

Studies on Medicinal Plant Resources of the Himalayas: GC-MS Analysis of Seed Fat of Chyuri (*Diploknema butyracea*) from Nepal

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ABSTRACT

Chyuri (*Diploknema butyracea* (Roxburgh) H. J. Lam) is widely used in Nepal for its medicinal properties and as a source of Chyuri ghee (fat) extracted from the seeds. The main purpose of the present study was the GC-MS analysis of fatty acids in seed fat of Chyuri after acidic methanolysis. Palmitic acid methyl ester (1), linoleic acid methyl ester (2), oleic acid methyl ester (3) and steric acid methyl ester (4) were identified as the main components. Ethnomedicinal use and distribution of Chyuri in Nepal is also discussed.

Key words: Chyuri, *Diploknema butyracea*, GC-MS, Nepal

INTRODUCTION

Chyuri (*Diploknema butyracea* (Roxburgh) H. J. Lam; *Bassia butyracea* Roxburgh; *Madhuca butyracea* (Roxburgh) Macbride; *Aesandra butyracea* (Roxburgh) Baehni), belonging to family Sapotaceae is a deciduous tree about 20 m high. It is distributed throughout Nepal mainly in the sub-Himalayan tracts on open hillsides of 300 to 1,500 meters, and also in northern India and Bhutan.^[2] It is also named as Indian butter tree and the main product of the tree is ghee or fat, extracted from the seeds and named as Chyuri ghee or Phulwara butter.

Various parts of Chyuri plant are used by different ethnic groups of Nepal for medicinal and other purposes. Seed fat is applied for headache, rheumatism, boils and pimples. It is also used as emollient for chapped hands and feet in winter. Juice of the bark is used for the treatment of indigestion, asthma, rheumatism and boils and also as anthelmintic. Juicy pulp of ripe fruit is eaten fresh. Juice of the corolla is boiled

into a syrupy liquid, which villagers use like syrupy sugar.^[2,3] Chyuri tree is socially, culturally and economically important to the *Chepangs*, an indigenous ethnic group of people living in dense forests of mid-Southern Chitwan district of Nepal, who call it *Yoshi* in their language. Fruit makes a dietary supplement for *Chepang* people. The fat extracted from seed is used for cooking and lighting lamps. Some people use it as hair oil and raw material for soap. Bark and oil cake are used as fish poison. Oil cake is also used as fertilizer to protect crops from harmful insects and worms.^[4]

Previous phytochemical studies have isolated butyraceol,^[5] MI-saponin A, 16 α -hydroxy MI-saponin A, butyrosides A, B,^[6] C, D,^[7] from the seeds of *D. butyracea*. Similarly, butyric acid and myricetin-3-*O*-rhamnoside were isolated from the leaves.^[8] α -Spinasterol, β -sitosterol glucoside, α -amyrin acetate, β -amyrin acetate and 3 β -palmitoxy-olea-12en-28-ol were isolated from bark and fruit pulp.^[9] Flavonoids, quercetin and dihydro-quercetin were isolated from the nut-shell^[10] and quercetin, quercetin-3-*O*-rhamnoside, myricetin and myricetin-3-*O*-rhamnoside from the flowers.^[11] Various studies have reported the triglyceride and fatty acid composition of Phulwara butter collected from India^[12-14] but no such studies are reported for the analysis of Chyuri ghee from Nepalese sources. Hence, present investigation was carried out to study the components in seed fat and distribution of Chyuri plant in Nepal.

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EXPERIMENTAL

Plant material and extraction of seed oil

Chyuri seeds used in this study were collected from a habitat of Nepalgunj, Nepal by the authors on the three times of expeditions in 2007 (Sample A), 2008 (Sample B) and 2009 (Sample C). The seed oil was extracted by the traditional method.^[4] The ripe Chyuri fruits were squeezed to remove their mesocarp. The seeds were then separated, cleaned, boiled, dried and then crushed to obtain seed flour. The flour was then steamed in a perforated bamboo basket and placed over a boiling metal pot for steaming. The steamed flour was then squeezed to extract the fat.

Sample preparation

Ten mg of each sample were subjected to methanolysis by refluxing with 1ml of freshly prepared 5% HCl-MeOH at 70 °C for 6 hours. The solvent was evaporated, and these samples were dissolved in hexane (1 ml) and subjected to the gas chromatography.

Seed fat analysis

The gas chromatographic analysis was performed on a Hewlett Packard Series II 5890 under the following conditions: Equity™-5 Fused silica capillary column (30 m × 0.25 mm × 0.25 μm); column temperature 150 °C (initial) to 250 °C at 5 °C/min; carrier gas He; flow rate 1.22 ml/min; volume injected 1 μl dissolved in hexane. Quantification of relative amount of each component was done according to the area percentage method. Gas chromatography-mass spectrometry (GC-MS) analysis was performed in a JEOL MS route (JMS 600W) mass spectrometer, operating at 70 v. Identification of individual components was based on the comparison of mass spectra with NIST/EPA/NIH Mass Spectral Library.

Distribution data

To study the distribution pattern of Chyuri, voucher specimens deposited in the National Herbarium and Plant Laboratories, Godawari, Kathmandu, Nepal were investigated. Reported locality, altitude and GPS data for the specimens studied are given in Table 2.

RESULTS AND DISCUSSION

Chyuri plant has medicinal and economical importance to various ethnic communities in Nepal. Chyuri seed fat is one of the main components used both as medicine for various ailments and commercial source for pharmaceutical, cosmetic and soap industries. The results of GC-MS analysis after methanolysis of seed fat showed the presence of palmitic acid methyl ester (1), linoleic acid methyl ester (2), oleic acid methyl ester (3) and steric acid methyl ester (4)

in all three samples A, B and C at almost similar composition (Table 1). Representative gas chromatogram of sample B is given in Figure 1. Palmitic acid methyl ester (1) was the main component in all three samples followed by oleic acid methyl ester (3). The relative composition of seed fat was found to be similar with the reported data for from previous studies collected from India.^[12-14] These studies suggested that the Chyuri ghee has high potential to be used in the manufacture of, cosmetics, soaps and in pharmaceutical and confectionery industry. The fatty acid composition is also important from the point that commercial ghee in Nepal is often adulterated with Chyuri seed oil.^[15]

Study of the voucher specimens deposited in the National Herbarium and Plant Laboratories, Godawari, Kathmandu, Nepal has revealed that it is widely distributed from eastern part to western part of Nepal at altitude from 150 m to 1620 m (Table 2.) The proper cultivation and utilization of Chyuri tree as a medicinal species and source of seed fat may improve the social and economic status of Nepalese people. Further bio-assay guided chemical analysis of the different plant parts of Chyuri may help in the development of evidence based medicines. Similarly, study on the altitude variation of components in seed oil would be interesting for the selection of proper biological source.

Table 1: Relative composition of Chyuri seed fat in samples A, B and C^a

Component	Sample A	Sample B	Sample C
palmitic acid methyl ester (1)	66.0%	58.5%	62.1%
linoleic acid methyl ester (2)	2.6%	3.4%	3.4%
oleic acid methyl ester (3)	29.0%	35.2%	30.6%
steric acid methyl ester (4)	2.4%	2.9%	3.9%

^apercentages were calculated from the peak area.

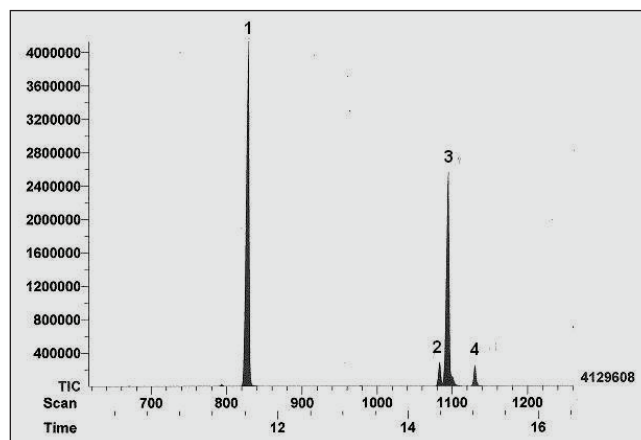


Figure 1: Gas chromatogram of Chyuri seed fat (Sample B).

Table 2: Distribution pattern of Chyuri tree in Nepal

S.N.	District	Locality	Altitude	Longitude (E)	Latitude (N)
1	Baglung	Raigha	850 m	83°02'	28°02'
2	Baitadi	Khateda - Patan	1200 m	80°03'	29°03'
3	Bajhang	Chin	1100 m	81°01'	29°04'
4	Banke	Khajura	180 m	83°04'	27°04'
5	Banke	Nepalgunj	150 m	81°37'	28°03'
6	Chitwan	Tungati	380 m	85°59'	27°42'
7	Chitwan	Lothar	150 m	84°45'	27°42'
8	Dadeldhura	Kalina - Gogan	1000 m	80°02'	29°02'
9	Dang	Ramche	1395 m	82°02'	28°01'
10	Dang	Dharampani	900 m	82°16'	28°00'
11	Dhankuta	Dharapani	1200 m	86°41'	27°18'
12	Jajarkot	Jhapra	1500 m	82°01'	28°05'
13	Kabhre	Amaltari	680 m	86°08'	27°38'
14	Kabhre	Mukpa	1460 m	83°40'	28°31'
15	Kaski	Pokhara	800 m	83°59'	28°13'
16	Makawanpur	Champ kharka	1450 m	85°01'	27°03'
17	Makwanpur	Kamle	1000 m	85°04'	27°01'
18	Myagdi	Myagdi	1080 m	83°31'	28°20'
19	Pyuthan	Bijuwar	780 m	82°01'	28°01'
20	Ramechhap	Hulak	1000 m	86°16'	27°44'
21	Rukum	Banphikot	1000 m	82°02'	28°04'
22	Salyan	Cherneta	1620 m	83°36'	28°19'
23	Sindhupalchok	Barabise	500 m	85°04'	27°05'
24	Sunsari	Barahchhetra	170 m	87°02'	26°05'
25	Surkhet	Babai	180 m	81°02'	28°03'
26	Surkhet	Kalyan	870 m	81°03'	28°04'
27	Udaypur	Trijuga	185 m	86°03'	26°05'

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