Proximate Composition, Carbohydrates and Lipids

The seeds of *Achyranthes bidentata* (AB) have been reported as excellent cereal grain substituents during periods of famine in both India and China. The chemical compositions of the seeds were determined and compared to those commonly found within the family Amaranthaceae, *i.e.* genus *Amaranthus* to those of more common cereal grains (Table 6). The study revealed that AB seeds were substantially higher in nutritional important minerals, such as P, K, Ca, Mg and Fe, as well as being substantially higher in total protein (1.6-2.4 times higher) than other grains. In addition to the higher edible oil content found in AB seeds, its lower saturated/unsaturated ratio make it potentially a superior source of nutritional oil. Although the study revealed that total vitamin E content in AB seeds was 1.6 times higher than that of *Amaranthus* seeds, only small quantities of squalene *i.e.* approximately 2.3 %

Table. 6 Proximate analysis of *Achyranthes bidentata* seed*

	Achyranthes bidentata	*Amaranthus* spp.	Cereals
Moisture (%)	9.0	11.3	11.6
Ash (%)	4.5	2.9	1.7
Protein (%)	22.0	14.5	11.0
Fat (%)	11.5	7.5	2.7
Carbohydrate (%) (by difference)	53.0	63.8	73.0

* (Marcone *et al*., 2003)

were found to be present in the oil (Marcone *et al*., 2003). Overall, the study revealed that AB seeds had high levels of nutritionally important components that may be of significant importance in the formulation of diets for humans and animal. Results of proximate analysis revealed that AB seeds was substantially higher in crude fat content than those previously reported for 21 *Amaranthus* accessions by Budin *et al*. (1996).

Glucose and fructose were identified from the roots of *Achyranthes fauriei* (Takemoto *et al.*, 1967b). The roots of *Achyranthes bidentata* contain a water-soluble oligosaccharide (composed of 6 glucose units and 3 mannose units) (Hui *et al*, 1989) and *n*-butyl-β-D-fructopyranoside (Wang *et al*., 2004). Also, a bioactive fructan was isolated from the roots of *Achyranthes bidentata* (Yu *et al*., 1995b). The fructan was shown to be a mixture of short-chain fructans with an average degree of polymerzation of 8, containing more (2→6) than (2→1) linked β-fructofuranosyl residues, with branching at *O*-6 or *O*-1 of the 18% of the D-fructofuranosyl residues. *n*-Butyl-β-D-fructopyranoside was also isolated from the roots (Chao *et al*., 1999b). A peptide-polysaccharide with immunological activity was isolated from *Achyranthes bidentata.* It is composed of D-glucuronic acid, D-galactose, D-galacturonic acid, L-arabinose and L-rhamnose in molar ratio of 12:2:3:1:1 (Fang *et al*., 1990).

The amino acid composition of *Achyranthses bidentata* seeds are: aspartic acid + asparagines, 8.2; threonine, 3.4; serine, 5.6; glutamic acid + glutamine, 19.4; glycine, 15.0; alanine 4; valine, 4.8; methionine, 1.7; proline, 6.3; isoleucine, 4.0; leucine, 7.1; tyrosine, 2.8; phenylalanine, 3.6; lysine, 3.7; histidine, 2.5; and arginine, 7.9 % mol (Marcone *et al*., 2003). Analysis of the amino acid content of total AB seed revealed that it was a richer source of the essential amino acid lysine which is usually the limiting amino acid found in cereal grains. AB seed was also found to contain substantial levels of the sulphur-containing amino acid methionine as well as arginine, an essential amino acid for infants. Further differences were also observed in the fatty acid profiles of AB as compared to the above-mentioned 21 *Amaranthus* species. AB was found to contain relatively less saturated fatty acid, i.e. palmitic (15.7%) and stearic (3.6%) but was higher in oleic (28.2%) and significantly higher in linoleic (51.5) fatty acids (Table 7). The amount of linolenic acid was very low (0.35%). These differences resulted in a lower saturated to unsaturated (S/U) ratio for AB than for other studied species and, therefore, would indicate that AB is potentially a better source of nutritional oil.. Further compositional analysis of the lipid material for the presence of important antioxidants indicates that AB was substantially rich in α-tocopherol. Comparison of the α-tocopherol content of AB with those found in the 21 *Amaranthus* accessions surveyed by Budin *et al*. (1996) revealed that AB contains significantly higher amounts, i.e. 1.6 times higher. Although the lower S/U ratio previously determined for AB oil would indicate a lower oxidation stability, its higher α-tocopherol content would help to stabilize the oil against oxidation during heating while serving as a much richer dietary source of vitamin.

Table: 7 Lipid composition of *Achyranthes bidentata* seed*

	Achyranthes bidentata	*Amaranthus* spp[a]
Fatty acid (%)		
Palmitic (16:0)	15.70	18.50
Palmitoleic (16:1)	0.60	NR[b]
Stearic (18:0)	3.60	3.20
Oleic (18:1)	28.20	22.00
Linoleic (18:2)	51.50	48.80
Linolenic (18:3)	0.35	0.20
S/U	0.22	0.33
Triacylglycerol (%)		
LnLnL	1.28	
LnLL	0.65	
LLL	15.90	
PLnL	0.92	
OLL	23.90	
PLL	17.00	
OOL + PoOO	12.70	
POL	15.10	
PPL	4.30	
OOO	2.97	
POP	0.85	
OOS	2.76	
SOS	1.40	
Fat soluble components		
α-Tocopherol (mg/g seed)	26.7	17.0
Mono and diglycerides	1.2%	
Squalene	2.3%	

L: Linoleic acid; Ln: Linolenic acid; O: oleic acid; P: palmitic acid; Po: palimitoleic acid; S: stearic acid.
[a] Budin *et al.* (1996); [b] NR, not reported. * Marcone *et al*. (2003).

The triacylglycerol composition (TAG) of AB showed that the majority of TAG are in tri and di-unsaturated form and no tri saturates were identified (Table 7). The major constituent was OLL (O = oleic, and L = linoleic acids) (23.9%) followed by PLL (P = palmitic acid) 17.0%, LLL (15.9%), POL (15.2%), OOL (12.7%) and PPL (4.3%) (Table 7). The amounts of disaturates (POP and SOS; S = stearic acid) were 0.83% and 1.37%, respectively, indicating that, despite the high (19.3% saturated fatty acids) TAG composition, only 2.2% of TAG are in the disaturated from. This characteristic provides a superior property, especially when the AB is oil used in products which require a low crystallization range. The amount of squalene was tentatively determined as 2.3%.

Ecdysterones and Sterols

Ecdysterone and inokosterone, which possess insect hormone activity have been identified from *Achyranthes* species specially the roots (radix) (Takemoto *et al*., 1967a,e; Kobayashi *et al*., 1967; Ogawa *et al*., 1974, 1977). The arthropod molting activity of some of these ecdysteroids have been reported (Hikino and Takemoto, 1972). Li *et al*. (2007a) studied the accumulation dynamic of ecdysterone in vegetative organs of *Achyranthes bidentata*. They found that ecdysterone could be detected in root, stem, and leaf, and the content had significant difference in different organs. The content of ecdysterone was higher in young organs, and it significantly changed at various growth periods. At the harvesting time (November), the content of ecdysterone in different vegetative organs decreased in the order of leaf > root > stem. Table 8 summarises the ecdysteroids isolated from some *Achyranthes* species. Takemoto *et al*. (1968a) reported that rubrosterone, isolated from *Achyranthes rubrofusca* is probably synthesized from the insect-molting steroids ecdysterone and inokosterone. The biosynthesis of ecdysterone and inokosterone from cholesterol and mevalonic acid in *Achyranthes fauriei* have been studied by Hikino *et al.* (1975). β-Sitosterol, daucosterol, α-spinasterol and α-spinasterol-3-*O*-β-D-glucoside were isolated from the roots of *Achyranthes bidentata* Bl. (Wei *et al*., 1997). β-Sitosterol-β-D-glucoside, stigmasteryl β-D-glucoside, stigmasterol and β-sitosterol were identified from *Achyranthes faureiei* (Takemoto *et al*., 1967b). The desmethylsterol composition of *Achyranthes bidentata* is shown in Table 2 (Patterson *et al*. 1991).

Saponins

Triterpenoid saponins have been isolated from *Achyranthes* species. The following saponins have been identified from *Achyranthes bidentata*: two kinds of achybidensaponin: 3-*O* [α-L-rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyl]-oleanolic acid-28-*O*-(β-D-glucopyranosyl) and 3-*O*-(β-D-glucuronopyranosyl)-oleanolic acid-28-*O*-(β-D-glucopyranosyl) (Wang and Zhu, 1996), bidentatoside I; an unusual triterpene saponin bearing an unusual dioxopropionic acid unit, bidentatoside II (**35**), chikusetsusaponin V, chikusetsusaponin V methyl ester (Marouf *et al*., 2001; Mitaine-Offer *et al*., 2001a,b), oleanolic acid α-L-rhamnopyranosyl-β-D-galactopyranoside (Nikolov *et al*., 1991), and other oleanolic acid saponins (Gedeon and Kincl, 1956; Li, 1988; Nguyen *et al*, 1995; Marouf *et al*., 2001). Two oleanolic acid saponins having a sialyl Lewis mimetic structures were isolated as methyl esters from the roots of *Achyranthes fauriei*. The two saponins are characterized as oleanolic acid glucuronides having unique substituents composed of $C_6H_9O_5$ and $C_9H_{15}O_9$ respectively (Ida *et al*., 1998). Also, the roots of *Achyranthes fauriei* contain the following saponins: achyranthosides A-H (e.g **36**-**40**) (Ida *et al*., 1994b,1995; Ando *et al*., 2008), achyranthoside C dimethyl ester, achyranthoside C butyl dimethyl ester, achyranthoside E dimethyl ester and achyranthoside E butyl methyl ester (Li *et al*., 2005). The roots also contain the following oleanolic acid glucuronides (chikusetsusaponins): IVa, V, 28-deglucosyl chikusetsusaponin V, pseudoginsenoside RT_1, and oleanolic acid 3-*O*-β-D-glucururonopyranoside (Ida *et al*., 1994a; Ando *et al*., 2008), and other oleanolic acid glycosides (e.g.18-(β-D-glucopyranosyl)-28-oxoolean-12-en-3β-yl-3-*O*-(β-D-glucopyranosyl)-β-D glucopyranosiduronic acid methyl ester, **41**) (Li *et al*., 2005). Oleanolic acid, oleanolic acid glucuronide and ursolic acid were identified from the saponin fraction of *Achyranthes longifolia* (Wu and Zhang, 1982).

Other Constituents

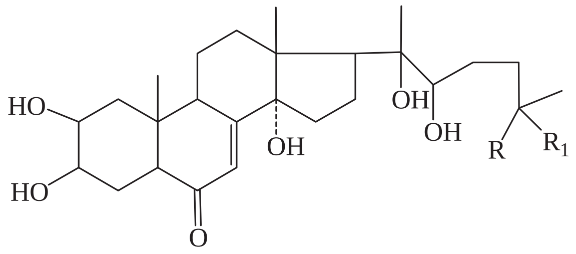
The alkaloid content (as achyranthin mg/100 g dry weight) of *Achyranthes bidentata* was observed at flowering (0.971) (Ratra, 1979). Khurshid Alam *et al*. (2003) reported the isolation of *N*-*trans*-feruloyl-4-methyldopamine from *Achyranthes ferruginea.*

The following compounds have been isolated from *Achyranthes bidentata* Blume: betaine

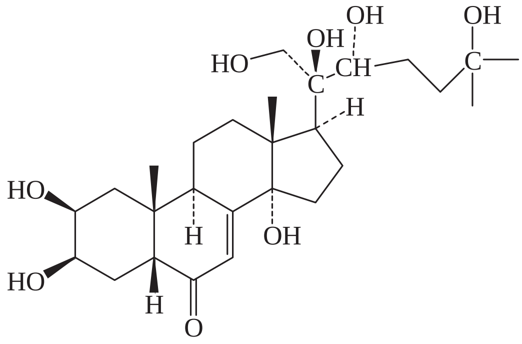
Table 8. Steroids and/or ecdysterones of some *Achyranthes* species

Species	Plant part	Ecdysterones	References
1. *Achyranthes bidentata*		Ecdysterone (**30**), stachysterone, podecdysone C, β-ecdysterone, 25-(*R*)-inokosterone (**31**), 25-(*S*)-inokosterone, polypodine B and 20-hydroxyecdysone	Wei *et al*. (1997); Meng *et al*. (2002); Wang *et al.* (2004); Zhao *et al*. (2007)
	R	Achyranthestrone (**32**), echdystrone, β-ecdysone, inokosterone, stachysterone D, polypodine B, (20*R*,22*R*)-2β,3β,20β,22α,25-pentahydroxycholesta-8,14-dien-6-one (**33**) and 2β,3β,14α, 20β,22α,25-hexahydroxycholest-7-en-6-one	Takemoto *et al*. (1968b); Ogawa *et al*. (1971); Meng *et al*. (2005), Lin *et al*. (2006); Li *et al*. (2007e)
2. *Achyranthes faurei*	R	Ecdysterone inokosterone, epiecdysterone, β-ecdysterone and polipodine B	Takemoto *et al*. (1967c, 1968b); Ogawa *et al.* (1971), Ando *et al.* (2008)
3. *Achyranthes japonica*	Wp	Ecdysterone and inokosterone	Takemoto *et al.* (1968b)
4. *Achyranthes japonica* var. *kachijoensis*	R	Ecdysterone and inokosterone	Ogawa *et al.* (1971)
5. *Achyranthes longifolia*	R	Ecdysterone and inokosterone	Takemoto *et al.* (1967d); Ogawa *et al*. (1971); Wu and Zhang (1982)
6. *Achyranthes mollicula*	R	Ecdysterone and inokosterone	Ogawa *et al.* (1971)
7. *Achyranthes obtusifolia*	R	Ecdysterone	Takemoto *et al*. (1967d,f); Ogawa *et al.* (1971)
8. *Achyranthes ogatai*	R	Ecdysterone	Ogawa *et al.* (1971)
9. *Achyranthes rubrofosca*	R	Ecdysterone, inokosterone and rubrosterone (**34**)	Takemoto *et al.* (1967d; 1968a,b); Ogawa *et al.* (1971)

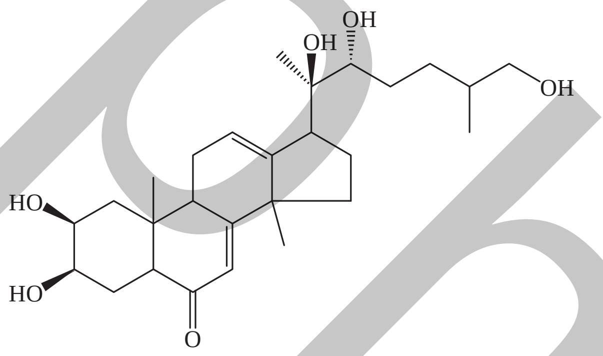
R: roots, Wp: whole plant



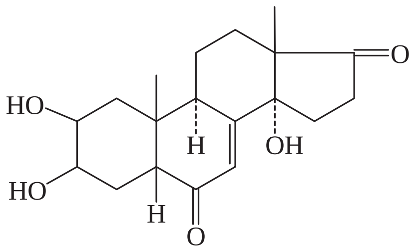
30 Ecdysterone R= Me, R_1 =OH
31 Inokosterone R=CH_2OH, R_1=H



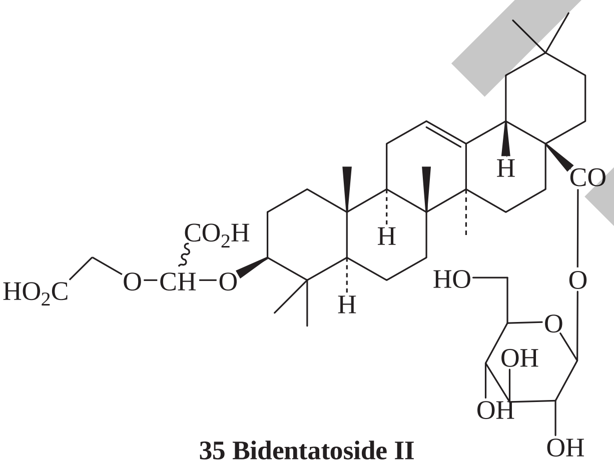
32 Achyranthesterone A



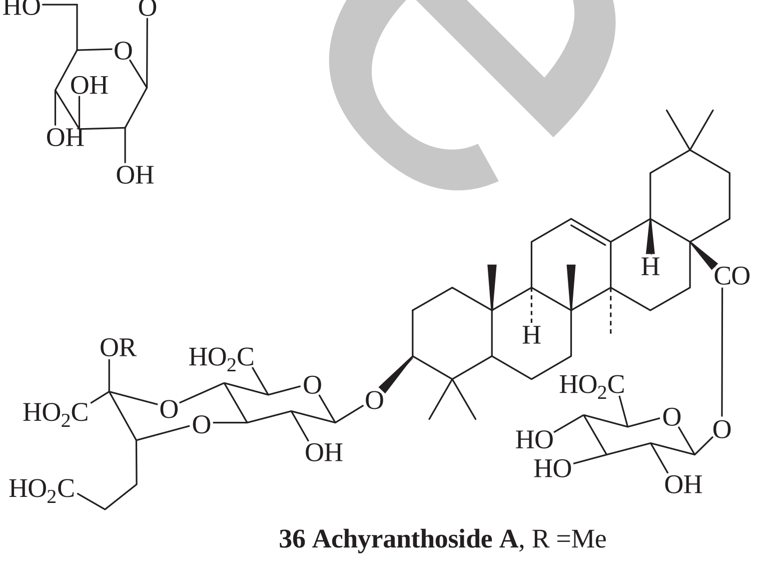
33



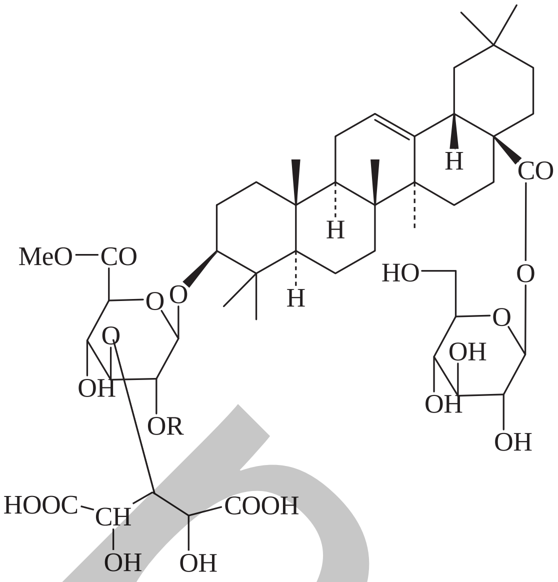
34 Rubrosterone



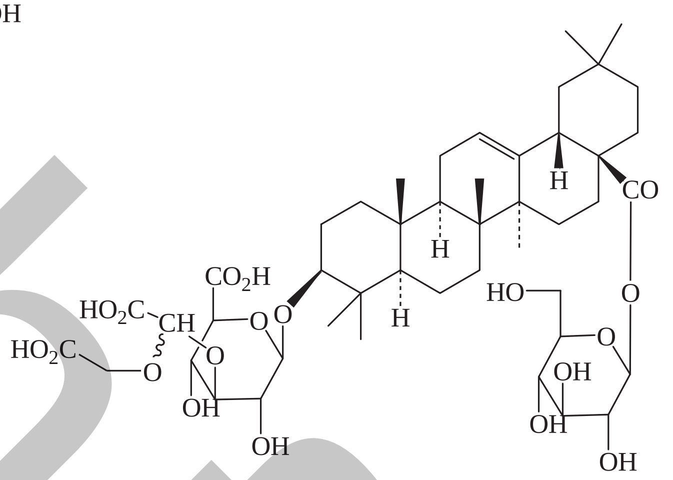
35 Bidentatoside II



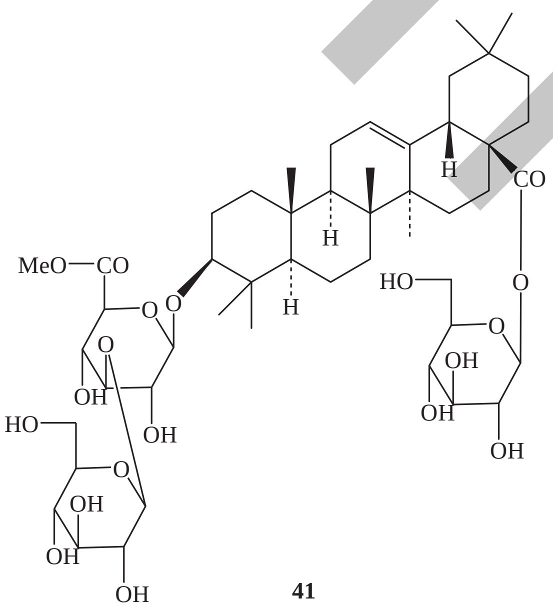
36 Achyranthoside A, R =Me
37 Achyranthoside B, R= H



38 Achyranthoside C R=H
39 Achyrantheside D R=β-O-glucopyranosyl



40 Achyranthoside E



41

(Takemoto *et al*., 1967b; Chao *et al*., 1995), γ-aminobutyric acid, organic acids (citric, malic, oxalic and succinic acids) (Takemoto *et al*., 1967b), anthraquinones (chrysophanol, emodin and physicon) (Bishit *et al*., 1993), rutin, isoquercetin, astragalin, caffeic acid (Nguyen *et al*., 1995; Nicolov *et al*., 1996), dibutyl phthalate (Wei *et al*., 1997), 6-hydroxymethyl-furfuraldehyde (Wei *et al*., 1997; Meng *et al*., 2002) and allantoin (Wang *et al*., 2004).

Folk Medicine, Pharmacological and Biological Activities

Achyranthes bidentata has wide application in traditional and folk medicine in several countries. It is used as expectorant, anti-inflammatory, antipyretic, antirheumatic and diuretic