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Comparison of seven genomic DNA isolation techniques from internal organs, gills and muscle tissues of *Notopterus notopterus* (Notopteridae) using PCR amplification and Nanodrop

Abstract. The techniques and the principles used in DNA isolation play a vital role in the obtaining of a purified genetic material. In present study, we investigated the efficiency of seven genomic DNA isolation techniques in terms of isolated DNA concentration, yield and purity from internal organs, gills and muscle tissues of *Notopterus notopterus*. Isolated DNA quality was analysed through Nanodrod and PCR using mitochondrial COI genetic markers. Results showed that GeneJET Genomic DNA Purification Kit was found significantly higher in terms of isolated DNA concentration (1200-1288 $ng.ul^{-1}$), yield (257.6 $ng.ul^{-1}$) and purity (1.91-2.00), and also successful in PCR amplification as compared to other evaluated six traditional DNA isolation methods. The nucleotide minimum bands range was observed 200 base pairs in heart sample and maximum 600 base pair was observed in intestine. There is no data on description of parameters analysed in this work to date, neither the evaluation of isolated DNA using PCR amplification of mitochondrial COI gene for the species *N. notopterus*. Present study also revealed that the traditional DNA isolation methods are the secondary choice for isolation of DNA. The data of present study also indicated that the GeneJET Genomic DNA Purification Kit is useful for DNA isolation and can be used best in genetic applications for fishes.
Key words: Comparative analysis, DNA isolation, NanoDrop, PCR amplification, *Notopterus notopterus.*

Introduction

The studies of molecular genetics depend completely on the viability of isolated DNA. From a small piece of tissue, isolation of high-quality DNA is difficult. Therefore, the DNA isolation method must be accurate, fast, effective, and productive with less contamination [1]. It must be economical based on costs and time. Mostly, for PCR amplification, the high quality of DNA is critically important because co-purifying inhibitors in presence of extraction reagents affect the PCR efficiency [2]. The hydrolytic enzymes and impurity of extraction buffer damaged the DNA during its extraction [3].

Traditional DNA isolation methods are laborious and time-consuming [4]. The reagents used in the traditional DNA extraction methods are considered risky for health and also cause contamination of extracted DNA [5].

Presently, DNA isolation kits are commercially available which considered useful and less hazardous to perform [6]. So, the selected DNA isolation method must be efficient and have less contamination [7].

Nanodrop is used to measure the concentration and purity of DNA, essentially to execute dilutions for PCR amplification. It is also used to analyse the increasing efficiency and quality of molecular studies [8].

The basic goal of the present study was to isolate DNA of high concentration with maximum yield and purity from the internal organs (intestine, liver, and heart), gills and muscle tissues of *Notopterus notopterus* (Notopteridae) that is effectively used for PCR amplification. For this purpose, we compared one commercially available GeneJET Genomic DNA Purification Kit with six traditional DNA extraction methods, including Phenol chloroform, TNES, Rapid MT, Urea SDS, Salt out and SNET methods and evaluated the effectiveness of the extracted DNA in terms of high concentration, maximum yield, and purity for PCR amplification from *N. notopterus* species available in Pakistan.

Materials and methods

Specimen collection. A total of 60 specimens of *N. notopterus* were collected from Marala Headworks, River Chenab, Punjab province, Pakistan. The Marala Headworks, River Chenab is located at 71°42'E, 31°63'N of Pakistan (Figure 1).

The collected specimens were instantly frozen and transported to the Fisheries Research Laboratory, Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan. Fish internal organs (intestine, liver, and heart), gills, and muscle tissues for DNA isolation were removed, put in an Eppendorf tube, and then used for DNA isolation.

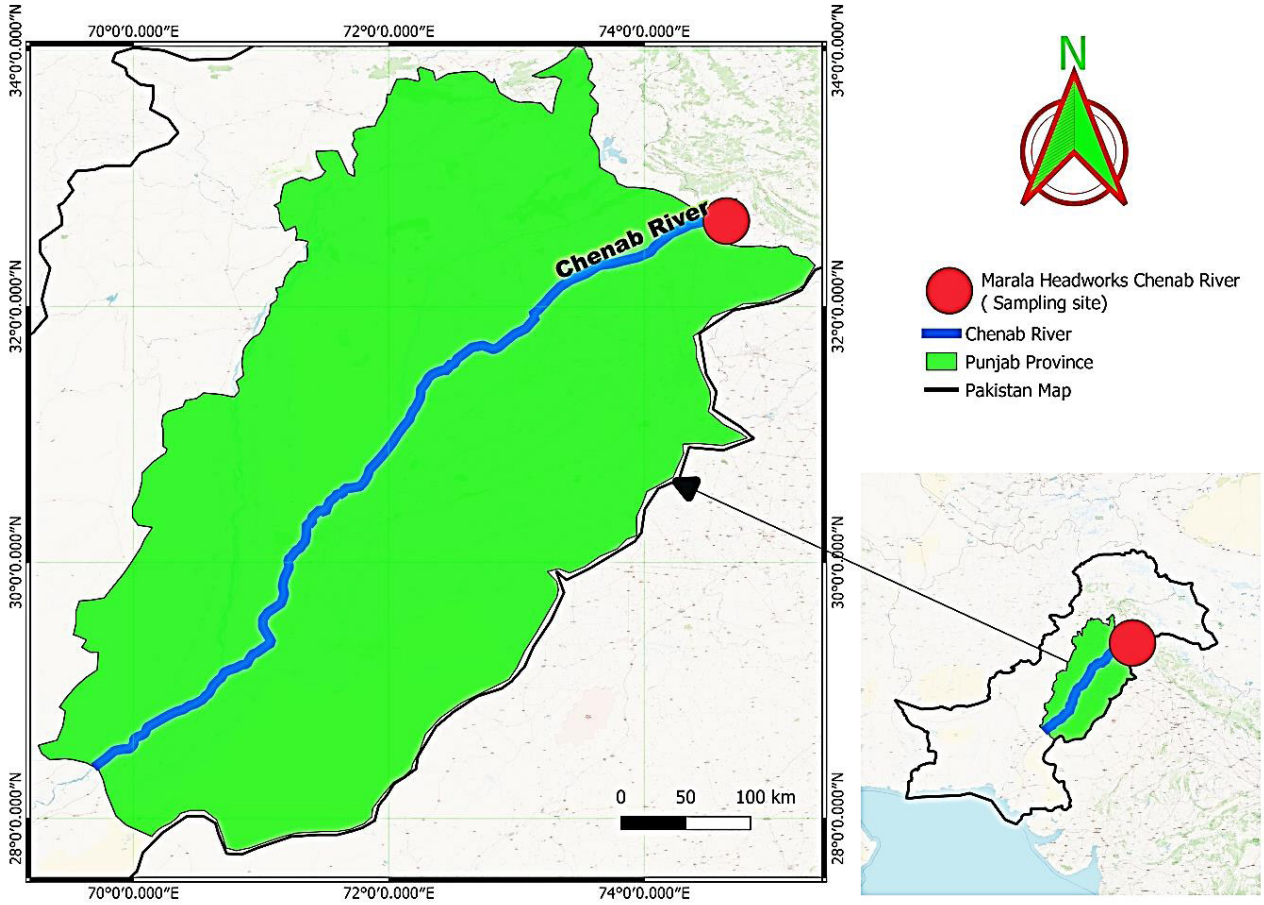

Figure 1 – Study area and sampling site of *Notopterus notopterus* fish. Map present the Marala Headworks of River Chenab, Punjab, Pakistan from where the specimens were collected

DNA isolation. Six traditional DNA isolation techniques whose methodologies had been described previously for fishes were selected from the literature and conditions of DNA isolation were intended to remain same for TNES [9], Phenol chloroform [10], Urea SDS [11], SNET [12], Rapid MT [13] and Salt out methods [12]. Seventh proposed, evaluated and compared method was a commercially available GeneJET Genomic DNA Purification Kit. Methodologies of seven DNA isolation techniques are described under.

GeneJET Genomic DNA Purification Kit. The fish tissues of 20 mg were clipped in liquid nitrogen of each sample. Fish tissues were then homogenized and the collected homogenized mixture was put into a micro-centrifuge tube. Then 180µl digestion solution was added to re-suspend. The 20 µl Proteinase K was also added and mixed the solution. It was incubated at 56°C and after that RNase solution 20 µl was added, mixed and incubated at room temperature for 10 minutes. Lysis solution 200 µl was added and after that 400µl ethanol (50%) was added and

pipetted. Then lysate was transferred into GeneJET Genomic DNA Purification column and centrifuged it at 6000 rpm for 1 minute. The GeneJET Genomic DNA Purification column was put into a new micro-centrifuge tube. The Wash Buffer I 500 µl was added containing ethanol; it was centrifuged at 8000 rpm for 1 minute; the 500 µl Wash Buffer II with ethanol was added into GeneJET Genomic DNA Purification column and centrifuged it at 12000 rpm for 3 minutes. The GeneJET Genomic DNA Purification column was transferred into micro-centrifuge tube and added 200µl Elution Buffer to elute genomic DNA and then it was incubated at room temperature for 2 minutes. After that it was centrifuged at 8000 rpm for 1 minute. The purified DNA was collected and stored at -20°C. Then 60 µl of nuclease free water was added for re-suspend DNA.

TNES method. 20 mg tissues sample put in 800 µl of buffer (Tris-HCl 10 mM, NaCl 125 mM, EDTA 10 mM, SDS 0.5%, urea 4 M), homogenized by adding 10 µl of RNase and incubated at 42ºC for 1 hour. 10 µl Proteinase K was added after incubation of 1 hour. Then incubate it overnight at 42ºC. Then 800 µl of phenol, chloroform and isoamyl alcohol were added with the ratio of 25:24:1 respectively. After that it was centrifuged at 10,000 rpm for 15 minutes. DNA pellets were formed in 1 M NaCl. Then 70% ethanol was added to wash DNA and left it for air dried. Then 60 µl of nuclease free water was added for re-suspend DNA [9].

Phenol chloroform method. The fish tissues 20 mg were taken and homogenized in DNA extraction buffer. 12 µl Proteinase K was added in paste mixed it with vortex mixer and incubate at 37ºC for 1 hour. Then again it was incubated at 55ºC for 1 hour. After that it was centrifuged at 5000 rpm for 10 minutes. Collect the supernatant and added phenol, chloroform and isoamyl alcohol with ratio 25:24:1 respectively. Then again it was centrifuged at 12000 rpm for 10 minutes and collected the supernatant. Add chloroform and isoamyl alcohol with ratio 24:1. Then again it was centrifuged at 12000 rpm for 10 minutes and collected the supernatant. 0.1 volume of 3 M sodium acetate and equal volume of 100% ice cold ethanol were added. Then put micro-centrifuged tube at -20ºC for 1 hour. After that it was centrifuged at 1000 rpm for 10 minutes. The pellets of DNA were formed at base of tube. DNA pellets were collected and added 100 µl of 70% ethanol and centrifuged at 1000 rpm for 10 minutes. Then 60 µl of nuclease free water was added for re-suspend DNA [10].

Urea SDS method. Fish tissue sample of 20 mg was homogenized in 100 µl of TESU6 buffer (Tris-HCL 10 mM with 8.0 pH, EDTA 20 mM with 8.0 pH, SDS 2%, Urea 6M), 12 µl proteinase K and mixed with vortex mixer. In a shaking incubator incubated at 55ºC for overnight with oscillation of 200 rpm. Then added 10 µl NaCl (5 M) and gently mixed. After that phenol, chloroform, isoamyl alcohol with ratio 25:24:1 was added and centrifuged it at 10,000 rpm for 5 minutes. Collect the supernatant and added equal volume of isopropyl alcohol (chilled). It was gently mixed and kept at -20ºC. Then centrifuge it at 10,000 rpm for 5 minutes. DNA pellets were formed at base of tube. Then DNA pellets were washed with 70% alcohol (chilled). 60 µl of nuclease free water was added to re-suspend the DNA [11].

SNET method. In 500 µl of buffer (Tris-Cl 20 mM, NaCl 400 mM, SDS 1%, Proteinase K 400 µg/ml EDTA5 mM) 50 mg fish tissues were homogenized and left it for overnight in a shaking incubator at 55ºC with oscillation of 200 rpm. The phenol, chloroform, isoamyl alcohol was added with ratio 25:24:1 respectively. After that it was placed at room temperature in shaking incubator for 30 minutes and centrifuged at 14000 rpm for 5 minutes. Then collect the supernatant and added isopropanol (chilled) with equal volume. After that it was centrifuged at 8000 rpm for 15 minutes and washed with 70% ethanol. Then 60 µl of nuclease free water was added for re-suspend the DNA [12].

Rapid MT method. Fish tissue 20 mg were taken and homogenized it in buffer (NaCl 200 mM, SDS 0.2%, EDTA 5 mM, Tris-HCl 100 mM). Then 10 µl of Proteinase K was added and mixed with vortex mixer. Then it was incubated at 55ºC overnight in a shaking incubator with oscillation of 200 rpm. Then the mixture was centrifuged at 12,000 rpm for 15 minutes. Collected the supernatant added the isopropanol 400 µl and mixed gently. Then centrifuged at 12,000 rpm for 20 seconds and pellets of DNA were formed at the base of micro-centrifuge tube. DNA pellets were washed with 70% alcohol and re-suspended the DNA pellets in 60 µl nuclease free water [13].

Salt out method. Fish tissues 20 mg were homogenized in 550 µl buffer (EDTA 50 mM, Tris-HCl 50 mM, SDS 1%, NaCl 100 mM) and 7 µl

proteinase K was added. Then incubated overnight in a shaking incubator at 50ºC with oscillation of 200 rpm and added 600µl NaCl (5M). After that it was centrifuged at 12,000 rpm for 10 minutes. Aqueous layer was collected and transferred into new micro-centrifuge tube. After that chilled 700µl ethanol was added and put micro-centrifuge tube at -20ºC for 2 hours. It was then centrifuged at 12,000 rpm for 10 minutes; DNA pellets were formed and washed with 70% ethanol. Then 60 µl of nuclease free water was added for re-suspend the DNA [12].

Quantification and visualization of extracted DNA. Isolated DNA concentration and purity was evaluated at OD A_{260}/A_{280} with NanoDrop. The success of PCR amplification was visualized using gel electrophoresis [14].

PCR amplification. The PCR amplification was successfully completed using the fish primes Fish F1 and Fish R1. The sequences of primers were used Fish F1 COI 5′- TCAACCAACCACAAAGA CATTGGCAC-3′ and Fish R1 COI 5′-TAGACTT CTGGGTGGCCAAAGAATCA-3′. PCR reaction volume was 25µl containing DNA template 1.5µl, PCR Master Mix 12.5µl (BLIRT S.A.), 0.1µl forward primer, 0.1µl reverse primer and 10.8µl nuclease free water. The condition for PCR thermal cycler, initial denaturation was set at 95°C for 2 minutes, further 30 complete rotations with denaturation was set at 95°C for 30 seconds, annealing at 54°C for 40 seconds and extension at 72°C for 1 minute. The final extension at 72°C was set for 7 minutes. The success of PCR amplification was checked on 2 % (w/v) agarose gel by running the PCR products.

Statistical analysis. The statistical analysis of seven different DNA isolation techniques was evaluated with SPSS software. The analysis of variance of DNA concentration and purity were calculated from fish internal organs, muscle tissues and gills of *N. notopterus*. The one-way ANOVA with LSD post hoc test was used to analyse the statistically significant differences among seven different DNA isolation techniques in terms of concentration and purity of isolated DNA at a level of 5% (P<0.05).

Results and discussion

Statistical analysis of isolated DNA concentration and purity. The results of One-way analysis of variance with LSD post hoc test revealed that the concentration of isolated DNA in internal organs, gills and muscle tissues with GeneJET Genomic DNA Purification Kit, were significantly (P<0.05) higher as compared to investigated six traditional methods. Among traditional DNA isolation methods; Urea SDS, SNET, Salt out and Rapid MT methods were found non-significantly (P>0.05) higher as compared to GeneJET Genomic DNA Purification Kit.

The isolated DNA purity of GeneJET Genomic DNA Purification Kit was obtained significantly (P<0.05) higher as compared to investigated six traditional methods. Among traditional DNA isolation methods; Phenol chloroform method, TNES method, Urea SDS, SNET, Salt out and Rapid MT methods were found non-significantly (P>0.05) higher as compared to GeneJET Genomic DNA Purification Kit.

Isolated DNA concentration and purity evaluation using NanoDrop. Isolated DNA with range of 1.7-2.0 at absorbance A_{260}/A_{280} is considered pure. Isolated DNA highest concentration was observed with GeneJET Genomic DNA Purification Kit (1288 $ng.\mu l^{-1}$) in muscle tissues of *N. notopterus* as compared to six investigated traditional methods. Among six traditional methods the high DNA concentration was observed with TNES method (1159 $ng.\mu l^{-}$) while the lowest DNA concentration was found with Rapid MT methods (61 $ng.\mu l^{-}$) (Table 1). Mean isolated DNA concentration of seven investigated methods are shown in Fig. 2a-e.

Isolated DNA analysis revealed that the highest proportion of purity was obtained with GeneJET Genomic DNA Purification Kit (1.71-2.0 $ng.\mu l^{-}$) as compared to other methods in internal organs (intestine, liver and heart), gills and muscle tissues (Table 1). Among six traditional methods the high DNA purity was observed with TNES method (1.71-2.0 $ng.\mu l^{-}$) while the lowest DNA purity was found with Salt out method (1.12 ng.µl) of seven investigated methods. Mean isolated DNA purity of seven investigated methods are shown in Fig. 3a-e.

Purity range of isolated DNA samples above, below and within satisfactory limits.

N. notopterus isolated DNA proportion from internal organs, muscle tissues and gills with seven DNA isolation methods showed that 100% samples of GeneJET Genomic DNA Purification Kit were within purity range (1.7-2.0) (Table 2).

Table 1 – Comparison of seven DNA isolation techniques in terms of concentrations and purities.

Species name	Organ	Method	DNA Conc. range ($ng.ul^{-1}$)±SD	DNA Purity A_{260}/A_{280} Range ± SD	PCR
Notopterus notopterus	Intestine	GeneJET Genomic DNA Purification Kit	805-891±29.81	1.91-2.00±0.03	+
		Phenol Chloroform method	205-541±115.95	1.19-1.89±0.27	+
		TNES method	521-793±100.50	1.73-1.99±0.08	+
		Urea SDS method	258-693±125.26	1.13-1.84±0.27	+
		SNET method	105-484±122.58	1.26-1.44±0.05	+
		Rapid MT method	61-193±48.85	1.21-1.40±0.07	+
		Salt out method	61.5-421±116.36	1.19-1.42±0.07	+
	Liver	GeneJET Genomic DNA Purification Kit	1116-1194±29.43	1.90-2.00±0.04	+
		Phenol Chloroform method	621-839±87.61	1.70-1.94±0.09	+
		TNES method	828-1002±57.76	1.75-1.99±0.10	+
		Urea SDS method	579-694±47.67	1.13-1.71±0.19	+
		SNET method	391-694±102.64	1.51-1.71±0.09	+
		Rapid MT method	402-628±63.78	1.19-1.71±0.24	+
		Salt out method	105-421±112.92	1.21-1.42±0.05	+
	Heart	GeneJET Genomic DNA Purification Kit	1126-1198±23.24	1.90-2.00±0.04	+
		Phenol Chloroform method	602-919±101.98	1.69-1.89±0.07	+
		TNES method	932-1093±50.46	1.75-1.98±0.09	+
		Urea SDS method	546-786±72.75	1.40-1.71±0.12	+
		SNET method	369-546±73.06	1.19-1.84±0.20	+
		Rapid MT method	432-693±92.04	1.13-1.45±0.12	+
		Salt out method	61.5-314±74.96	1.21-1.33±0.05	+
	Muscle Tissue	GeneJET Genomic DNA Purification Kit	1200-1288±27.63	1.90-2.00±0.03	+
		Phenol Chloroform method	521-681±63.34	1.65-1.88±0.07	+
		TNES method	1069-1159±30.73	1.76-1.99±0.09	+
		Urea SDS method	521-693±65.20	1.13-1.73±0.18	+
		SNET method	185-484±125.24	1.27-1.39±0.03	+
		Rapid MT method	261-407±51.45	1.35-1.42±0.03	+
		Salt out method	61.5-193±34.16	1.19-1.40±0.06	+
	Gills	GeneJET Genomic DNA Purification Kit	1138-1193±22.44	1.91-2.00±0.03	+
		Phenol Chloroform method	445-876±133.74	1.53-1.99±0.14	+
		TNES method	786-1100±124.52	1.73-1.99±0.09	+
		Urea SDS method	391-694±110.15	1.51-1.71±0.08	+
		SNET method	105-421±129.42	1.21-1.42±0.07	+
		Rapid MT method	369-546±80.00	1.51-1.84±0.14	+
		Salt out method	166-421±84.99	1.12-1.42±0.08	+

SD = Standard Deviation

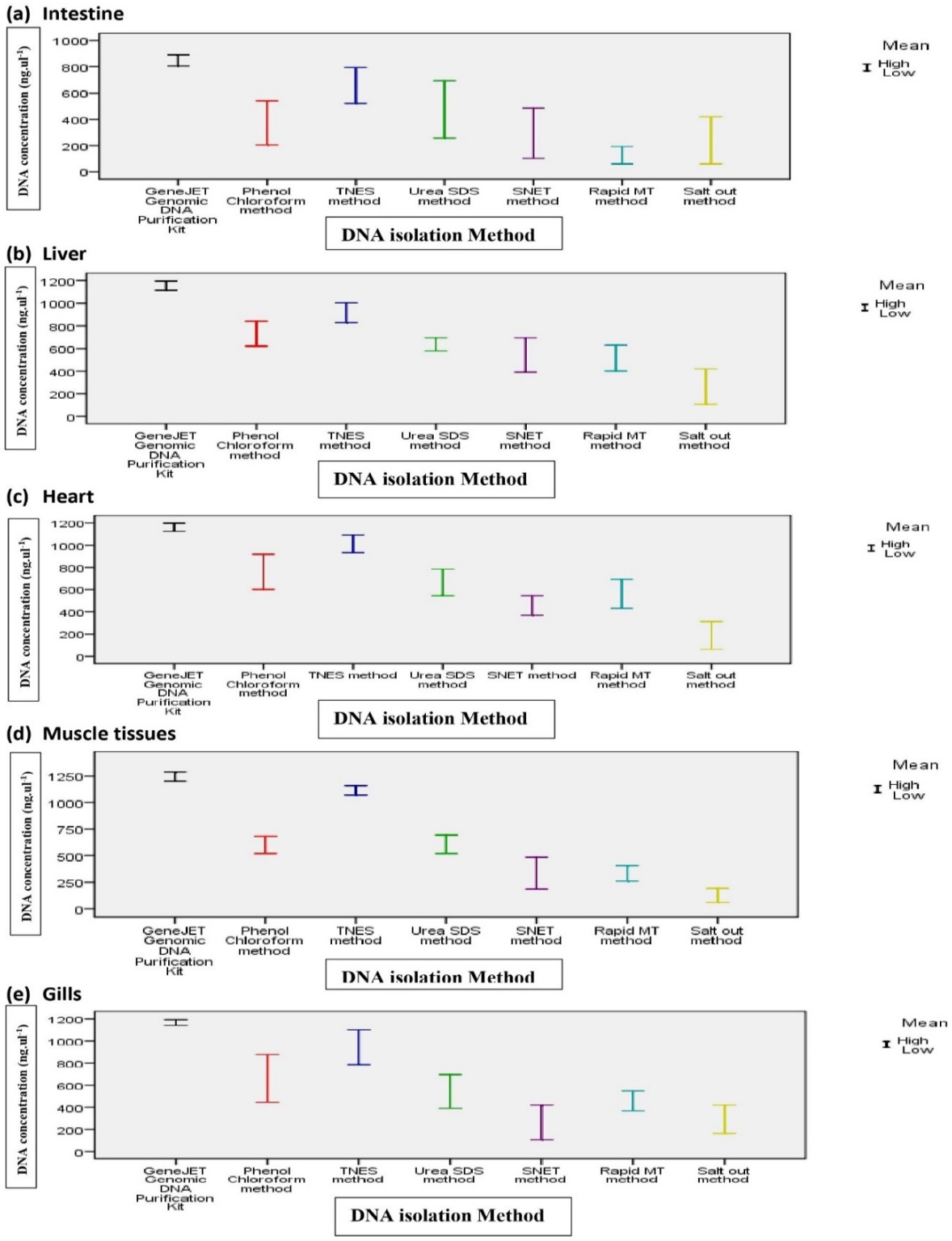

Figure 2 – The comparison of mean DNA concentration ($ng.ul^{-1}$) obtained with seven different methods (GeneJET Genomic DNA Purification Kit, Phenol chloroform method, TNES method, Urea SDS method, SNET method, Rapid MT method and Salt out method) from (a) intestine; (b) liver; (c) heart; (d) muscle tissues; (e) gills of *Notopterus notopterus*. High and low bars indicate the mean DNA concentration values.

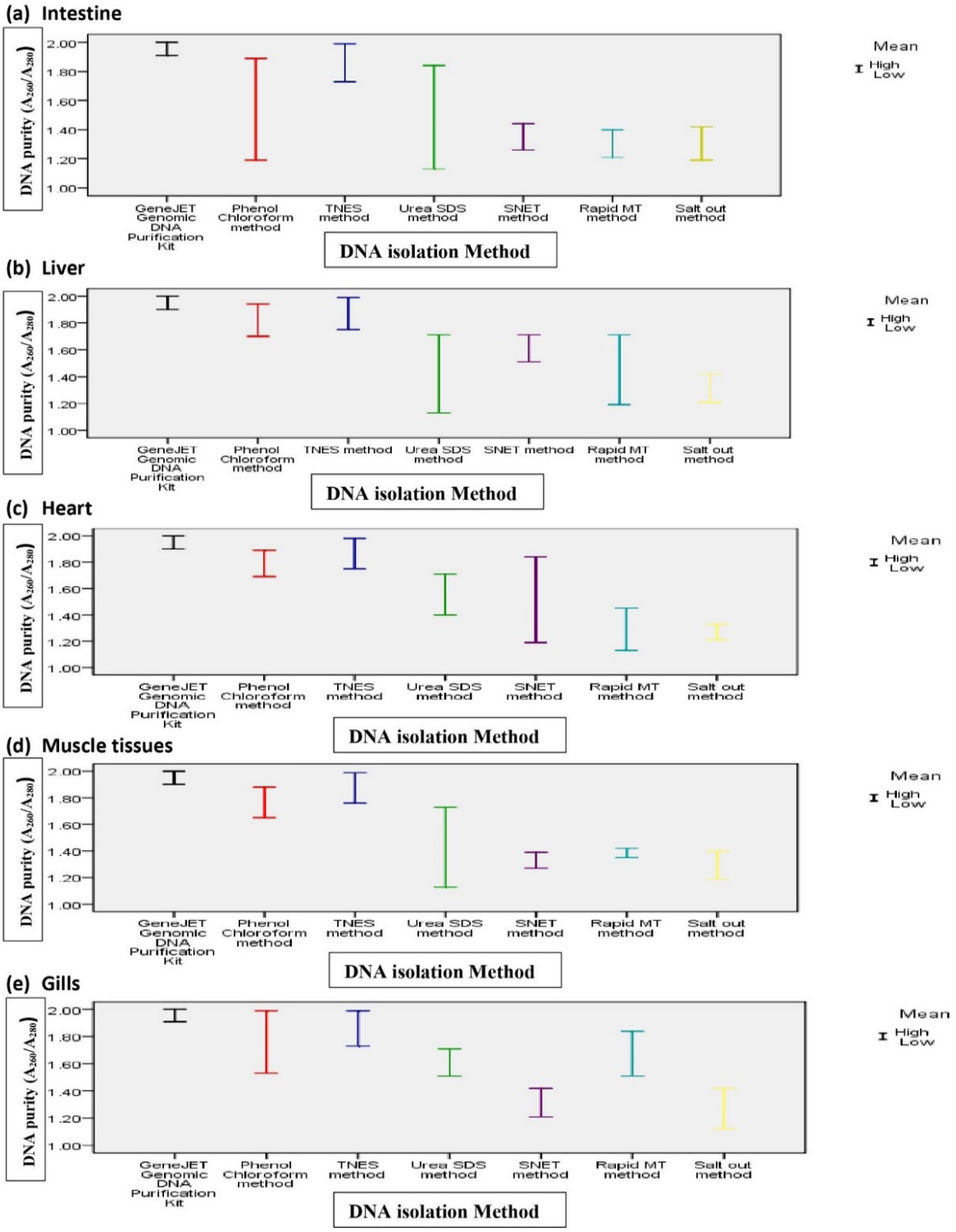

Figure 3 – The comparison of mean DNA purity (A_{260}/A_{280}) obtained with seven different methods (GeneJET Genomic DNA Purification Kit, Phenol chloroform method, TNES method, Urea SDS method, SNET method, Rapid MT method and Salt out method) from (a) intestine; (b) liver; (c) heart; (d) muscle tissues; (e) gills of *Notopterus notopterus*. High and low bars indicate the mean DNA purity values.

Table 2 – Isolated DNA sample range of purity within, below and above satisfactory limits

Method of DNA isolation	Total samples	Samples within purity range (1.7-2.0)	Samples below purity (1.7)	Samples above purity (2.0)	Total %age of samples in purity 1.7-2.0 range
GeneJET Genomic DNA Purification Kit	60	60	0	0	100%
Phenol Chloroform method	60	54	06	0	90%
TNES method	60	59	01	0	98%
Urea SDS method	60	21	39	0	35%
SNET method	60	11	49	0	18%
Rapid MT method	60	11	49	0	18%
Salt out method	60	0	60	0	0%

Among six traditional methods the TNES methods 100% samples were observed within purity range (1.7-2.0) while none of the Salt out method sample was found within purity range (Table 2).

Success of PCR amplification. Isolated DNA two best samples of high concentration and purity were used for PCR amplification of each method from each organ (intestine, liver and heart), muscle tissues and gills of *N. notopterus*. PCR gel illustration indicated a successful amplification of PCR using mitochondrial COI genetic marker. Fig. 4a-e revealed that GeneJET Genomic DNA Purification Kit was found exclusively successful and better performed in terms of quality of PCR amplification in internal organs (intestine, liver and heart), muscle tissues and gills of *N. notopterus* as compared to six traditional methods. The nucleotide minimum bands range was observed 200 base pairs in heart samples (Fig. 4c) and maximum 600 base pair band range was observed in intestine (Fig. 4a) while in liver 400 base pairs band range (Fig. 4b), muscle 500 (Fig. 4d) and gills 500 base pairs (Fig. 4e).

Comparative analysis of DNA extraction techniques feasibility. Seven DNA extraction methods were different in terms of processing, time and labour. The incubation period, addition of reagents and supernatant transfer into new micro-centrifuge tube were also different. The GeneJET Genomic DNA Purification Kit was preferred because incubation time was short, less laborious and necessary reagents for extraction of DNA had already been added in commercially available kits in ready-to-use form. So, the commercially available kits are extremely suitable for DNA extraction of high concentration, yield and purity.

Economic feasibility. The relative cost of seven different DNA isolation comparisons was presented in Table 3. The maximum yield of DNA from single sample was obtained with GeneJET Genomic DNA Purification Kit technique as compared to other traditional DNA isolation techniques. The DNA yield must be considered when cost of DNA isolation method comes into mind. The cost of the GeneJET Genomic DNA Purification Kit was comparable with the other evaluated traditional methods and assessed to be a cost-effective technique in terms of yield and concentration of extracted DNA per microgram and per individual sample, are shown in Table 3.

The considerations of safety in isolation of DNA. Many reagents which were used during seven DNA isolation methods are harmful to eyes and skin. The GeneJET Genomic DNA Purification Kit was proved highly safe and has no serious health issues out of seven assessed DNA isolation techniques, while phenol and chloroform have serious safety and health concerns, which used in traditional methods like Phenol chloroform method. The phenol damages the skin if accidentally exposed to skin due to its high corrosive nature and listed as very dangerous. The phenol also poisons and damages the eyes which eventually lead to death. The chloroform is carcinogen and damage reproductive system as well. So, due to these safety concerns, many laboratories worldwide no longer use phenol chloroform DNA isolation technique for the DNA isolation while the use of commercially available GeneJET Genomic DNA Purification Kits does not cause any serious safety and health dangers, conferring to information provided by the manufacturers.

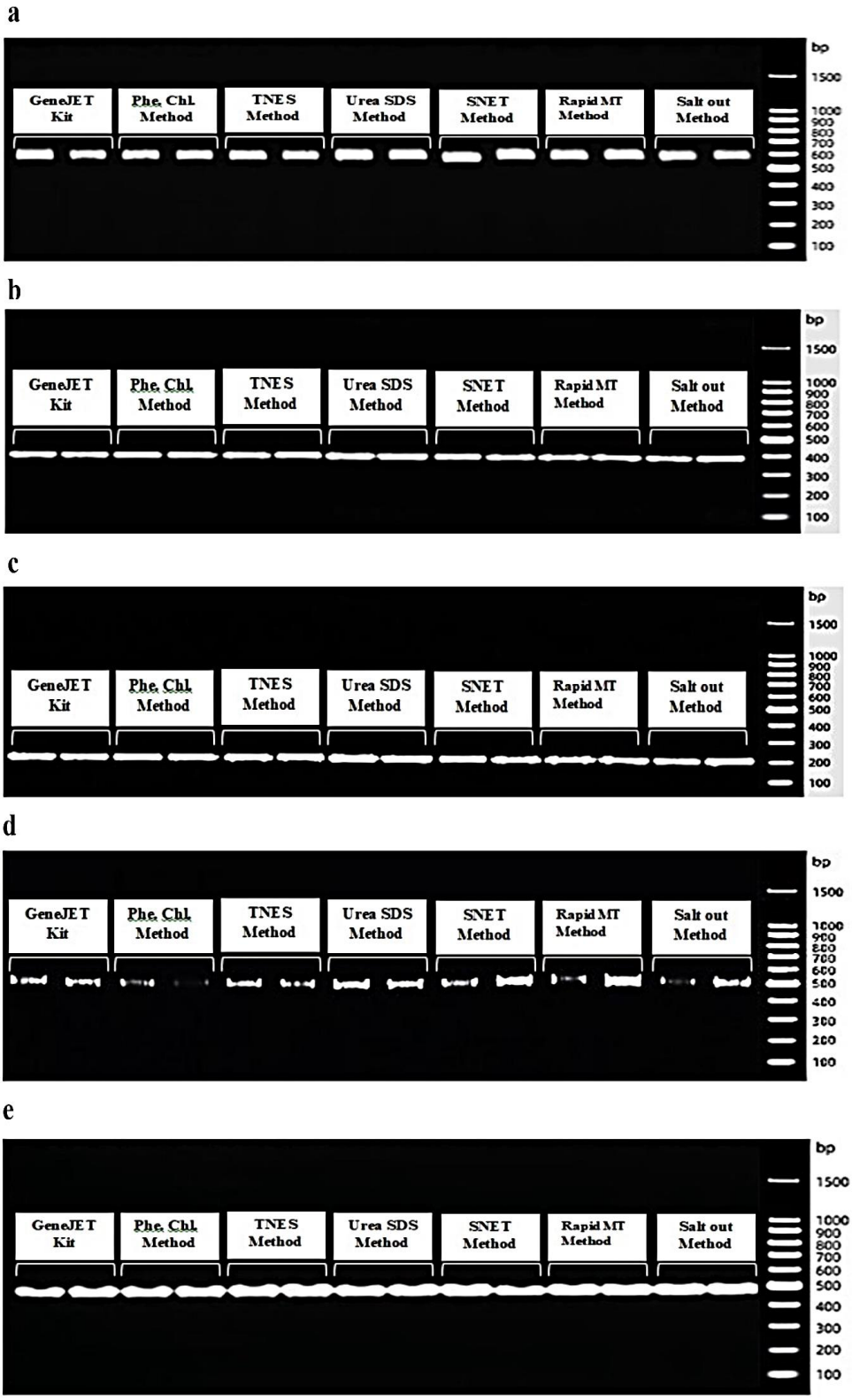

Figure 4 – PCR amplification comparison between seven different DNA isolation techniques GeneJET Genomic DNA Purification Kit, TNES method, Phenol chloroform method, Urea SDS method, SNET method, Rapid MT method and Salt out method in (a) intestine; (b) liver; (c) heart; (d) muscle tissues; (e) gills of *Notopterus notopterus*

Table 3 – Comparison of seven DNA isolation techniques relative to the costs per microgram of isolated DNA per sample

DNA isolation Method	Per DNA extraction cost	Mean DNA yield (ug) extracted	Cost per ug of extracted DNA
GeneJET Genomic DNA Purification Kit	2.00 USD	257.6	0.20 USD
Phenol Chloroform method	2.01 USD	183.8	0.20 USD
TNES method	2.00 USD	231.8	0.20 USD
Urea SDS method	1.66 USD	157.2	0.16 USD
SNET method	1.86 USD	138.8	0.18 USD
Rapid MT method	1.68 USD	138.6	0.16 USD
Salt out method	1.66 USD	84.2	0.16 USD

Genetic studies required good quality DNA. Isolated DNA concentration and purity depends upon its isolation technique [1]. Mostly soft tissues i.e., muscles, liver, heart and gills are selected for isolation of DNA [15]. In present study, we used internal organs (intestine, liver, and heart), gills and muscle tissues of *N. notopterus* for isolation of DNA as used by Wasko et al. [15]. We compared and evaluated the efficiency of six traditional DNA extraction techniques with one commercially available GeneJET Genomic DNA Purification Kit.

Mean concentration and purity of investigated seven DNA isolation techniques are presented in Fig. 2 and Fig. 3 respectively. Fig. 2 mean value indicated that GeneJET Genomic DNA Purification Kit has highest DNA concentration (GeneJET 1114.9; Phenol Chloroform 618.7; TNES method 928.3; Urea SDS method 585.5; SNET method 378.4; Rapid MT method 399.2; Salt out method 222.55) $ng.ul^{-1}$ while Salt out method has lowest concentration. Fig. 3 indicated that the GeneJET Genomic DNA Purification Kit has highest purity (GeneJET 1.95; Phenol Chloroform 1.73; TNES method 1.86; Urea SDS method 1.5; SNET method 1.27; Rapid MT method 1.4; Salt out method 1.37) while SNET method has lowest purity. These results obtained using Nanodrop spectrophotometer. The best purity value ranges from 1.7 to 2.0 as reported by Parpinelli & Ribeiro [16]; and Lagass-Pereira et al. [17]. In present study, considering the seven DNA isolation techniques, GeneJET Genomic DNA Purification Kit has the highest value of 1.95 while the lowest value was observed with SNET method 1.27 as reported by Parpinelli & Ribeiro [16].

In present study Post hoc test (ANOVA) was used to evaluate the statistical significance of each DNA isolation method followed Pereira et al. [7]. The GeneJET Genomic DNA Purification Kit was found significantly ($P<0.05$) higher in terms of DNA concentration and purity as compared to examined traditional six DNA isolation methods. Results also revealed that the concentration and purity of isolated DNA differ significantly among all methods at 5% ($p<0.05$) level as reported by Jasbeer et al. [18].

Table 1 of present study showed that the concentration of isolated DNA varied from 61 to 1288 with the highest concentration GeneJET Genomic DNA purification Kit and the lowest in Rapid MT method. These variations in concentration and purity revealed the significant difference among seven investigated DNA isolation techniques. So, it is said that these credible variation in concentrations of DNA purity depends on the efficiency of DNA isolation technique which important for collection of highly pure DNA as reported by Oosting et al. [19].

The purity of isolated DNA varied from 1.12 to 2.0 in present study. GeneJET Genomic DNA purification Kit has highest purity level 2.0 while Salt out method has lowest level of purity 1.12 provided in Table 1. Results also revealed that the concentration and purity of isolated DNA differ significantly among all methods at 5% ($p>0.05$) level [18].

The suitability of isolated DNA was also analysed using gene amplification [18]. In present study mitochondrial COI gene was used to amplify the isolated DNA. The mitochondrial COI gene has a size 600 bp. Fig. 4a-e of present study indicated that the minimum bands range was observed 200 base pairs in heart samples and maximum 600 base pair was observed in intestine while in liver 400 base pairs, muscle 500 and gills 500 base pairs as Jasbeer et al. [18] reported the amplification of mitochondrial COI gene with size 1227 base pairs. The amplified gene region indicated a clear band range on the gel

picture. These proofs confirmed that the DNA isolated through GeneJET Genomic DNA Purification Kit and TNES method is suitable for downstream analysis.

The considerable variations were observed relative to isolated DNA yield and reagents cost. The GeneJET Genomic DNA Purification Kit was found cheapest and economic as reported by Marsal et al. [20], which have per sample cost of 2.00 USD (Table 3) while a cost of 31.2 USD per sample was reported by Manen et al. [21]. However, this cost of GeneJET Genomic DNA Purification Kit per sample (2.00 USD) is reasonable and economic [21].

In terms of time and labour commercial DNA isolation kits are consider faster as compared to traditional DNA isolation methods. The estimated DNA extraction time of GeneJET Genomic DNA Purification Kit is around 2 hours while traditional DNA isolation methods have estimated time is around 15 hours as reported by *Marsal* et al. [20]. So, the GeneJET Genomic DNA Purification Kit is found the best in terms of processing time and labour.

DNA concentration and purity are critical for PCR amplification. It is also desirable that DNA should contain less contaminant and inhibitors for PCR amplifications. DNA isolation techniques are laborious and time-consuming. Different DNA extraction methods have variation in time to complete the process [2]. GeneJET Genomic DNA Purification Kit needs just 1 hour of incubation for tissue digestion which is very short and beneficial while in the Phenol chloroform method the incubation time for tissue digestion is of two hours and other investigated traditional methods (Urea SDS method, Phenol chloroform method, Rapid MT method, Salt out method and SNET method require overnight incubation as reported by Chowdhury et al. [10]. The GeneJET Genomic DNA Purification Kit is less laborious and completed shortly in terms of phase separation and centrifugation steps while Phenol chloroform method is laborious and time-consuming in terms of phase separation and centrifugation steps [9]. The centrifugations require phenol, chloroform and isoamyl alcohol which are dangerous for skin if accidently exposed to skin [22]. In Salt out method, protein is extracted from tissue lysate with concentrated NaCl. Salt and absolute ethanol was used for DNA pellets. The overnight incubation, centrifugation multiple times and absolute ethanol for DNA pellets are also needed in Salt out method [23].

Proteinase K is used in each method for digestion of proteins while DNA extraction buffer vary in different methods as described by Desjardins & Conklin [24]. Proteinase K activity is best at 20-60°C. The 20-60°C is broad range of temperature widely used for extraction of good quality DNA. In SDS 0.5% (w/v) Proteinase K is completely activated and often used in buffer containing SDS (sodium dodecyl sulphate). Proteinase K digests and separate proteins from DNA, and then phenol remove the dislodged proteins easily [25]. The use of Proteinase K less than 30µl did not feasible for production of high quantity and purity of DNA while in present study we just used 7-20µl of Proteinase K which produced high quantity and purified DNA [26]. Isolated DNA with range of 1.7-2.0 at absorbance A_{260}/A_{280} is considered pure [27]. Present study showed that the GeneJET Genomic DNA Purification Kit has short incubation time and economic, as compared to analysed six traditional methods. So, GeneJET Genomic DNA Purification Kit not only produced high quantity, maximum yield and purified, DNA but also saved time and money.

Conclusion

The efficiency of seven DNA extraction techniques subjected to different technological processes were assessed and compared in present study. The best results were obtained with GeneJET Genomic DNA Purification Kit from internal organs (intestine, liver and heart), gills and muscle tissues of *N. notopterus*. The commercially available GeneJET Genomic DNA Purification Kit was found the best in terms of isolated DNA concentration, yield, and quality of PCR amplification as compared to all investigated six traditional methods. Among traditional DNA isolation methods, both TNES method and Phenol chloroform method were found better in terms of concentration, yield and purity of isolated DNA for PCR amplification while Salt out method yielded lowest in concentration, purity and yield of DNA. Present study proved that the GeneJET Genomic DNA Purification Kit has short incubation time and economic, as compared to analysed six traditional methods. GeneJET Genomic DNA Purification Kit not only produced high quantity, maximum yield and purified, DNA but also saved time and money. According to best of our knowledge this study was the first attempt in which one commercially available GeneJET Genomic DNA Purification Kit was analysed with six traditional DNA isolation methods from internal organs (intestine, liver and heart), gills and muscle tissues of *N. notopterus*. Moreover, present study also revealed that the traditional DNA isolation methods are the secondary

choice for isolation of DNA. The data of present study also indicated that the GeneJET Genomic DNA Purification Kit is useful for DNA isolation and can be used best in genetic applications for fishes.

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References

1. Mezzomoa P., Mielniczki-Pereira A.A., Sausenb T.L., Marinho J.R., Cansian R.L. (2021) Evaluation of eight protocols for genomic DNA extraction of Hypostomus commersoni Valenciennes, 1836 (Loricariidae: Siluriformes). *Brazilian Journal of Biology*, vol. 81, no. 3, pp. 674-683. https://doi:10.1590/1519-6984.229278.

2. Muhammad H., Iqbal Z., Iqbal M.U., Younas T. Bashir Q. (2016) An efficient method for DNA isolation from fish fin. *Pakistan Journal of Agricultural Sciences*, vol. 53, no. 4, pp. 843-850. https://doi:10.21162/Pakjas/16.3998

3. Smith D.S., Maxwell P.W., Boer S.H. (2005) Comparison of several methods for the extraction of DNA from potatoes and potato-derived products. *Journal of Agriculture and Food Chemistry*, vol. 53, no. 26, pp. 9848–9859. https://doi:10.1021/jf051201v

4. Tongeren S.P.V., Degener J.E., Harmsen H.J. (2011) Comparison of three rapid and easy bacterial DNA extraction methods for use with quantitative real-time PCR. *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 30, no. 9, pp. 1053-1061. https://doi:10.1007/s10096-011-1191-4

5. Zora P., Eliska P., Gabriela B. (2017) Comparative study of DNA extraction methods from fresh and processed yellowfin tuna muscle tissue. *International Journal of Food Properties*, vol. 20, no. 1, pp. 430-443. https://doi:10.1080/10942912.2017.1297953

6. Mega N.O., Revers L.F. (2011) Developing a rapid, efficient and low-cost method for rapid DNA extraction from arthropods. *Ciência Rural*, vol. 41, no. 2, pp. 1563-1570. https://doi:10.1590/s0103-84782011005000112

7. Pereira C.J., Chaves, Bastos E., Leitão A., Guedes-Pinto H. (2011) An efficient method for genomic DNA extraction from different mollusc species. *International Journal of Molecular Sciences*, vol. 12, no. 11, pp. 8086–8095. https://doi:10.3390/ijms12118086.

8. Rana N., Jain S. (2019) DNA quantification of wild and cultured *Labeo Rohita* (Hamilton, 1822) using Nanophotometer. *Journal of Experimental Zoology India*, vol. 22, no. 1, pp. 453–455.

9. Zhong L.I., Hong-Wei L., Gui-Wei A.Z. (2012) A rapid PCR quality DNA extraction method in fish. *Acta Hydrobiologia Sinca*, vol. 36, no. 2, pp. 365–367. https://doi:10. 3724/SP.J.1035.2012.00365

10. Chowdhury M.M., Rahman S.A., Nahar L., Rahman M., Reza H., Ahmed S. (2016) Efficiency of different DNA extraction methods for fish tissues: A comparative analysis. *IOSR Journal of Pharmacy and Biological Science*, vol. 11, no. 3, pp. 11-15. https://doi: 10.9790/3008-1103041115

11. Naeem M., Hassan S., Masud S., Ali Q., Hayat S., Naeem A.D., Naeem Z. (2020) Comparative analysis of genomic DNA extraction protocols: Maxi-preparation of quality DNA for genetic evaluation and phylogenetic studies. *Genetics and Molecular Research*, vol. 19, no. 1, pp. 1-9.

12. Hofkar M.H., Deursen, J.M.V. (2011) Transgenic mouse methods and protocols. *Springer*, no. 6, pp. 26–31.

13. Mukhopadhyay T., Bhattachatjee S. (2014) Standardization of genomic DNA isolation from minute quantities of fish scales and intestine amenable to RAPD-PCR. *Proceedings of Zoological Society*, vol. 67, no. 1, pp. 28–32. https://doi:10.1007/s12595-013-0065-4

14. Downs T.R., Wilfinger W.W. (1983) Fluorometric quantification of DNA in cells and tissue. *Analytical Biochemistry*, vol. 131, no. 2, pp. 538-47. https://doi:10.1016/0003-2697(83)90212-9

15. Wasko A.P., Martin C., Oliveira C., Forresti. (2003) Non-destructive genetic sampling in fish. An improved method for DNA extraction from fish fins and scales. *Hereditas*, vol. 138, no. 3, pp. 161-165. https://doi:10.1034/j.1601-5223.2003.01503.x

16. Parpinelli R.S., Ribeiro R.P. (2009) Estudocomparativo de protocolos de extraçãode DNA em diferentes tecidos de tilápia do nilo (*Oreochromis niloticus*). *Global Science and Technology*, vol. 2, no. 1, pp. 22-33.

17. Lagass-Pereira L., Damasceno J.D.S., Da-Vitoria E.L., Silva M.H., Farro, A.P.C. (2016) Comparison of DNA extraction protocols for different marine fish tissues. *Pan-American Journal of Aquatic Sciences*, vol. 11, no. 2, pp. 135-142.

18. Jasbeer K., Son R., Ghazali M.F., Cheah Y.K. (2009) Real-time PCR evaluation of seven

DNA extraction methods for the purpose of GMO analysis. *International Food Research Journal,* vol. 16, no. 2, pp. 329-341. https://doi:10.1002/ece3.6558.

19. Oosting T., Hilario E., Wellenreuther M., Ritchie P.A. (2020) DNA degradation in fish: Practical solutions and guidelines to improve DNA preservation for genomic research. *Ecology and Evolution*, vol. 1, no. 1, pp. 1–9. https://doi:10.1002/ece3.6558

20. Marsal G., Baiges I., Canals J.M., Zamora F., Fort F. (2011) A fast, efficient method for extracting DNA from leaves, stems, and seeds of *Vitis vinifera*. *American Journal of Entomology and Viticulture*, vol. 62, no. 1, pp. 376-381. https://doi:10.5344/ajev.2011.10082

21. Manen J.F., Bouby L., Dalnoki O., Marinval P., Turgay M., Schlumbaum A. (2003) Microsatellites from archaeological Vitis vinifera seeds allow a tentative assignment of the geographical origin of ancient cultivars. *Journal of Archaeological Science*, vol. 30, no. 3, pp. 721-729. https://doi:10.1016/S0305-4403(02)00244-3

22. Triant D.A.A., Whitehead. (2009) Simultaneous extraction of high-quality RNA and DNA from small tissue samples. *Journal of Heredity*, vol. 10, no. 2, pp. 246–250. https://doi.org/10.1093/jhered/esn083

23. Giampaolo Z., Nikolaos A. (2012) Detection of pathogens in water using micro and Nano technology. (The International Water Association, UK).

24. Desjardins P.D., Conklin. (2010) NanoDrop microvolume quantitation of nucleic acids. *Journal of Visual Experiments JVE*, vol. 22, no. 5, pp. 25–65. https://doi:10.3791/2565.

25. Goldenberger D., Perschil I., Ritzler M.M., Altwegg. (1995) A simple “universal” DNA extraction procedure using SDS and proteinase K is compatible with direct PCR amplification. *PCR Methods and Applications*, vol. 4, no. 6, pp. 368–70.

26. Kumar R.P.J., Singh N.S., Nagpure B., Kushwaha S.K., Srivastava W.S., Lakra. (2007) A non-invasive technique for rapid extraction of DNA from fish scales. *Indian Journal of Experimental Biology*, vol. 45, no. 11, pp. 992–997.

27. Cawthorn D., Steinman H., Witthuhn R.C. (2011) Comparative study of different methods for the extraction of DNA from fish species commercially available in South Africa. *Food Control*, vol. 22, no. 2, pp. 231-244. https://doi:10.1016/j.foodcont.2010.07.003