provides preliminary information on the quality of the drug. This study would provide preliminary scientific evidence for *Cassia sp* as potent drug; because of *Cassia* leaves have secondary metabolites like alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides, carboxylic acid, Resin and anthraquinonoid. Hence phyto-chemical study of three species of *Cassia* (*C. fistula*, *C. siamea*, *C. tora*) is essential in order to evaluate active constituents responsible for its medicinal actions and the manufacturing of new drugs. The documentation of ethnomedicinal properties and the variation in the plant pigments among the three studied species can suggest their effective plantation in Jharkhand and bringing these underutilised plants with immense ethnomedicinal potential into mainstream for commercial utilisation..

Table 1: Documentation of ethnomedicinal properties by different tribal groups

S.N.	Name of the plant	<i>Cassia fistula</i>	<i>Cassia tora</i>	<i>Cassia siamea</i>
	General	According to the Munda vidyas the plant is of pitta hark nature () The root is used against tuberculosis glands ,diabetes ,as a tonic, the root and bark paste mixed in equal amount is used against snake bite()	According to the local munda and birhor baidyas the leaves of <i>Cassia</i> It is generally used in the teething problems Fracture (If)	Dropsy-bk decoct. Alcoholic addiction (If. Decoct.) Diabetes (sd, fl. Decoct.)
	Digestive system	The fruit pulp purgative value and is mostly used against constipation Bark extract and fruit is used against mouth ulcer And stomach pain. Bark decoction is used for gargle	Jaundice (If) Stomach-ache (If) Piles (If) Liver tonic	----
	Respiratory system	The leaf extract is used against asthma and cold	----	----
	Genital system	----	----	----
	Urinary system	-----	----	----
	Eyes ears and throat	The seed is used	----	----
	skin	Fruit	Skin diseases, psoriasis, tumours Leprosy, ulcers, irritation, itching, eruptions Ring worm (Root paste with lemon juice) Hastens suppuration (Leaves poultice) Eczema (oil)	Ulcers, Boils – Apply bk paste
	Heart disease		Cardiac disorders (If)	----
	joints	Rheumatism (rub with leaves)	Joint pains Gout	----
	Nervous	Paralysis (facial) (rub the leaves) Epilepsy	Sciatica Headache (oil)	----

(All information is based on the local Vaidya's and the use of 100 different tribal people)

Table 2: Quantitative estimation

S.N.	Plant Name	Chl-A ($\mu\text{g/ml}$)	Chl-B ($\mu\text{g/ml}$)	Ratioa/b	Total Chl. ($\mu\text{g/ml}$)	Carotenoid ($\mu\text{g/ml}$)
1	<i>Cassia fistula</i>	254.516	305.741	.8332	533.7	10.6
2	<i>Cassia siamea</i>	257.88	393.16	.6559	630.2	9.395
3	<i>Cassia tora</i>	266.36	271.96	.9794	538.32	16.3

Table 3: Phytochemical screening for the presence of secondary metabolite

S.N.	Phytochemical test	Chemical	Observation	Result		
				<i>Cassia fistula</i>	<i>Cassia siamea</i>	<i>Cassia tora</i>
1	Tannins	FeCl ₃ (0.1%)	Appearance of blue black color	+++	+++	+++
2	Saponins	Olive oil	Emulsion formation	++	++	+++
3	Terpenoids	CHCl ₃ , Conc H ₂ SO ₄	Reddish brown colour	++	+++	++
4	Alkaloids	Mayers reagent	Ppt was not formed	-	-	-
5	Phyto sterols	CHCl ₃ , Conc H ₂ SO ₄	Cherry red colour	+++	+++	+++
6	Resins	Acetone	Turbid was formed	++	++	+

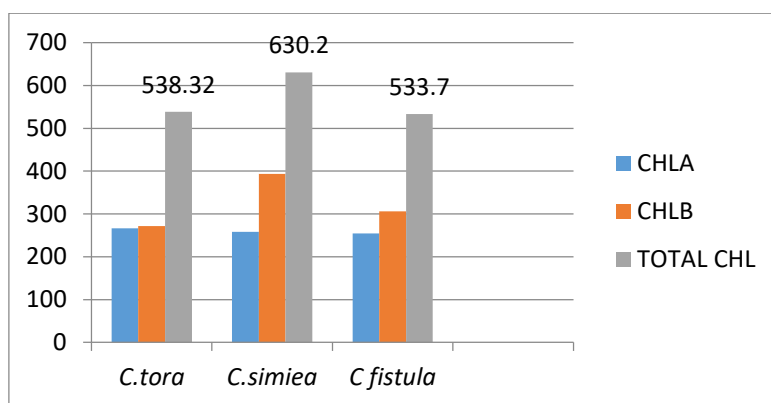


Fig. 2: Comparison of *Chl-a*, *chl-b* and total *chl* of three species of *Cassia*

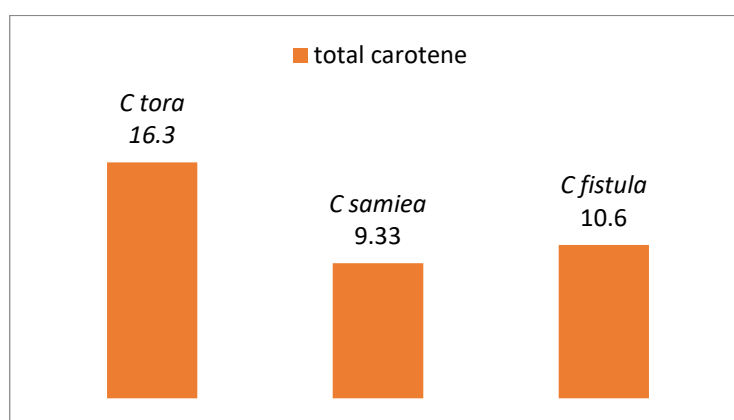


Fig 3.: Comparative study of carotenoid of the three species of *Cassia*

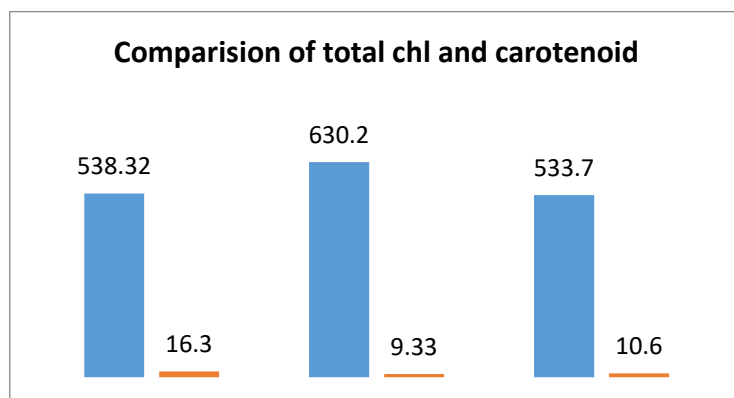


Fig 4.: Comparative study of total Chl-and carotenoids of the three species of *Cassia*

Author's Contribution

All authors contributed equally in all stages from designing of the research work to the finalization of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

References

- Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant physiology*. 24(1): 1. DOI: [10.1104/pp.24.1.1](https://doi.org/10.1104/pp.24.1.1)
- Butnariu M (2016) Methods of Analysis (Extraction, Separation, Identification and Quantification) of Carotenoids from Natural Products, *Journal of Ecosystem and Echography* 6(2): 2157-7625. DOI: [10.4172/2157-7625.1000193](https://doi.org/10.4172/2157-7625.1000193)

- Campeanu G and Neata G (2012) Studies concerning the extraction of Chlorophyll and total Carotenoids from vegetables, *Romanian iotechnological Letters* **17**(5): 7702-7708.
- Costache MA, Campeanu GH, Neata G (2012) Studies concerning the extraction of chlorophyll and total carotenoids from vegetables. *Romanian Biotechnological Letters* **17**(5): 7702-7708.
- Dabriyal RM, Narayana DBA (1998) Ayurveda Herbal Raw Material, *The Eastern Pharmacist*: 31-35.
- Deori C, Begum SS, Mao AA (2007) Ethnobotany of Sujen---A local rice beer of Deori tribe of Assam.
- Devlin RM and Witham FH (1986) *Plant Physiology* (Eds 4). CBS Publishers & Distributors, New Delhi, India
- Eric B, Fredric M, Lucien H, Elmar K and Torsten B (2016): Comparison of 3 Spectrophotometric Methods for Carotenoids Determination in Frequently Consumed Fruits and Vegetables, *Journal of Food Sciences*
- Ghosh TK (1971) Studies on Flora of Ranchi District. *Ph.D. Thesis, Vols I-II. Ranchi University, Ranchi*
- Ghosh TK and Sahu SC (1986). Plants used by Mundas of Chotanagpur for preparation of country liquor (Handia). *Mendel* **3**(2): 79-82.
- Haines HH (1921-25). The Botany of Bihar and Orissa, Vol I-IV. BSI, Calcutta.
- Hemadri K and Rao SS, 1984. Jaundice: Tribal Medicine. *Ancient Sci Life* **3**: 209-212. DOI: [10.1139/b79-163](https://doi.org/10.1139/b79-163)
- Hiscox JD and Israelite GF (1979) Different methods of chlorophyll extraction. *Can J Bot* **57**: 1332-1332.
- Indira P, Shamsad AS and John PM 2015: *The Effect of Air Pollution on Some Biochemical Factors of Some Plant Species Growing in Hyderabad, Int J Pharm Bio Sci* **6**: 1349-1359.
- Iqbal M, Shafiq M, Zaidi S and Athar M (2015) Effect of automobile pollution on chlorophyll content of roadside urban trees. *Global Journal of Environmental Science and Management* **1**(4): 283-296.
- Keshari BP (1983) Jharkhand Andolan Ki Vastavikta (In Hindi). Ranchi: Prakash.
- Kumar S and Kumari B. (2010). *Identification of the adulterant plants being used ignorantly in rice beer (Handia) making in Jharkhand. Biospectra* **5** (2) Sept. (Spl. Issue) p.p. 477-480.
- Maity TK, Mandal SC, Mukherjee PK, Saha K, Das J, Pal M, Saha BP. (1998) Studies on antiinflammatory effect of *Cassia tora* leaf extract (fam. Leguminosae). *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* **12**(3): 221-223. DOI: [10.1002/\(SICI\)1099-1573\(199805\)12:3<221::AID-PTR221>3.0.CO;2-L](https://doi.org/10.1002/(SICI)1099-1573(199805)12:3<221::AID-PTR221>3.0.CO;2-L)
- Markwell J (2002) Nondestructive chlorophyll assessment. *New Phytologist*, **153**: 7-8. DOI: [10.1046/j.0028-646X.2001.00309.x](https://doi.org/10.1046/j.0028-646X.2001.00309.x)
- Oudhia P (2001). Traditional medicinal knowledge about Pod borer *Heliocoverpa armigera* in Chhattisgarh. India. *International Chickpea and pigeonpea Newsletter*. **I**: 14-15.
- Patil UK, Saraf S and Dixit VK. (2004) Hypolipidemic activity of seeds of *Cassia tora* Linn. *J Ethnopharmacol* **90**: 249-52. DOI: [10.1016/j.jep.2003.10.007](https://doi.org/10.1016/j.jep.2003.10.007)
- Pereyra MS, Davidenco V, Nunez SB & Argüello JA (2014) Chlorophyll content estimation in oregano leaves using a portable chlorophyll meter: relationship with mesophyll thickness and leaf age. *Rev. Agronomía & Ambiente* **34**(1-2): 77-84.
- Seyyednejad SM and Motamedi H. (2010) A review on native medicinal plants in Khuzestan, Iran with antibacterial properties. *International journal of Pharmacology*. **6**(5): 551-560. DOI: [10.3923/ijp.2010.551.560](https://doi.org/10.3923/ijp.2010.551.560)
- Shibghatallah MAH, Khotimah SN, Suhandono S, Viridi S & Kesuma T (2013) Measuring Leaf Chlorophyll Concentration from Its Color: A Way in Monitoring Environment Change to Plantations. Available from: <https://arxiv.org/ftp/arxiv/papers/1305/1305.1148.pdf> (accessed: 27 Jan. 2018). DOI: [10.1063/1.4820322](https://doi.org/10.1063/1.4820322)
- Singh K, Singh DK and Singh VK (2017) Chlorophyllin Treatment against the Snail *Lymnaea acuminata*: A new tool in Fasciolosis Control, *Pharmacognosy Journal* **9**(5): 594-598. DOI: [10.5530/pj.2017.5.94](https://doi.org/10.5530/pj.2017.5.94)
- Singh NP, Mudgal V, Khanna KK, Srivastava SC, Sahoo AK, Bandopadhyay S, Aziz N, Das M, Bhattacharya RP and Hazra PK (2001) *Flora of Bihar Analysis, Botanical Survey of India, Calcutta*
- Vimala T and Poonghuzhali TV (2015) Estimation of Pigments from Seaweeds by Using Acetone and DMSO, *International Journal of Science and Research* **4**(10).
- Wakefield JM and Bhattacharjee J (2011) Effect of air pollution on chlorophyll content and lichen morphology in Northeastern Louisiana, *Evansia* **28**(4): 104-114. DOI: [10.5530/pj.2017.5.94](https://doi.org/10.5530/pj.2017.5.94)
- Yen GC and Chung DY (1999) Antioxidant effects of extracts from *Cassia tora* Linn. Prepared under different degrees of roasting on the oxidative damage to biomolecules, *Journal of Agricultural Food Chemistry* **47**: 1326-1332. DOI: [10.1021/jf9810618](https://doi.org/10.1021/jf9810618)