Malaysian Journal of Science 29 (3): 280-283 (2010)

Alkaloids From Roots of *Alseodaphne Corneri* Kosterm

[1]Mohd Azlan Nafiah, [2]Mat Ropi Mukhtar, [3]Hiroshi Morita, [1]Kartini Ahmad, [2]Khalijah Awang, [2]A. Hamid A. Hadi

[1]Department of Chemistry, Faculty of Science and Technology, University Pendidikan Sultan Idris, 35900, Tg. Malim, Perak.
[2]Department of Chemistry, Faculty of Science, University Malaya, 50603 Kuala Lumpur.
[3]Faculty of Pharmaceutical Sciences, Hoshi University, Shinagawa-ku, Tokyo 142-8501, Japan
Received in 15th September 2010, accepted in revised form 25th November 2010.

ABSTRACT This is the first report on the occurrence of bisbenzylisoquinoline alkaloids in *Alseodaphne corneri* which belongs to the family of Lauraceae. Chemical studies on the roots of this species have yielded four bisbenzylisoquinoline alkaloids; (-)-gyrolidine **1**, norstephasubine **2**, (+)-2-norlimacusine **3** and (+)-stephasubine **4**. The isolation was achieved by chromatographic techniques and the structural elucidation was performed via spectral methods; namely 1D and 2D NMR, IR, UV and MS, and in comparison with published literature.

(**Keywords:** bisbenzylisoquinoline, *alseodaphne corneri*, spectroscopy)

INTRODUCTION

Alseodaphne corneri Kosterm (KL 4928) of Lauraceae, grows as a wild plant, 6-8 m high. The plant is also known as Medang and the genus includes more than 50 species, distributed through the Yunnan to West Malaysia and 23 species are found in Malaysia [1]. This paper reports the isolation and identification of four bisbenzylisoquinoline from root extract of the plant species. Structural elucidation was performed with the aid of spectroscopic methods; $^{1}H/^{13}C$-NMR, IR, UV, MS.

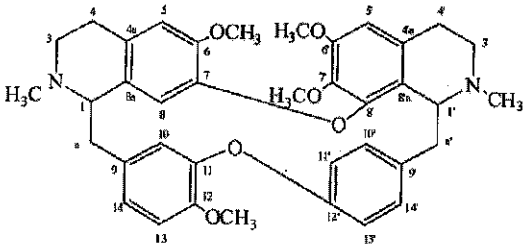
MATHERIAL AND METHODS

Plant material

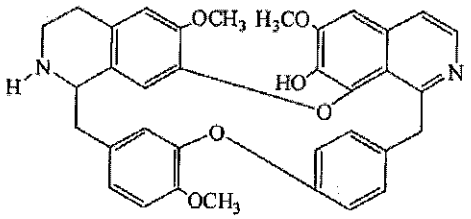
The roots of *Alseodaphne corneri* were obtained from University Malaya Herbarium. 3 Kg of the air-dried roots of *Alseodaphne corneri* were moistened with 25% NH_4OH and soaked in CH_2Cl_2 for 3 days (cold extraction). The CH_2Cl_2 extract was evaporated to 500ml followed by extraction using 5% HCl until Mayer's test is negative. The HCl extract was basified with concentrated ammonia to pH 11 and re-extracted
with CH_2Cl_2. The CH_2Cl_2 was washed with distilled H_2O and dried over anhydrous sodium sulphate. Finally, the extract was evaporated to dryness to give crude alkaloid (6.8g). The extract was subjected to the gradient elution column chromatography, using mixtures of hexane, hexane/CH_2Cl_2, CH_2Cl_2 and CH_2Cl_2/MeOH as eluants. A total of 90 fractions were obtained and fraction 29-30 were further purified using the preparative TLC (Silica gel 60 F_{254}, CH_2Cl_2:MeOH; 98:2, 97:3, 96:4) afforded (-)-gyrolidine **1**, norstephasubine **2**, (+)-2-norlimacusine **3** and (+)-stephasubine **4**, respectively.

(-)-Gyrolidine (**1**): UV λ_{max} (MeOH) nm: 261 and 282; IR υ_{max} cm^{-1}: 3401 (OH), 1507 and 1228. Mass spectrum m/e (%): 622; $[\alpha]_D^{26}$ -115° (*c* 1.1, MeOH); ^{1}H NMR ($CDCl_3$) ppm: 3.88 (3H, *s*, 12-OCH_3), 3.78 (3H, *s*, 6′-OCH_3), 3.62 (3H, *s*, 6-OCH_3), 3.18 (3H, *s*, 7′-OCH_3), 2.66 (3H, *s*, *N*′-CH_3), 2.57 (3H, *s*, *N*-CH_3), 7.42 (1H, *dd*, *J*= 8.2 and 1.4 Hz, H-14′), 6.95 (1H, *d*, *J*= 6.95 Hz, H-13′), 6.93 (1H, *d*, *J*= 2.3 Hz, H-10′), 6.80 (1H, *d*, *J*= 9.0 Hz, H-14), 6.76 (1H, *d*, *J*= 8.2 Hz, H-13), 6.63 (1H, *s*, H-8), 6.37 (1H, *d*, *J*= 5.4 Hz, H-11′), 6.35 (1H, *s*, H-5′), 6.31 (1H, *s*, H-5), 5.44 (1H, *d*, *J*= 1.8 Hz, H-10), 4.21 (1H, *d*, *J*= 5.4 Hz, H-1′), 3.64 (1H, *m*, H-1), 2.42 (1H, *m*, H_{ax}-3), 2.76 (1H, *m*, H_{eq}-3), 2.34 (2H, *m*, H_{ax}-4 and H_{eq}-4), 2.86 (1H, *dd*, *J*= 14.6 and 3.6 Hz, H_{ax}-α), 3.15 (1H, *m*, H_{eq}-α), 4.21 (1H, *d*, *J*= 5.4 Hz, H-1′), 2.93 (1H, *m*, H_{ax}-3′), 3.21 (1H, *m*, H_{eq}-3′), 2.70 (1H, *m*, H_{ax}-4′), 3.03 (1H, *m*, H_{eq}-4′), 2.80 (1H, *dd*, *J*= 14.6 and 5.9 Hz, H_{ax}-α′), 3.35 (1H, *d*, *J*= 17.8 Hz, H_{eq}-α′).

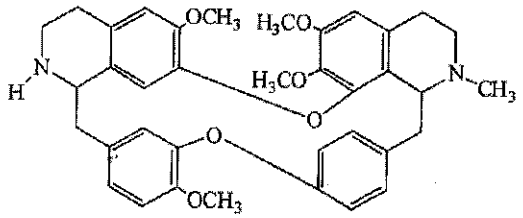
Norstephasubine (**2**): UV λ_{max} (MeOH) nm: 240, 286 and 338; IR υ_{max} cm^{-1}: 2950 (*N*-H). Mass spectrum m/e (%): 576; $[\alpha]_D^{26}$ +309° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$) ppm: 3.87 (3H, *s*, 12-OCH_3), 4.04 (3H, *s*, 6′-OCH_3), 4.05 (3H, *s*, 6-



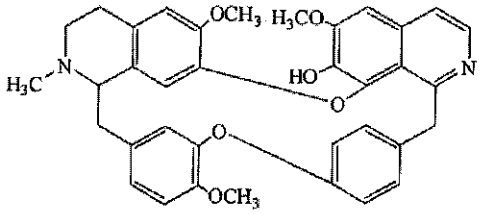
1



2



3



4

OCH_3), 8.42 (1H, *d*, *J*= 8.4 Hz, H-3′), 7.46 (1H, *d*, *J*= 5.6 Hz, H-4′), 7.35 (1H, *d*, *J*= 7.0 Hz, H-14′), 7.04 (1H, *d*, *J*= 8.0 Hz, H-10′), 6.97 (1H, *s*, H-5′), 6.71 (2H, *s*, H-14 and H-13), 6.64 (1H, *dd*, *J*= 1.9 and 8.2 Hz, H-11′), 6.55 (1H, *s*, H-5), 6.43 (1H, *dd*, *J*= 1.9 and 8.2 Hz, H-13′), 6.04 (1H, *s*, H-8), 4.89 (1H, *d*, *J*= 1.7 Hz, H-10), 5.37 (1H, *d*, *J*= 13.9 Hz, H-α′), 4.51 (1H, *d*, *J*= 13.6, H-α′), 4.08 (1H, *d*, *J*= 3.9 Hz, H-1), 2.55 (1H, *m*, H_{ax}-3), 2.92 (1H, *d*, *J*= 11.2 Hz, H_{eq}-3), 2.20 (2H, *m*, H_{ax}-4), 2.38 (1H, *d*, *J*= 15.8 Hz, H_{eq}-4), 2.70 (1H, *m*, H_{ax}-α), 2.73 (1H, *m*, H_{eq}-α).

(+)-2-Norobaberine (**3**): UV λ_{max} (MeOH) nm: 240, 286 and 338; IR υ_{max} cm^{-1}: 3401. Mass spectrum m/e (%): 576; $[\alpha]_D^{26}$ -170° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$) ppm: 7.46 (1H, *d*, *J*= 8.0 Hz, H-14′), 6.96 (1H, *d*, *J*= 8.0 Hz, H-13′), 6.95 (1H, *d*, *J*= 8.0 Hz, H-11′), 6.90 (1H, *d*, *J*= 8.0 Hz, H-10′), 6.84 (1H, *d*, *J*= 8.0 Hz, H-13), 6.79 (1H, *d*, *J*= 8.0 Hz, H-14), 6.67 (1H, *s*, H-8), 6.34 (1H, *s*, H-5′), 6.33 (1H, *s*, H-5), 5.57 (1H, *br s*, H-10), 4.24 (1H, *br s*, H-1′), 4.21 (1H, *br s*, H-1), 3.89 (3H, *s*, 12-OCH_3), 3.76 (3H, *s*, 6′-OCH_3), 3.60 (3H, *s*, 6-OCH_3), 3.18 (3H, *s*, 7′-OCH_3), 2.66 (3H, *s*, *N*′-CH_3).

(+)-Stephasubine (**4**): UV λ_{max} (MeOH) nm: 240, 287 and 337; IR υ_{max} cm^{-1}: 3400, 1460. Mass spectrum m/e (%): 590; $[\alpha]_D^{26}$ +339° (*c* 0.09, MeOH); ^{1}H NMR ($CDCl_3$) ppm: 4.04 (3H, *s*, 6′-OCH_3), 4.01 (3H, *s*, 6-OCH_3), 3.83 (3H, *s*, 12-OCH_3), 2.47 (3H, *s*, *N*-CH_3), 8.40 (1H, *d*, *J*= 5.6 Hz, H-3′), 7.44 (1H, *d*, *J*= 5.3 Hz, H-4′), 7.38 (1H, *d*, *J*= 7.0 Hz, H-14′), 6.95 (1H, *s*, H-5′), 6.94 (1H, *d*, *J*= 4.2 Hz, H-10′), 6.67 (1H, *s*, H-13), 6.60 (1H, *d*, J= 8.5 Hz, H-11′), 6.51 (1H, *s*, H-5), 6.45 (1H, *d*, *J*= 8.0 Hz, H-13′), 5.95 (1H, *s*, H-8), 4.74 (1H, *s*, H-10), 5.33 (1H, *d*, *J*= 13.9 Hz, H_{eq}-α′), 4.47 (1H, *d*, *J*= 14.1 Hz, H_{ax}-α′), 3.64 (1H, *br s*, H-1), 2.44-2.53 (2H, *m*, H-3), 2.98 (1H, *dd*, *J*= 14.1 and 3.6 Hz, H_{eq}- α), 2.23 (1H, *m*, H_{ax}-α), 2.42 (1H, *m*, H_{eq}-4), 2.18 (1H, *m*, Hax-4).

RESULTS AND DISCUSSION

Compound (**1**) was isolated as yellow amorphous from CH_2Cl_2. the UV spectrum showed bands at λ_{max} (MeOH) 261 and 282 nm which showed typical of bisbenzylisoquinoline moiety [2, 3]. The IR spectrum revealed the presence of OH group. Peaks at 1507 cm^{-1} indicated the presence of conjugated doubled bond, while peaks at 1228 cm^{-1} corresponded to C-O group [4]. Mass spectrum of the compound gave molecular at m/e 622 that corresponded to molecular formular $C_{38}H_{42}N_2O_6$.

The ^{1}H NMR spectrum displayed mutually coupled signals at δ 7.44 (*J*= 5.4 Hz) and δ 8.40 (*J*= 5.6 Hz) due to the presence of a substituted pyridine system. Conspicuously present were two doublets at δ 4.47 and δ 5.33, with a large coupling constant at 14.1 Hz and 13.9 Hz, respectively, which represented the two geminal protons of the benzylic methylene adjacent to the pyridine ring. The presence of H-1 broad singlet upfield at δ 3.64, accompanied by an *N*-methyl signal at δ2.47, argued convincingly in favor of placing the pyridine system on the right hand-side of the dimmer [5, 6]. The peaks for three methoxyls singlet appeared at δ 4.00, 4.01 and 3.83 corresponding to C-6′, C-6 and C-12 respectively. The resonances for H-10′, H-11′, H-13′ and H-14′ appeared as a doublet at δ 6.94 (*J* =8.0 Hz), 6.60 (*J* =

8.5 Hz), 6.45 (J = 8.0 Hz) and 7.38 (J= 8.0 Hz). Six singlet aromatic protons (each 1H) observed at δ 4.74, 5.95, 6.51, 6.67, 6.67 and 6.94 were attributable to H-10, H-8, H-5, H-13, H-14 and H-5′, respectively. Based on the above data, compound (**1**) was elucidated as (-)-gyrolidine [7] which were from VI type.

The second alkaloid, compound (**2**) was isolated as pale yellow amorphous solid. Its UV spectrum showed absorption bands at λ_{max} (MeOH) 240, 286 and 338 nm typical of the conjugated quinonoid moiety [8]. In addition, the IR spectrum gave broad band at 2950 cm^{-1} due to presence of C-H group.

The ^{1}H NMR spectrum exhibited a relative symmetry of the chemical shift pattern of the aromatic protons resonated as doublet signals at δ 8.42 and 7.46 corresponding to H-3′ and H-4′, respectively, with coupling constant J = 5.6 Hz which is an *ortho* disubstituted aromatic ring. The former peak was assigned in the downfield region due to the fact that it was attached to the carbon adjacent to nitrogen atom and as a result it was more deshielded compared to the latter. Another set of doublet signals presence at δ 7.35 (J= 7.0 Hz) and 7.04 (J= 8.0 Hz) corresponding to two aromatic protons, H-14′ and H-10′. H-13′ and H-11′ were resonated as two doublet doublets signals at δ 6.43 (J= 2.4 and 8.2 Hz) and 6.64 (J= 1.9 and 8.2 Hz), respectively. A doublet which consists of two protons resonated at δ 6.72 with coupling constant 8.3 Hz referred to H-14 and H-13. In addition, a doublet signal with small coupling constant 1.7 Hz at high field region δ 4.89 were referred to H-10. The ^{1}H- NMR also showed three singlet signals at δ 6.97, 6.55 and 6.04 corresponding to H-5′, H-5 and H-8, respectively. In addition, another three singlet signals at δ 4.05, 4.04 and 3.87 were referred to three methoxyl groups at C-6, C-6′ and C-12, respectively. On the basis of the spectroscopic studies and data obtained from the literature reviews alkaloid (**2**) (VI type) was assigned as a norstephasubine [9].

The third alkaloid, compound (**3**) was isolated as yellow amorphous from CH_2Cl_2. The UV spectrum showed absorption bands at λ_{max} (MeOH) 295 nm. The IR spectrum showed absorption at 3401cm^{-1} indicating the presence of *N*-H group. The mass spectrum exhibited a molecular ion peak at m/e 608 suggesting a molecular formula of $C_{37}H_{40}N_2O_6$. Other significant fragmentation peaks were revealed at m/e 577 $[M-31]^+$ indicating the loss of methoxyl group.

The ^{1}H-NMR spectrum showed four methoxyl singlet signals at δ 3.18, 3.60, 3.76 and 3.89 attached to C-7′, C-6, C-6′ and C-12, respectively. The singlet proton signal at δ 2.66 indicated presence of the methyl group at B′ ring attached to nitrogen atom. Six doublet aromatic protons were observed at δ 7.46, 6.96, 6.95, 6.90, 6.84 and 6.79 with coupling constant 8.0 Hz which attributable to H-14′, H-13′, H-11′, H-10′, H-13 and H-14, respectively. Another 4 aromatic protons were observed as a singlet at δ 6.67 (H-8), 6.34 (H-5′), 6.33 (H-5) and 5.57 (H-10). In addition two broad singlet signals which attributable to H-1 and H-1′ were observed at δ 4.21 and 4.24, respectively. Comparison of the empirical data with the literature values of the known compound [8, 10] was deduced as (+)-2-norobaberine (VI type).

The last alkaloid, compound (**4**) was isolated as yellow amorphous. Its tend to darken when exposed to air or light which showed unstable condition of the compound. Alkaloid (**4**) showed a peak at 3400 cm^{-1} typical of the stretching of a hydroxyl group [4]. The other significant peaks were also observed at 1460 cm^{-1} which showed the presence of the imine chromophore of a dihydroisoquinoline moiety [5]. The UV spectrum display maxima absorption at 337 nm confirmed the presence of bisbenzylisoquinoline moiety [2]. The ^{1}H-NMR spectrum displayed mutually coupled signals at δ 7.44 (J= 5.4 Hz) and δ 8.40 (J= 5.6 Hz) due to the presence of a substituted pyridine system. Conspicuously present were two doublets at δ 4.47 and δ 5.33, with a large coupling constant at 14.1 Hz and 13.9 Hz, respectively, which represented the two geminal protons of the benzylic methylene adjacent to the pyridine ring. The presence of H-1 broad singlet upfield at δ 3.64, accompanied by an *N*-methyl signal at δ2.47, argued convincingly in favor of placing the pyridine system on the right hand-side of the dimmer [5, 6]. The peaks for three methoxyls singlet appeared at δ 4.00, 4.01 and 3.83 corresponding to C-6′, C-6 and C-12 respectively. The resonances for H-10′, H-11′, H-13′ and H-14′ appeared as a doublet at δ 6.94 (J= 8.0 Hz), 6.60 (J = 8.5 Hz), 6.45 (J = 8.0 Hz) and 7.38 (J= 8.0 Hz). Six singlet aromatic protons (each 1H) observed at δ 4.74, 5.95, 6.51, 6.67, 6.67 and 6.94 were attributable to H-10, H-8, H-5, H-13, H-14 and H-5′, respectively. As a result, the author deduced that compound (**4**) is indeed (+)-stephasubine which isolated from species of *Stephania suberosa* Forman [11].

ACKNOWLEDGEMENT

The study was supported by University Malaya Research Grant (PS147/2007B). The author also wishes to thank to Teo Leong Eng, Pak Din, Hazry and Rafly for their excellent technical assistance.

REFERENCES

1. Ng, F.S.P., *Tree Flora of Malaya, A Manual for Foresters*. Vol. 4. 1989: Longman Malaysia Sdn. Bhd. 109-117.
2. Sangster, A.W. and K.L. Stuart, (1965). *Chemical Reviews*, **65**: p. 69-130.
3. Kanyinda, B., R. Vanhaelen-Fastre, M. Vanhaelen and R. Ottinger, (1997). Two New Isochondodendrine-Type Alkaloids from the Roots of *Anisocycla jollyana*., *Journal of Natural Products*, **60**: p. 1121-1124.
4. Williams, D.H. and I. Fleming, *Spectroscopic Methods in Organic Chemistry*. 4th Ed ed. 1989: McGraw-Hill Book Company.
5. Amarendra, P., K.M. Tarun, K.M. Prabir and C.R. Brindaban, (1988). (+)-3',4'-dihydrostephasubine, a bisbenzylisoquinoline alkaloid from *Stephania hernandifolia*, *Phytochemistry*, **27(2)**: p. 653-655.
6. Guinaudeau, H., M. Lebeouf and A. Cave, (1975). *Lloydia*, **38**: p. 294.
7. Chalandre, M.C., J. Bruneton, P. Cabalion and H. Guinaudeau, (1986). Alcaloides de *Gyrocarpus americanus Journal of Natural Products*, **49(1)**: p. 101-105.
8. Damas, P., J. Bruneton, A. Fournet and H. Guinaudeau, (1985). (+)-3',4'-dihydrostephasubine, a bisbenzylisoquinoline alkaloid from *Stephania hernandifolia*, *Journal of Natural Products*, **48(1)**: p. 69-71.
9. Patra, A., Freyer, A. J., Guinaudeau, H, Shamma, M., Tantisewie, B. , Pharadai, K., (1986). The Bisbenzylisoquinoline Alkaloids is *Stephania suberosa.*, *Journal of Natural Products* **49**: p. 424.
10. Bamrung, T., A. Susan, G. Helene and S. Maurice, (1989). New Bisbenzylisoquinoline from *Stephania pierrii*, *Journal of Natural Products*, **52(4)**: p. 846-851.
11. Patra, A., (1987). Promorphinane and hasubanane alkaloids of *stephania suberosa*, *Phytochemistry* **26**: p. 2391.

ERRATUM [Vol 29 (1)]

Inadvertently the following figures and tables were missed in the final printing for paper by Kumar D. *et al.* (page 52 to 61).

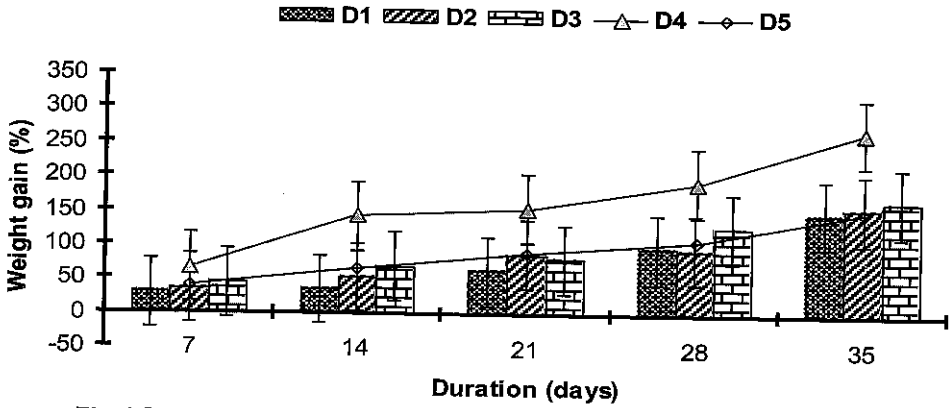


Fig 1.Percentage weight gain of *Channa striatus* fry fed on different protein level diets

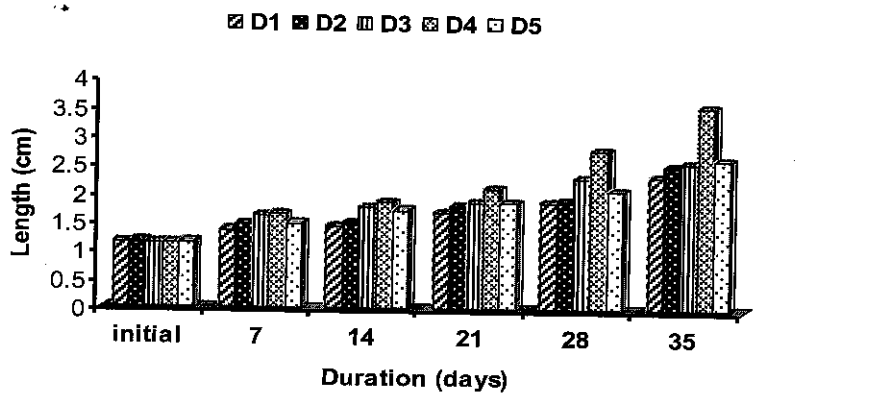


Fig 2. Length increment of *Channa striatus* fry fed on different levels of proteins

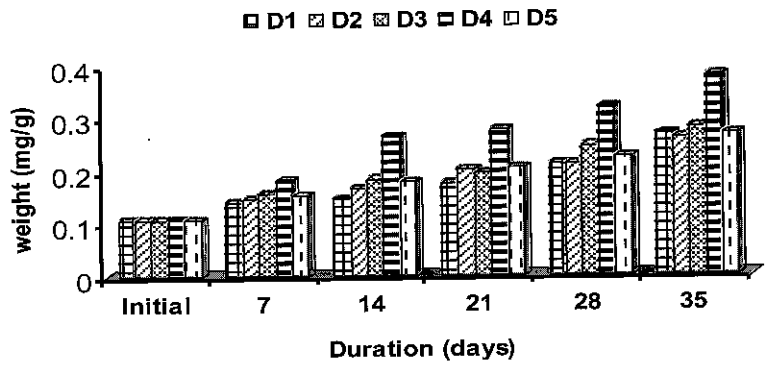


Fig 3. Weight increment of *Channa striatus* fry fed on different dietary protein levels

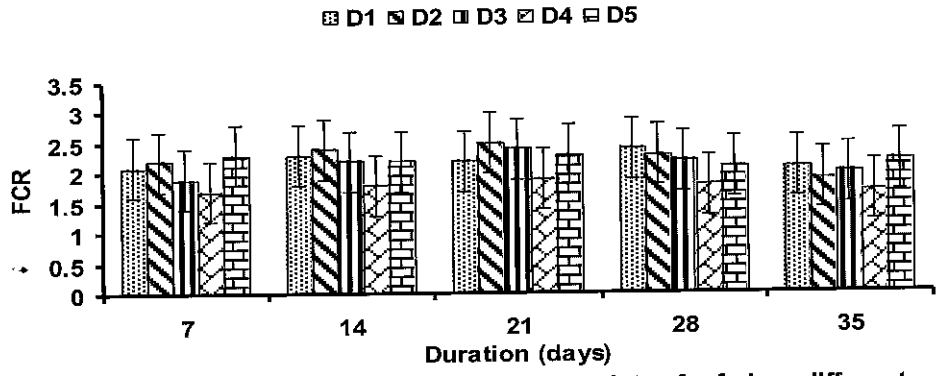


Fig. 4. Food conversion ratio of *Channa striatus* fry fed on different dietary protein levels.

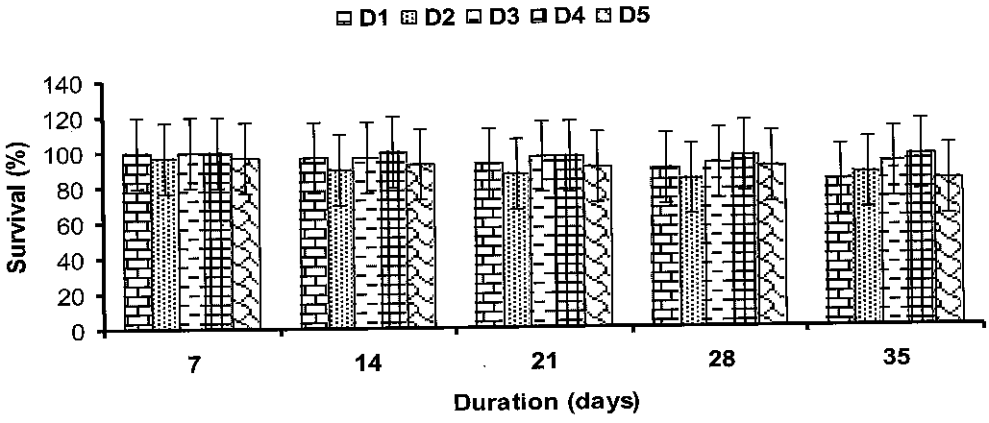


Fig 5.Survival of *Channa striatus* fry fed on different dietary protein levels

Table 1: Bio chemical composition of bio wastes* and feed ingredients

Ingredient	Protein (%)	Carbohydrate %	Lipid (%)	Ash (%)	GE/kcal/100g[a]	E/P**
Chicken intestine*	68.45	3.93	10.12	15.72	407.70	5.95
Fish waste*	58.06	3.69	6.18	24.21	326.59	5.62
Silkworm pupae*	55.02	1.00	17.12	22.01	396.73	7.21
Soya bean meal	49.64	9.20	9.70	5.20	337.66	6.80
Ground nut oilcake	48.04	6.90	10.9	11.64	332.74	6.92
Rice bran	15.90	20.4	3.90	7.90	175.11	11.01
Tapioca	14.60	43.7	0.20	8.60	218.16	14.94

Note:

*bio waste

**Energy/protein

[a] Gross energy

Table 2: Percentage and proximate composition of formulated diets

	Crude protein level %				
Ingredients	**40**	**45**	**50**	**55**	**60**
Chicken intestine	13.8	22.9	32.8	40.0	47.0
Fish waste	18.0	18.0	16.8	20.0	20.0
Silk worm pupae	19.0	19.0	20.0	20.0	20.0
Ground nut oil cake	6.0	6.0	6.0	6.0	3.0
Soya bean flour	5.0	5.0	6.0	5.0	2.0
Rice bran	31.2	22.1	11.4	2	1.0
Cod liver oil	3	3	3	3	3
Tapioca flour	3	3	3	3	3.0
Vit.Min.mix [a]	1.0	1.0	1.0	1.0	1.0
Proximate composition	**Nutrient content (%)**				
Protein %	39.08	43.35	49.20	54.72	59.36
Carbohydrate %	10.50	8.88	7.239	5.248	3.77
Lipid %	7.971	8.181	8.569	12.732	13.25
Gross Energy (Kcal/100g)	279.94	304.29	319.32	372.69	392.89
E/P ratio (g/Kcal)	7.162	6.709	6.489	6.810	6.618

[a] Vitamin-mineral per 100g premix contained: Vitamin A 200,000 IU, Cholecalciferol 40,000 IU, Vitamin B_{12} 80 mg Vitamin E 30 units,Vitamin K 40 mg,calcium pantochenate 100mg,nicotinamide 400mg,vitamin B_{12} 240 mg, choline chloride 6 g, calcium 30g,manganese 1.1 g, idodine 40mg,iron mg, zinc 600mg,copper 80mg,cobalt 18 mg

Table 3: Growth performance of *Channa striatus* fry fed on different levels of protein for a period of 35 days

	Protein %				
	40 (D1)	**45 (D2)**	**50 (D3)**	**55 (D4)**	**60 (D5)**
Initial length (cm)	1.15±0.01 [a]	1.16±0.01 [a]	1.14±0.01 [a]	1.15±0.01 [a]	1.16±0.01 [a]
Initial weight (g)	0.108±0.01 [a]	0.107±0.01 [a]	0.107±0.01 [a]	0.109±0.01 [a]	0.108±0.01 [a]
Final length (cm)	2.31±0.015 [f]	2.56±0.02 [d]	2.61±0.054 [c]	3.57±0.02 [a]	2.63±0.002 [b]
Final weight (g)	0.268±0.005 [c]	0.27±0.002 [b,c]	0.286±0.003 [b]	0.396±0.015 [a]	0.271±0.001 [c]
SGR (%/day)	1.13±0.011 [d]	1.163±0.002 [c]	1.209±0.003 [b]	1.613±0.036 [a]	1.14±0.004 [c,d]
Weight gain (%)	148.77±2.370 [c]	155.55±0.514 [c]	165.94±1.99 [b]	267.21±10.85 [a]	151.54±0.974 [c]
ADG (%)	0.458±0.002 [c]	0.479±0.002 [b,c]	0.509±0.006 [b]	0.824±0.040 [a]	0.467±0.001 [c]
FCR	2.26 ± 0.040 [c]	2.37±0.025 [d]	2.18±0.02 [b]	1.526±0.020 [a]	2.46±0.017 [e]
Survival (%)	83.33±5.77 [a]	86.66±5.773 [a]	93.33±5.773 [a]	96.66±5.773 [a]	83.33±5.773 [a]

The mean values having different superscripts in the same row are significant difference at $p<0.05\%$ level

Table 4: Body composition of *Channa striatus* fry (dry weight basis) fed on different protein diets.

	Protein %					
	Initial	**40 (D1)**	**45 (D2)**	**50 (D3)**	**55 (D4)**	**60 (D5)**
Protein (%)	52.16±0.03 ^{f}	54.39±0.03 ^{d}	56.34±0.03 ^{c}	58.36±0.041 ^{a}	60.32±0.032 ^{a}	53.38±0.02 ^{e}
CHO (%)	1.036±0.025 ^{b}	1.06±0.015 ^{b}	1.046±0.041 ^{b}	0.976±0.02b ^{a}	1.08±0.02 ^{b}	0.98±0.02 ^{a}
Lipid (%)	5.86±0.0152 ^{d}	6.22±0.02 ^{b}	7.55±0.03 ^{a}	6.56±0.02 ^{c}	5.45±0.025 ^{c}	6.03±0.026 ^{c}
Ash (%)	21.66±0.02 ^{d}	23.83±0.025 ^{a}	22.48±0.02 ^{c}	22.56±0.03 ^{b}	21.67±0.02 ^{d}	20.51±0.02 ^{e}
Moisture (%)	78.56±0.104 ^{a}	76.83±0.025 ^{c}	75.28±0.02 ^{e}	76.67±0.12 ^{d}	77.65±0.023 ^{b}	75.16±0.041 ^{e}
GE(Kcal/100g)	287.48±0.009 ^{e}	301.01±0.015 ^{d}	321.02±0.026 ^{b}	317.47±0.025 ^{c}	321.27±0.005 ^{a}	277.46±0.015 ^{f}
E/P	5.512±0.002 ^{c}	5.53±0.001 ^{b}	5.70±0.03 ^{a}	5.46±0.02 ^{d}	5.326±0.002 ^{e}	5.2±0.01 ^{f}

The mean values having different superscripts in the same row are significant difference at $p<0.05\%$ level

Table 5: Summary of ANOVA treatments of the effect of different levels of dietary protein on the growth performance of *Channa striatus* fry (the means were compared using Duncan multiple range test).

Parameters	Source of variation	SS	df	MS	F-value	Significance
Initial length (cm)	Between groups	0.001	4	0.001	0.001	1.000
	Within groups	0.001	10	0.001		
	Total	0.001	14			
Initial weight (g)	Between groups	0.001	4	0.001	0.001	1.000
	Within groups	0.001	10	0.001		
	Total	0.001	14			
Final length (cm)	Between groups	2.808	4	0.702	3169.128	0.05*
	Within groups	0.002	10	0.001		
	Total	2.810	14			
Final weight (g)	Between groups	0.036	4	0.009	178.852	0.05*
	Within groups	0.001	10	0.001		
	Total	0.036	14			
SGR (%/day)	Between groups	0.499	4	0.125	408.586	0.05*
	Within groups	0.003	10	0.001		
	Total	0.502	14			
ADG (%)	Between groups	0.292	4	0.073	211.838	0.05*
	Within groups	0.003	10	0.001		
	Total	0.295	14			
Weight gain (%)	Between groups	30486.997	4	7621.749	296.393	0.05*
	Within groups	257.150	10	25.715		
	Total	30744.147	14			
FCR	Between groups	1.551	4	0.388	677.768	0.05*
	Within groups	0.006	10	0.001		
	Total	1.556	14			
Survival (%)	Between groups	360.000	4	90.000	0.794	0.555
	Within groups	1133.333	10	113.333		
	Total	1493.333	14			

* - Statistically significant difference at ($p < 0.05$ % level).

Table 6: Summary of ANOVA body composition of *Channa striatus* fry fed on different levels of dietary protein (the means were compared using Duncan multiple range test).

Parameters	Source of variation	SS	df	MS	F-value	Significance
Protein (%)	Between groups	145.124	5	29.025	27789.74	0.05*
	Within groups	0.013	12	0.001		
	Total	145.137	7			
Carbohydrate (%)	Between groups	0.031	5	0.006	8.634	0.05*
	Within groups	0.009	12	0.001		
	Total	0.040	7			
Lipid (%)	Between groups	7.740	5	1.548	2786.512	0.05*
	Within groups	0.007	12	0.001		
	Total	7.747	7			
Ash (%)	Between groups	18.329	5	3.666	4428.458	0.05*
	Within groups	0.010	12	0.001		
	Total	18.339	7			
Moisture (%)	Between groups	26.340	5	5.268	1054.767	0.05*
	Within groups	0.060	12	0.005		
	Total	26.400	7			
Gross energy	Between groups	5265.227	5	1053.045	3303960	0.05*
	Within groups	0.004	12	0.001		
	Total	5265.231	7			
Energy/protein	Between groups	0.463	5	0.093	1058.118	0.05*
	Within groups	0.001	12	0.001		
	Total	0.464	7			

* - Statistically significant difference at ($p < 0.05$ % level).