Int. J. Chem. Sci.: 6(4), 2008, 2000-2007

PHYTOCHEMICAL INVESTIGATION OF FERMENTED LEAVES OF *CASSIA OBTUSIFOLIA* LINN.

A. N. MISHRA* and M. H. ANSARI

Department of Chemistry, S.G.R.P.G. College, Dobhi, JAUNPUR – 222149 (U. P.) INDIA

ABSTRACT

The chemical composition of the methylene chloride extract of the fermented leaves of *Cassia obtusifolia* legume was analysed for the first time by GC and GC/MC. The major components are found to be aliphatic acids and identified as hexonic acid (26%), palmitic acid (10.4) and valeric acid (6.3%) with lesser amounts of p-ethyl phenol (17.2%) and p-methylphenol (14%). Examination of the protein fraction from leaves indicated 20.3% in the crude leaves and 12.9% in the fermented leaves. Moreover this traditional legume had a high content of potassium and calcium element.

Key words: *Cassia obtusifolia,* Fabaceae, Fermented leaves, Volatiles, Amino acids, Trace elements.

INTRODUCTION

Cassia obtusifolia Linn (Fabaceae) is commonaly known as 'chakwar' in Hindi. It is widely distributed along road side and other tropical and temperate regions of Asia, Africa and America.[1] Due to the presence of several anthraqinone glycosides, aliphatic acids and amino acids, its leaves, seeds and roots were used for antiplasmodic,[2] antinflammatory[3], laxative[4], purgative[5], psoriosis[6], anthelminatic[7] and in the treatment of half headache,[8]. The last reported study on the fermented leaves of *C. obtusifolia* which described the phytochemical study was done by Dirar et al.[9]. We now investigated the volatiles from the methylene chloride extract of the fermented leaves of *C. obtusifoila* and analysed the composition of amino acids and trace elements from the test plant of *Cassia obtusifolia.*

EXPERIMENTAL

Plant material

The fresh mature leaves of *Cassia obtusifolia* were collected from the rural area of

* Author for correspondence

Jaunpur district and identified by Taxonomist of Botany Department, S.G.R.P.G. College, Dobhi, Jaunpur (U.P.) and voucher specimen was deposited at herbarium and assigned that voucher specimen No. 124. The leaves are pounded into paste without releasing the juice. The paste is placed in an earthenware and covered with sorghum leaves. The whole jar is buried in the ground up to the neck. Every three days ,the contents are mixed by hand. The fermentation takes about fourteen days. After fourteen days, the strongly smelling black fermented paste was removed and sun dried.

Preparation of extract

Air dried fermented leaf samples (500 g) were soaked in dichloromethane for 50 h. The solvent was removed by filtration, and the fresh methylene chloride (CH_2Cl_2) was then added to the sample for another 50 h. The CH_2Cl_2 extracts were combined and evaporated under reduce pressure to give 2.9 g of a greenish viscous residue (yield 1.8%).

Mineral solution from the fermented leaves

Fermented leaves (10 g) oven dried at 110ºC and ground to fine powder were incinerated at 550ºC according to the processing previously described method[10]. The ash (1 g) was then dissolved in 1 liter of water and acidified with 15% H_2SO_4.

Extraction and isolation of fermented leaves (Analytical technique)

The volatiles were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Apparatus types and analytical conditions were as previously reported[11]. The percentage composition of the extract was computed from the GC peak areas without using correction factors. The volatile constituents were identified by their retention indices and by comparison of the mass spectra with those of authentic compounds or with those from literature[12].

Amino acid analyses

Air-dried and powdered leaves of *C. obtusifolia* were hydrolysed, under nitrogen in HCl at 120ºC for 24 h using a Pico-Tag work station (Waters). Along with 2-β-merceptoethanol (4%), to preserve sulphur-containing amino acids, 200 µL of 6 N HCl were placed in the hydrolysis tank. After hydrolysis, 10 n mole of glocosamic acid per mg of sample were added as an internal standard. The samples were dried under vacuum in a speedvac apparatus (Savant instrument Inc., Farmingdale) and taken up with 0.05 M lithium citrate buffer, pH 2.2. The samples were submitted to ion exchange chromatography on an automatic amino acid analyser (Beckman 3600). Amino acids were

detected by the ninhydrin reaction, identified by their retention time and wavelength ratio, and quantified by their absorption at 570 nm (440 nm for proline).

Mineral contents

The element sodium and potassium were determined with a 410 flame spectrophotometer using butane under the pressure of 2.1 kg/cm^2, the debit was 0.4 L/min. Calcium and magnesium were determined with an atomic absorption spectrophotometer. (Spectro Varian 20 BQ). S-2501. Colorimeter method was used for the determination of iron and phosphorus[13]. The chemical constituents of fermented leaves of *C. obtusifolia* such as different aliphatic acids and carbohydrates, amino acids, and trace elements are reported in Tables 1, 2 and 3, respectively.

Table 1. Constituents of fermented leaves of *Cassia obtusifolia* Linn.

R. I.	Chemical constituents	Amount (%)
725	Propionic acid	0.4
770	Isobutyric acid	3.1
849	Stigmasterol	0.1
855	Palmitic acid	10.4
860	3-Methyl butyric acid	5.5
865	2-Methyl butyric acid	2.5
952	Valeric acid	6.3
956	Cassiaside	0.4
970	Kaeimpferol	1.0
975	Trimethyl pyrazine	0.3
1030	d-Ononital	0.1
1048	Hexonic acid	26.00
1049	5-Hexonic acid	0.3
1066	p-Methyl phenol	14.00

Cont…

R. I.	Chemical constituents	Amount (%)
1069	Cis-Linalyl oxide	0.4
1071	Uridine	0.3
1075	Butyl isovalerate	1.9
1078	Isobutyl valerate	2.6
1105	Phenyl ethanol	1.3
1158	p-Ethylphenol	17.2
1171	(Z)-3- Hexenylisobutyrate	0.1
1238	2,3- Dihydrobenzo furane	0.1
1246	Phenyl ethyl acetate	0.4
1280	Isopentyl hexanoate	0.5
1283	2-Methyl butyl hexanoate	0.2
1338	Benzyl butanoate	0.2
1341	Phenyl ethyl propionate	0.1
1352	Quercitrin	0.2
1437	Phenyl –ethyl butyrate	0.9
1460	Mannotiose	0.7
1480	Epimelibiose	0.9
1490	Mannose	0.5
1634	Galactose	0.8
Total		99.97

Table 2. Amino acids composition of *Cassia obtusifolia* Linn.

Amino acid	Crude leaves (mg / 100 g weight)	Fermented leaves (mg / 100 g weight)	Requirement (mg /50 kg weight Adult / Day)	Percentage of the daily requirement
Threonine	980	522	350	14.91
Valine	1606	1282	500	25.64
Cystine	13	4	650 (Cyst + Met)	0.33 (Cys + Met)
Methionine	11	18	-	-
Isoleucine	1181	987	500	19.74
Leucine	2234	1803	700	25.75
Tyrosine	435	239	700 (Tyr + Phe)	18.01 (Tyr + Phe)
Phenylalanine	1562	1022	-	-
Lysine	1477	790	600	13.16
Histidine	664	356	500	7.12
Arginine	1314	601	-	-
Aspartic acid	3786	1345	-	-
Serine	863	505	-	-
Glutamic acid	-	-	-	-
Proline	1769	858	-	-
Glycine	1428	1074	-	-
Alanine	1595	1638	-	-
Total amount of Amino acids	20.30%	12.90%	945/0.2 Decimil	

Table 3. Elemental composition of the fermented leaves of *Cassia obtusifolia* Linn.

Element	Amount (mg/kg)
Na	1310
K	31500
Ca	27250
Mg	1490
P	2800
Fe	0.4
Total ash	18%

RESULTS AND DISCUSSION

The examination of our results given by the GC and GC-MS (which are summarized in Table 1) show that the extract of fermented leaves of *C. obtusifolia* was essentially made up of aliphatic acids (48.20%). The corresponding esters are 7%, while phenolics account for 33%. The large content of hexonic acid (26%) and palmitic acid (10.4%) could be identified as responsible for the goat-like and the cheese-like odour of the fermented leaves of *C. obtusifolia*. The amount of other aliphatic acids such as 2-methyl butyric acid (5.48%) and 3-methyl butyric acids (5.5%) seems to be noteworthy. The account for carbohydrate glycosides are 3.0%. In fact, it is well known that these compounds give a fruity odour. The occurance of p-methyl phenol (14%) and p-ethyl phenol (17.2%) suggests the attack of the bulk phenolic constituents of the raw materials by the yeast during semi-anaerobic fermentation.

The total protein concentration in the mature dried leaves of *C. obtusifolia* is 20.3% but falls to 12.9% after fermentation. The loss of proteins may result because of their use for the growth of the micro-orgrnisms during fermentation. It could be noted in Table 2 that the fermentation modifies the relative amount of amino acids also. In all amino acids, only the amounts of methionine and alanine increases. The participation of 10 g of fermented *C. obtussifolia* leaves to the daily requirements in essential amino acids of an adult is from 13 to 25% of the needed amounts. However, the amount of histidine is only 7% and the sulfur amino acid are negligible, but these constituents are available in the cereals which are consumed with fermented leaves of *C. obtusifolia*. Although

fermentation lowers the protein value, this process seems to be necessary to the palatability and the non-toxicity of these leaves.

The fermented leaves of *C. obtusifolia* contain the major elements needed by human body (Table-3). It appears that locations of Cassia species and their environments have effects on the chemical constituents.[13]

In conclusion, these results show that elemental and amino acid compositions of the fermented leaves of *C. obtusifolia* corroborated the nutritional value of this traditional legume (food) which is used by many poor peoples in South Africa as meat substitute. Their nutritional value is enhanced, when this legume is eaten with cereals. This strongly smelling legume is also used as flavour whose volatiles are reported here for the first time.

ACKNOWLEDGEMENT

The authors are thankful to Director, N.B.R.I. Lucknow, (U.P.) and Principal, S.G.R.P.G. College, Dobhi, Jaunpur (U.P.) for providing analytical techniques and necessary facilities to complete the work.

REFERENCES

1. K. M. Nadkarni, Indian Matteria Medica, Dhootapapeshwar Prakashan, Bombay, (1954) p. 291.
2. A. N. Mishra and M. H. Ansari, Int. J. Mendal, **23**, 47 (2006).
3. K. N. Mishra and A. N. Mishra, Int. J. Mendal, **22**, 81 (2005).
4. A. N. Mishra, M. H. Ansari and R. K. Pandey, J. P. A. S., **12,** 25 (2006).
5. A. N. Mishra and M. H. Ansari, Ind. J. Not. Prod., **3**, 13 (2007).
6. A. N. Mishra, M. H. Ansari and K. N. Mishra, J. P. A. S., **14**, 96 (2005).
7. A. N. Mishra and M. H. Ansari, Int. J. Plant Archives, **7**, 241 (2007).
8. R. Zafar, Medicinal Plant of India, C. B. S. Publishers and Distributors, New Delhi, (2005) p. 53.
9. H. A. Dirar, D. B. Harper and M. A. Collins, J. Sci. Food and Agric. **36**, 881 (1985).
10. Afnor Norme, Determination des cendres, methode per incineration a 550ºC, NF V **30** 760 (1981).
11. Y. Mahmout, M. Mbaiguinam and J. M. Bessiere, L. J. Soc. Ouest- Afr. Chim, **11.,** 137 (2001).

12. R. P. Adams, Identification of Essential Oils by Ion Trap Mass Spectroscopy, Academic Press, Inc. New York, (1989).

13. M. C. Buoso, D. Ceccato, R. Cherubini, G. Moschini, S. O. Olabanji and O. R. Ombuwajo, PIXE-PIGE, Elemental Evaluation on Medicinal Plants (2002).

Accepted : 03.07.2008