

## Chemical composition, mineral and nutritional value of wild *Bischofia javanica* seed

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### Abstract

The present study investigates chemical composition, mineral and nutritional value of *Bischofia javanica* seed cultivated in northern India. The seed contains protein 18.69%; carbohydrates 18.91%, crude fiber 5.32% and ash 6.83%. The seed contains phosphorous 391.4 mg/100 gm, calcium 710 mg/100 gm, magnesium 610 mg/100 gm, copper 2.43 mg/100 gm, Iron 2.33 mg/100 gm, potassium 1.25 mg/100 gm, zinc 1.4 mg/100 gm and sodium: 0.08 mg/100 gm. The physico-chemical characteristics of seed oil measured include saponification value: 289.3, iodine value 178.3, acid value 6.59 (mg KOH/gm) refractive index: 1.48. The yield of seed oil was 20.1%. The oil extracted from the seeds of *Bischofia javanica* was analysed for its chemical composition by Gas chromatograph mass spectroscopy (GCMS), The fatty acids in seed oil were identified as- Linolenic acid 56.76%, Palmitic acid 12.28%, Linoleic acid 12.90%, Oleic acid 12.19%, and Stearic acid 3.86%. This study concludes that *Bischofia javanica* seeds are good source of food nutrients such as: minerals, proteins and carbohydrates. The seed oil of *Bischofia javanica* is an important source of essential omega 3 fatty acid i.e. linolenic acid.

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### Introduction

*Bischofia javanica* belongs to family Euphorbiaceae and is commonly known as Bishop wood found in the sub-Himalayan forests from Kumaun eastwards throughout eastern India, is a medium to fairly large in size, usually a deciduous tree, 30-50 m tall, bole straight or poorly shaped. Its bark is dark-grey, fairly smooth, fleshy, juicy and astringent inside exfoliating in angular scales. Leaves are arranged spirally, pinnately 3-foliolate, glabrous; petiole 8-20 cm long; stipules oblong-triangular, papery, 7-22 mm long. Flowers are unisexual, actinomorphic, 5-merous, small, greenish, apetalous. Male flowers in an axillary, many-flowered, 9-20 cm long panicle, sepals united at base, hooded; stamens 5, free, opposite to the calyx lobes. Flowers are green or greenish yellow, berries reddish brown to blue-black, fleshy, 3-4 seeded, seed dark brown, smooth, shining, embedded in a soft, colourless, edible pulp. Fruits of *Bischofia javanica* are harvested from August to October. The major phyto-constituents which have been isolated from *Bischofia javanica* are tannin,  $\beta$  amyryns, betulinic acid, friedelan-3 $\alpha$ -ol, epifriedelinol, friedelin, luteolin and glucoside, quercetin, beta-sitosterol, stigmosterol, ursolic acid (Cambie, 1984; Gupta, 1988; Whistler, 1992). Its bark yields tannins used in toughening of nets and ropes and a red dye used to stain rattan baskets, bark is also

used in high fever and burns, fruits are used in wine-making, seeds yield a dry-ing oil useful in surface coating and lubrication, leaves as an astringent for toothache and for treatment of eye diseases, plant having anti-ulcer, antihelminthic and antidysentric properties (Shu *et al.*, 2008; Seed Leaflet, 2012). Stem bark paste is used as an external application in skin diseases (Prasad *et al.*, 2008).

*Bischofia javanica* has been reported for its antitussive activity (WHO, 1990; Gairola *et al.*, 2010) and is also ethnomedicinally used for the treatment of tuberculosis, ulcers, fracture, dislocation and other inflammatory conditions due to its free radical scavenging property (Perry and Metzger, 1980; George, 1989; Sutharson *et al.*, 2009). Leaves of *Bischofia javanica* are used in treatment of Lactagogue, painful or hard breasts and to improve flow of milk (Lamxay *et al.*, 2011). Bark and leaves are used for treatment of sore throat, diarrhea, Nervous disorder and to stimulate hair growth (Ignacimuthu *et al.*, 2006; Pradhan and Badola, 2008). *Bischofia javanica*, has exhibited strong antiparasitic activity (Alen *et al.*, 2000) and antimicrobial activity (Khan *et al.*, 2001), antileukemic activity (Sutharson *et al.*, 2011), anti-inflammatory and antinociceptive activities (Sutharson *et al.*, 2007). The bioassay-guided fractionated compounds including betulinic acid and its derivatives, betulonic acid, 3 $\beta$ -O-(Z)-coumaroyl betulonic acid, and 3 $\beta$ -O-(E)-coumaroyl

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betulinic acid, from the chloroform extract of the bark of *Bischofia javanica* have been found to act as catalytic inhibitors of topoisomerase II activity with IC<sub>50</sub> values ranging from 0.38 to 58 µM (Wada and Tanaka, 2005). Earliest occurrence of fossil *Bischofia* woods in China, has contributed to our scant knowledge of Chinese Paleogene angiosperm woods (Feng *et al.*, 2012).

Oils from seed are often available as raw materials for chemical and industrial applications. Fats and oils are important food source for all human beings, and are supplying essential fatty acids such as Linoleic acid, Linolenic acid commonly known as Omega 3 fatty acid that cannot synthesize by our biological system. Fats and oils are also used for producing drug dispersants in therapeutics (Rauken and Kill, 1993). These oils are often available as raw materials for chemical and industrial applications. Due to their high demand, economic importance and natural source of these oil seeds to the chemical industry, attention has therefore been focused on underutilized such type of wild varieties *Bischofia javanica* seeds for their possible development and use.

## Materials and Methods

### Collection of seeds

The seeds of *Bischofia javanica* were collected from Ramnagar near Kalagarh adjoining of Garhwal and Kunmau forest region (Uttarakhand, India) at Latitude. 29.75° and Longitude. 78.53°. The voucher specimens were identified and authenticated by Dr. H.B. Singh, National Institute of Science Communication and Information Resources, New Delhi, India. Dried fruits were collected in polythene bags and brought to the laboratory. Seeds were separated from the fruit pod and stored in airtight amber colored glass bottles and kept in a refrigerator prior to analysis.

### Extraction of seed oil

A known weight of *Bischofia javanica* seeds were grinded into powder with high speed blender and dried in an air circulating oven at 50°C for 1 h. Oil was extracted from the seeds powder with petroleum ether (boiling point 60-80°C) using a Soxhlet extractor. The solvent was distilled off at 80°C and oil was dried over anhydrous sodium sulphate.

### Physical and Chemical analysis of seed oil

Physico-chemical analyses of the extracted oil was carried out by using AOAC methods (AOAC, 1990). Density of oil was determined picnometrically, refractive index was determined at 25°C using Abbey Refractometer, viscosity was determined by

Ostwald method (Standard Base, 2010), moisture was estimated by heating in oven at 105°C. Iodine value was determined using Wiji's method as reported in AOAC methods (AOAC, 1990). The procedures of Egan *et al.* (1981) were adopted for the estimation of Saponification values, Unsaponifiable matter content and acid value of the oil sample.

### Determination of metal/ mineral compositions

After digesting of the seed sample zinc, Iron and Copper concentration were determined using an atomic Absorption Spectrophotometer (Model no. –Varian 240FS+GTA120). Calcium and magnesium was determined by complexometric titration using 0.1M EDTA and Erichrome black T as indicator. Phosphorus was determined by titration method after precipitation with ammonium molybdate reagent (IS-7874-1975 method) Sodium and potassium were determined by using a flame Photometer (model No. ESICO 1381).

### Gas chromatography analysis (GC)

The Fatty acids were derivatized by using the boron trifluoride method as described by Hisil (1988). Samples were injected as 2 µl into a Nucon model 5700 equipped with 10% DEGS (Diethylene Glycol Succinate) + 1% H<sub>3</sub>PO<sub>4</sub> constant phase, a flame ionization detector (FID) and chromosorb G (100/120 mesh) support matter, internal diameter (2mm) and stainless steel (190 cm) column. Column temperature was programmed from 70°C to 200°C with the increasing rate of temperature 6°C/Minute. Injector and detector temperatures was set at 225°C. Nitrogen (N<sub>2</sub>) (25 ml/min) was used as the carrier gas. Hydrogen (40 ml/min) and Air (60 ml/min) were used as burnt and dry gas respectively. Fatty acid methyl esters were identified by comparison with fatty acid internal standards. Individual fatty acid concentration was expressed as percent.

### Gas chromatography/mass spectrometry analysis (GC/MS)

Derivatized fatty acids methyl esters were analysed by using a Shimadzu GC-2010 equipped with a Shimadzu GCMS-QP2010 Plus mass selective detector having HP-MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). The column oven initial temperature was 140°C, programmed at 4°C/min to final oven temperature 240°C and held for 10 min at this temperature, injector temperature was 270°C. Helium was used as carrier gas with column flow rate 1.21 ml/min and the split ratio 1:20. For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Ion source temperature was 230°C and Interface temperature

was 280°C. The components were identified by comparing their relative retention times and mass spectra with those of standards (main components), Wiley 8 library data of the main system.

## Results and Discussion

The nutritional status of seeds is presented in Table 1, the yield of seed oil is (20.1%) that is comparable with Soyabean seed oil (19.5%), Cotton seed oil (19.5%). Protein content of seed is (18.69%) which is near to other conventionally used oils e.g. Mustard seed oil (20.0%), Cotton seed oil (19.4%), Linseed seed oil (20.3%), Sunflower seed oil (19.8%), Coconut seed oil (23.9%), Almond seed oil (20.8%), reported in Nutritive value of Indian foods (Gopalan et al., 1971, Anonymous, 2001). Carbohydrate content (18.91%) also lies in the range of other oils which are used for food purposes and other applications.

Table 1. Proximate analysis of *B. javanica* seeds

Parameter	Result
Oil yield %	20.10
Carbohydrate content, %	18.91
Protein content, %	18.69
Moisture content, %	5.93
Ash content, %	6.83
Crude Fiber, %	5.32

Oil extracted from *Bischofia javanica* seeds had agreeable and unobjectionable colour and odour, comparable with other conventionally used seed oils. The moisture content is 5.93%, which lies in the range of 3.0-9.9% of other used oils, this suggests that oil can be stored for a long period. Acid value of oil is 6.59, which falls within the recommended codex of 0.6 and 10 for virgin and non-virgin edible fats and oil, respectively (Dawodu, 2009). This essence suggests that the *Bischofia javanica* seed oil is suitable for edible purposes and also in the manufacture of paints and varnishes (William, 1966). The iodine value of the oil is 178.3, which lies in the category of drying oils. Iodine value of the oil is 178.3, near to those for drying oils, Iodine value shows the degree of unsaturation of fatty acids in an oil or fat and is thus a relative measure of the unsaturation. Drying oil dry rapidly on contact with atmospheric oxygen and makes the oil useful on commercial scale for paint and varnishes, oil paints. Saponification value of *Bischofia javanica* oil is 289.3, Saponification value is used in checking adulteration. The low saponification value of any oil suggests that the oil may not be industrially useful. The high saponification value suggests that the oils contain high molecular weight fatty acids and low level of impurities. This suggests

Table 2. Metal/minerals contents of *B. javanica* seed (mg/100 gm) dry weights

Metal/Minerals	Result
Calcium(Ca)	710
Phosphorous(P)	391.4
Potassium (K)	1.25
Sodium(Na)	0.08
Iron(Fe)	2.33
Zinc(Zn)	1.40
Copper(Cu)	2.43
Magnesium(Mg)	610.0

Table 3. Physico-chemical properties of *B. javanica* seed oil

Physico-chemical properties	Result
State at room temperature	Slight viscous liquid
Colour	Yellow
Refractive index (at 40°C)	1.4863
Specific gravity (at 25°C)	0.9256
Acid value (mg KOH/gm)	6.59
Iodine Value	178.3
Unsaponifiable matter( % w/w)	0.48
Saponification value	289.3

that the oil could be used in soap making industry (Kirshenbauer, 1965; Amoo et al., 2004).

The mineral composition of the seeds are listed in Table 2. The level of phosphorous, calcium and magnesium are quite high. Calcium, potassium and magnesium are required for repair of worn out cells, strong bones and teeth in humans, building of red blood cells and for body mechanisms (WHO, 1996). Iron and Zinc are among the essential elements for humans and their daily requirements for an adult are 15 and 18 mg, respectively (Kampali and Pali, 2004). Levels of Zinc and Iron are low in seed but can be utilized for medicinal purposes. Total unsaturated fatty acid is 81.85%, Mono-unsaturated Fatty acids, (MUFA) : 12.19% while Poly-unsaturated fatty acids (PUFA) : 69.66%. Unsaturated fatty acids help to reduce cholesterol formation or deposition and hence to decrease the risks of atherosclerosis and other heart disease (David and Mehar, 1983).

Seed oils are composed primarily of triglycerides which are glycerol esters of fatty acids. GC-MS examination of *Bischofia javanica* seed oil (Table 4 and Figure 1) shows that it contains higher percentage of Omega-3 fatty acids also known as polyunsaturated fatty acids (PUFAs) like linoleic and linolenic acids are considered essential fatty acids, that is supported by higher iodine value of oil, because higher unsaturated fatty acids absorb more iodine. Omega 3 fatty acids are necessary for human health but the body can't make them you have to get them through food, they play a crucial role in brain function (brain memory and performance), as well as normal growth

Table 4. Fatty acid profile by gas chromatography mass spectroscopy of *B. javanica* seed oil

Fatty acids	Peak Area %
Palmitic acid(C16:0)	12.28
Stearic acid (C18:0)	3.86
Oleic acid(C18:1)	12.19
Linoleic acid(C18:2)	12.90
Linolenic acid(C18:3)	56.76

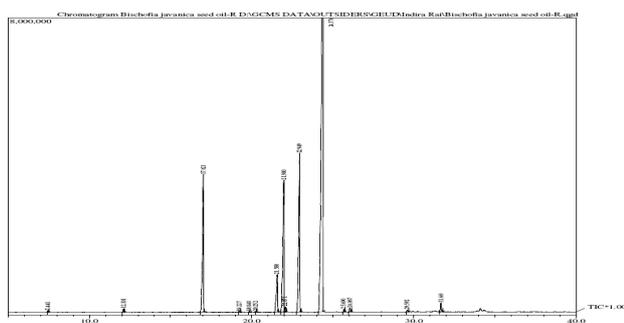


Figure 1. Chromatogram of *Bischofia javanica* seed oil by GC-MS

Peak#	R.Time	Area	Area%	Name
1	7.411	139957	0.10	Butanoic acid, 3-methyl-, methyl ester
2	12.101	305771	0.21	Tetradecanoic acid, 12-methyl-, methyl ester
3	17.021	17580718	12.28	n-Hexadecanoic acid
4	19.227	55929	0.04	Octanoic acid, methyl ester
5	19.848	60411	0.04	1-Pentene, 3-Methyl
6	20.252	40799	0.03	4-Hexen-2-one, 3-methyl-
7	21.580	5521676	3.86	Octadecanoic acid
8	21.980	17460911	12.19	9-Octadecenoic acid, methyl ester,
9	22.071	430354	0.30	11-Octadecenoic acid, methyl ester
10	22.949	18479015	12.90	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
11	24.376	81282988	56.76	9,12,15-Octadecatrienoic acid, methyl ester, C
12	25.690	298951	0.21	Heptacosanoic acid, methyl ester
13	26.067	394157	0.28	Methyl 9-Octadecenoate
14	29.592	207677	0.15	Eicosanoic acid, methyl ester
15	31.669	944507	0.66	11,14,17-Eicosatrienoic acid, methyl ester
		143203821	100.00	

Peak report of *Bischofia javanica* seed oil

and development and behavioral function. Research shows that omega-3 fatty acids reduce inflammation and may help lower risk of chronic diseases such as heart disease, cancer, and arthritis. In fact, infants who do not get enough omega-3 fatty acids from their mothers during pregnancy are at risk for developing vision and nerve problems. Symptoms of omega-3 fatty acid deficiency include fatigue, poor memory, dry skin, heart problems, mood swings or depression, and poor circulation (Harris & Isley. 2001, Von Schacky 2000, Omega-3 fatty acids downloaded from internet). It is important to have the proper ratio of omega-3 and omega-6 (another essential fatty acid) in the diet. Fatty acid profile of *Bischofia javanica* seed oil provide an important source of Omega 3 fatty acids for food supplement and medicinal purposes on commercial scale.

## Conclusion

Proximate and mineral composition of *Bischofia*

*javanica* seeds indicates their high food energy value and they could be an alternative source of human food and find application in mixed animal feed. Many of the physico-chemical properties studied of *Bischofia javanica* seed oil have very close similarity with other conventionally used oils and it is an important source of essential omega 3 fatty acid linolenic acid. The present study provides as baseline data to develop *Bischofia javanica* seed oil for both domestic as well as industrial purposes and also for proper cultivation of this tree with an organized manner in northern India for higher production of oil. This plant can be utilized to cure number of diseases that are mainly caused due to the deficiency of these minerals.

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