

## Evaluation Isolation and Characterization of Chemical constituents from *C. bonducella* L. seed

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### Abstract:

The objective of the present work is to study the different Pharmacognostic parameters of the seeds of *C. bonducella* and to isolate and characterize the chemical constituents from the seeds that are responsible for the activity. In pharmacognostic study of seeds of *C. bonducella*, macroscopy, microscopy, powder characteristic, and physical parameters were studied. Column chromatography of active extract; Structure elucidation of active fraction was done to isolate and characterize various chemical constituents. The alcohol soluble extractive value was found to be greater than water soluble extractive value. Petroleum ether extract showed the presence of steroids and terpenes. Ethanol extract showed positive test for flavonoids, alkaloids, glycosides, and tannins. By GC-MS analysis of saponified matter of petroleum ether extract contains fatty acid viz. hexadecanoic acid and 9-methyl-8-tridecen-2-ol, acetate. The unsaponified matter contains colour pigments namely lycoxanthin and carotene.

**Keywords:** *Caesalpinia bonducella*, seeds, extract, Thin layer Chromatography, GC-MS.

### Introduction:

In present study *Caesalpinia bonducella* used as a drug candidate derived from Kingdom: Plantae, Order: Fabales, Family: Caesalpiniaceae, Genus: *Caesalpinia*, Species: *C. bonducella*; Part used: Seeds of *C. bonducella* linn. Commonly known as Kakachika, Karanja. Seed Used in the treatment of intermittent fever, asthma, colic. Also used as antiperiodic, in dyspepsia, dentrifice, filariasis [Nadkarni, K.M., 1986; Kirtikar, K.R., Basu, B.D., 1987; Anonymous, 1992]. The seed kernel of plant *C. bonducella* mainly contains bonducin [Elizabeth, M. Williamson], natin [Anonymous, 1996] and sulphur compounds [Ghatak NG, 1934]. The seed also contains fatty acids [Elizabeth, M. Williamson]. The root mainly shows the presence of diosgenin as chemical constituent [Elizabeth, M. Williamson, 2002]. The leaf also contains

proteins [Elizabeth, M. Williamson]. The fruit contains saponins viz, saponin C and saponin D [Puri HS, 1980].

### 2. Materials and methods:

#### 2.1: Plant Material

Mature seeds of *C. bonducella* was collected and authenticated by Dr. T. Chakraborty, Joint Director, Botanical Survey of India (Voucher number: BSI/CAEB7PRAK).

#### 2.2 Pharmacognostic evaluation:

##### 2.2.1 Macroscopic evaluation:

Different parameters were studied in macroscopic evaluation of *C. bonducella* seeds that are color, odor, size and shape [Khandelwal, K.R., 2005].

### 2.2.2 Microscopic evaluation:

Microchemical and powdered characteristic of fresh seed was taken for microscopical evaluation [Khandelwal, K.R., 2005].

### 2.3 Determination of foreign organic matter:

The foreign organic matter was separated manually as completely as possible. Sample was weighed and percentage of foreign organic matter was determined from the weight of the drug taken [Anonymous, 1996].

### 2.4 Determination of moisture content:

Sample was dried to constant weight. After drying it was collected to room temperature in a desiccator. Weighed and calculated moisture content in terms of percent w/w [Anonymous, 1996].

### 2.5 Determination of ash values:

Ash value contains inorganic radicals like phosphates carbonates and silicates of sodium, potassium, magnesium, calcium etc. Such radicals are then removed by treating with acid and then acid insoluble ash value is determined [Khandelwal K.R., 2005].

### 2.6 Determination of extractive values:

Different extractive values like alcohol soluble extractive, water soluble extractive values were performed by standard method [Anonymous, 1996].

### 2.7 Extraction:

The seeds of *C. bonducella* was collected and dried in the shade and then powdered in a grinder. The powdered seeds were utilized for extraction. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction. A method described in [Chakrabarti et.al, 2003] was used for extraction of powdered plant.

**Technique:** Soxhlet Extraction.

**Procedure:** The extraction was carried out in several batches by using solvents like petroleum ether and 70% ethanol in water. The extraction was carried using petroleum ether in Soxhlet extractor till all the constituents were extracted. Absence of colored spot on plate indicates complete extraction. After completion of extraction, solvent was distilled off and concentrated extract was air-dried. The extract was stored in airtight container. The same procedure was followed using 70% ethanol [Chakrabarti et.al, 2003; Chakrabarti et.al, 2004].

**2.8. Preliminary phytochemical test** [Khandelwal, K.R., 2005]: The extracts obtained after extraction were characterized by preliminary phytochemical test for rough ideas of constituents present in extract.

**2.9. Thin layer chromatography** [Stahl, 1969]:

**1. Detection of Sugar:** Stationary phase=Silica gel-G, Mobile phase=Benzene: Glacial Acetic Acid: Methanol (20:20:60), Chamber saturation=30 min, Visualization= Anisaldehyde sulphuric acid, Extract=70% Ethanol

**2. Detection of Alkaloids:** Stationary phase=Silica gel-G, Mobile phase =Toluene: Ethyl acetate: Diethylamine (70:20:10), Chamber saturation=30 min, Visualization= Dragendorffs, Extract=70% Ethanol

**3. Detection of flavonoids:** Stationary phase=Silica gel-GS, Mobile phase= Ethylacetate: Formic acid: Glacial acetic acid: water (100:11:11:26), Chamber saturation=30 min, Visualization= Anisaldehyde- sulphuric acid, Extract=70% Ethanol

**4. Detection of Glycoside:** Stationary phase=Silica gel-G, Mobile phase=Chloroform: Methanol: Water (65:35:10), Chamber saturation=30 min,

Visualization=Sodium Nitroprusside reagent, Extract=70% Ethanol

**5. Detection of Terpene:** Stationary phase= Silica gel-G, Mobile phase=Toluene: Ethylacetate (9:1), Chamber saturation=30 min, Visualization=Vanillin – Sulphuric Acid, Extract=Petroleum ether.

### 2.10 Column chromatography of active extract:

Chemical constituents from 70% ethanol extract were isolated using column chromatography.

### Experimental column chromatography:

Height=55 cm, Diameter of column= 2 cm, Mobile phases= i) Ethyl acetate (100%), ii) Ethyl acetate: Methanol (9:1), iii) Ethyl acetate: Methanol (7:3), iv) Ethyl acetate: Methanol (5:5), v) Ethyl acetate Methanol: (3:7), vi) Ethyl acetate Methanol: (2:8), vii) Ethyl acetate Methanol: (1:9), Number of fractions collected= Seven.

### 2.11: Saponification of petroleum ether extract of *C. bonducella* seeds-

Reflux the petroleum ether extract with 25 ml of ethanolic KOH for about 30 min. Took proportionate quantity of water and transferred to the separating funnel. It was shaking with solvent ether and the ethereal layer was collected and evaporated which contains unsaponified matter. Now the remaining hydro alcoholic layer was made acidic with 10% HCl and transferred it to the separating funnel and again shaken with solvent ether. This ethereal layer contains fatty acid. Unsaponifiable matter and fatty acids were analyzed by GC-MS.

### 2.12 Structure elucidation of active fraction:

Fraction no. 1 eluted with ethyl acetate was found in more significant amount and pure in TLC hence it was subjected to GCMS and FTIR studies.

**Ethyl acetate (100%) fraction:**  
**Description:** White, **Solubility:** Soluble in methanol.

**Melting point :** 136-140<sup>0</sup> C, **Instrumental condition:** 1.FT-IR, 2.GC-MS Analysis.

## 3. Results

### 3.1. Macroscopic evaluation: Table no.1

Sr. No.	Parameter	Features of seed
1	Color	Green
2	Odor	Characteristic
3	Taste	Bitter
4	Size	2-4 cm long, 1-2 cm diameter
5	Shape	circular

### 3.1.1. Microscopic evaluation: Structure of the seed

The seed has a thick cylindrical 1 and straight funicle attached on the hilar part of the seed & near the micropyle (fig 1). The outer surface of the palisade layer has the cuticle (fig 2). Less distinct vascular strands are seen spread in the cotyledenous tissue (fig 3).

**3.1.2. Powder microscopy of the seed: -** (fig 4) columnar sclereids, lobed sclereids and thin walled small parenchyma cells are seen scattered in the powder.

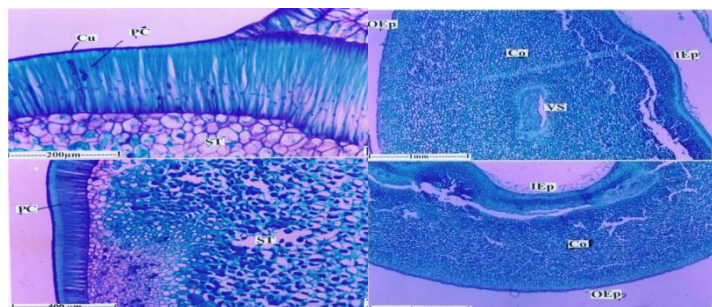


Figure (1)

Figure (2)

Figure (3)

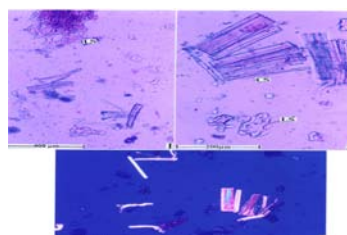


Figure (4)

### 3.2: Pharmacognostic constants: Table no.2

Sr. No.	Evaluation parameters	Value (%w/w)
1	Foreign organic matter	1
2	Moisture content	5.5
3	Total ash value	4.9
4	Water-soluble ash value	1
5	Acid-insoluble ash value	1
6	Water soluble extractive value	4.45
7	Alcohol soluble extractive value	10

## 3.3: Preliminary phytochemical test: Table no.3.

Sr.no	Chemical Test	Petroleum ether Extract	70% Ethanol Extract
1	<b>Carbohydrate</b> a) Molisch test b) Fehlings test c) Benedicts test d) Barfoed's test	- - - -	- - - -
2	<b>Protein</b> a) Biuret test b) Millions test	- -	- -
3	<b>Amino acid</b> a) Ninhydrin test	-	-
4	<b>Steroid</b> a) Salkowski test b) Liebermann-Burchard reaction c) Liebermann's reaction	+ + +	- - -
5	<b>Glycoside</b> a) Deoxysugares (Keller-killani test) b) Borntrager's test	- -	+ +
6	<b>Alkaloid</b> a) Dragendroff's test b) Mayer's test c) Hager's test d) Wagner's test	- - + -	+ + + -
7	<b>Test for Flavonoids</b> a) Lead acetate b) Sodium hydroxide c) Shinoda test	- - -	+ - +
8	<b>Test for Saponins</b> a) Foam formation test	-	-
9	<b>Tannin(Phenolic compounds)</b> a) 5% Ferric chloride test b) Lead acetate test c) Dilute Iodine test d) Dilute nitric acid test e) Potassium-dichromate test f) Acetic acid	- - - - - -	+ + - - - +

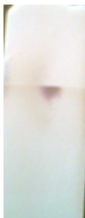
+: indicates presence of constituents

-: indicates absence of constituents


### 3.4: Thin layer chromatography profiles

Thin layer chromatography technique was carried out for characterization of different extracts.


**Table No. 4: TLC profile of Sugars**

Mobile Phase	Spraying reagent	Color of spot	Rf values for extract	
Benzene: Glacial acetic acid: Methanol (20:20:60)	Anisaldehyde-sulphuric acid	violet	70% Ethanol = 0.81	 <p>Figure: 5</p>


**Table No. 5: TLC profile of terpenes**

Mobile Phase	Spraying reagent	Color of spot	Rf values for extract	
Toluene:Ethyl acetate (9:1)	Vanillin- H <sub>2</sub> SO <sub>4</sub>	violet	Petroleum ether= 0.5	 <p>Figure: 6</p>

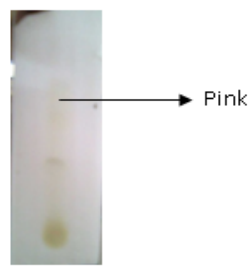
**Table No.6: TLC profile of alkaloids**

Mobile Phase	Spraying reagent	Color of spot	Rf values for extract	
Toulene: Ethylacetate: Diethylmine	Dragendorffs Reagent	Orange Brown	70%Ethanol = 0.42	 <p>Figure: 7</p>

**Table No.7: TLC profile of glycosides**

Mobile Phase	Spraying reagent	Color of spot	Rf values for extract	
Chloroform: Methanol: Water (65:35:10)	Sodium Nitroprusside (5%)	Pink	70%Ethanol = 0.8	 <p>Figure:8</p>

**Table No.8 : TLC profile of flavonoids**

Phase	Mobile	Spraying reagent	Color of spot	Rf values for extract	
Ethyl acetate: Formic acid: Glacial acetic acid: water(100:11:11:26)		Anisaldehyde-sulphuric acid	Green	70% Ethanol = 0.5	 <p><b>Figure:9</b></p>

**3.5. Column Chromatography: Table No. 9**

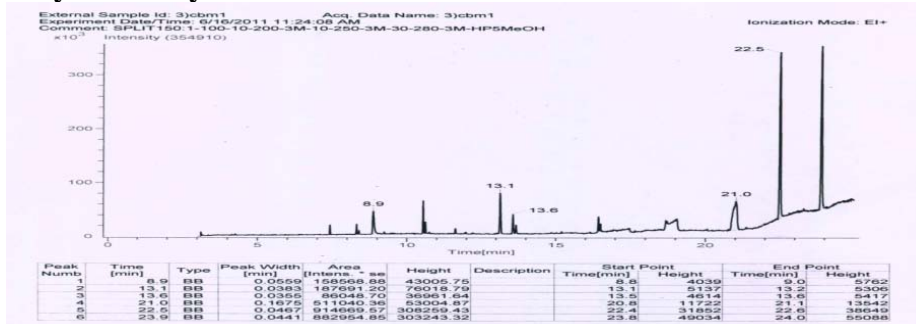
Fraction	Mobile phase	Concentration	Physical appearance	% Yield (gram)
1	EA	100%	White	1.1 gm
2	EA : MEOH	(9:1)	Brown	0.8 gm
3	EA : MEOH	(7:3)	Black brown	0.7 gm
4	EA : MEOH	(5:5)	Black brown	0.7 gm
5	EA : MEOH	(3:7)	Brown	0.5 gm
6	EA : MEOH	(2:8)	Brown	0.4 gm
7	EA : MEOH	(1:9)	Brown	0.4 gm

**3.6. Saponification results: Table no. 10**

Constituent	Rt (min)	Amount (%)
9-methyl-8-tridec an-2-ol-acetate	11.0	5.78
Hexadecanoic acid	13.0	74.8

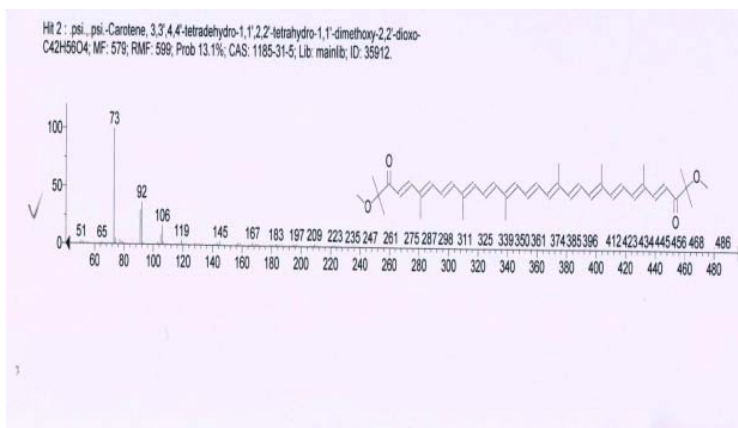
**3.7 Structure elucidation of active fraction:**

**1) GC-MS analysis of ethyl acetate fraction-**

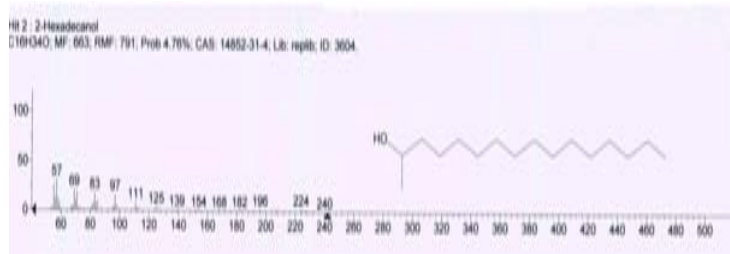


Peak No.	Identified Compound	Base peak	Major Peaks
1. (Rt 8.9)	Carotene,3,3',4,4-tetrahydro-1,1,2,2-tetrahydro-1,1-dimethoxy-2,2-dioxo	73	73,92,106

2) Carotene, 3, 3', 4, 4'- tetrahydro-1, 1, 2, 2-tetrahydro-1, 1-dimethoxy-2, 2-dioxo



3) 2-Hexadecanol



Peak No.	Identified Compound	Base peak	Major Peaks
2 (Rt 10.5)	2-Hexadecanol	57	55,69,83,97,111

4) FT-IR analysis of ethyl acetate (100%) fraction: Figure:10.





#### 4. Discussion:

Seeds of *Caesalpinia bonducella* (Caesalpinaceae) were used for studying pharmacognostical, phytochemical characters and evaluation of chemical constituents.

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