Research Article *http://dx.doi.org/10.4314/mejs.v16i2.10* ***Open Access***

Efficiency Evaluation of DNA Isolation Techniques in Fins of *Channa marulius* Using PCR Amplification and NanoDrop

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ABSTRACT

Highly pure and good-quality extracted DNA is vital for PCR amplification, gene sequencing, and species identification. Seven genomic DNA isolation techniques were used to isolate the DNA from the fins of *Channa marulius*. The concentration and purity of isolated DNA were assessed with PCR amplification of the mitochondrial COI gene and NanoDrop. The phenol-chloroform method was found significantly ($P<0.05$) higher in concentration (1001 $ng.ul^{-1}$) as compared to all other six evaluated DNA isolation methods. In terms of purity of isolated DNA GeneJET, the Genomic DNA Purification Kit was found significantly ($P<0.05$) higher among all analyzed methods. Both the Phenol chloroform method and GeneJET Genomic DNA Purification Kit were successfully used in PCR amplification. Concluding that the Phenol chloroform method and GeneJET Genomic DNA Purification Kit may be successfully used in PCR amplification. The phenol-chloroform method and GeneJET Genomic DNA Purification Kit proved the best in the extraction of DNA in high concentration with maximum yield and purity from fins of *C. marulius* and can be helpful to isolate DNA from fish species because a small quantity of fin tissues is required for DNA isolation without harm to fish. Moreover, data reported from this study about DNA isolation techniques may be used for molecular approaches like PCR amplification and gene sequencing of fishes.

Keywords: GeneJET genomic DNA purification kit, Molecular techniques, NanoDrop, Quantification, *Channa marulius.*

1. INTRODUCTION

High-quality DNA isolation is a critical step for molecular biology (Ahmad and Naeem, 2023). Deoxyribonucleic acid (DNA) stores and transmits hereditary information from parents to the new offspring generation after generation. In all living species, it is known as hereditary material, and it is widely used in molecular research. The different DNA extraction techniques play a vital role in the useful isolation of purified and significant amounts of DNA. DNA is helpful in scientific investigations for diagnostic, forensic, genetic, and medicinal applications (Mezzomoa et al., 2021). Impurities in extracted DNA, such as proteins, lipids, and various organic and inorganic chemicals, obstruct further examination of the DNA, particularly by PCR, and reduce the quality of stored DNA (Ahmad and Naeem,

Submitted :28th November 2023 Accepted : 11th February 2024 Published : 14th September 2024

2022). Many factors influence the amount of DNA extraction and purity, including sample size, extraction procedures, and sample storage.

In the case of fish, it is preferable to isolate DNA from fins and scales because both tissues are attractive sources of DNA isolation and are non-destructive to fish (Ahmad and Naeem, 2023). Because scales are not present in all fish species, fins are preferred for DNA isolation over scales (Oosting et al., 2020). Many researchers have attempted to separate DNA from animals kept in natural history museums, but they have not been successful in recovering significant amounts of DNA from the preserved specimens. These species were most likely captured before the development of molecular biology. By successfully extracting DNA from preserved specimens, one can gain a deeper understanding of genetic and molecular evolution of diverse species (Oosting et al., 2020; Mezzomoa et al., 2021; Ahmad and Naeem, 2022; Ahmad and Naeem, 2023).

The goal of this study was to extract substantial amounts of DNA from fish fins that could be used for efficient PCR amplification. Furthermore, removing small fins (a few centimeters) has no negative impact on fish. Therefore, seven genomic DNA isolation techniques used to isolate the DNA from fins of *Channa marulius*. The concentration and purity of isolated DNA were assessed with PCR amplification of mitochondrial COI gene and NanoDrop.

2. MATERIALS AND METHODS

2.1. Study Area and Sample Collection

A total of seventy *Channa marulius* specimens were collected from Sulemanki Headworks, River Satluj, Punjab, Pakistan (73° 51' 58.6"E, 30° 22' 40.3"N). Fish dorsal fins, pelvic fins, pectoral fins, anal fins, and caudal fins were removed and put in a sterile Eppendorf tube (2 mL) for DNA isolation. The seven different DNA extraction techniques, Urea SDS method, Phenol chloroform method, SNET method, TNES method, Salt out method, Rapid MT method, and GeneJET Genomic DNA Purification Kit were used for DNA extraction.

2.2. DNA Isolation

2.2.1. GeneJET Genomic DNA Purification Kit

GeneJET Genomic DNA Purification Kit (Thermo Scientific #K0721. Pub. No. MAN0012663) was used to isolate DNA. Each fin sample of 20mg was ground in liquid nitrogen. The homogenized mixture was put into micro-centrifuge tube. Then added a 180µl digestion solution. After that Proteinase K 20µl was added and mixed in the mixture with a vortex mixer. Then incubated it at 56°C. Then RNase solution 20µl was added and incubated

at room temperature for 10 minutes. Then lysis solution 200µl was added and mixed for 15 seconds. Then 400µl ethanol (50%) was added and mixed. After that lysate was transferred into GeneJET Genomic DNA Purification column. Then it was centrifuged at 6000 rpm for 1 minute. Then the mixture was transferred into a new micro-centrifuge tube (2 ml). Then Wash Buffer I 500µl was added and centrifuged at 8000 rpm for 1 minute. After that 500µl Wash Buffer II was added to GeneJET Genomic DNA Purification Column. Then it was centrifuged at 12000 rpm for 3 minutes and after centrifugation, it was transferred into micro-centrifuge tube and added 200µl elution buffer. Then it was incubated at room temperature for 2 minutes and centrifuged at 8000 rpm for 1 minute. Then purified DNA was collected and stored at -20°C (Ahmad and Naeem 2022).

2.2.2. DNA Extraction (Urea-SDS Method)

Take 20 mg of fish sample in 100µl TESU 6 Buffer and mix until homogenized. Add 30µl Proteinase K and mix by vortex and incubate at 55^{o}C for 1-2 hours. Add 10µl of 5M NaCl and mix with inversion and Centrifuge at 7000 rpm and take supernatant. Add an equal volume of phenol: chloroform: isoamyl alcohol i.e. 25: 24: 1 ratio respectively and mix with inversion then centrifuge mixture at 1000 rpm for 10 min. Take supernatant. Add an equal volume of chilled isopropyl alcohol (isopropanol) and invert several times. Keep at -20^{o}C overnight and then centrifuge at 1000 rpm for 10 min. DNA precipitates are washed with 70% ethanol and suspended in 60µl distilled H_2O (Li et al., 2012).

2.2.3. DNA Extraction (SNET Method)

Take 20mg of tissue in 500µl buffer and homogenize the mixture. Add 30µl Proteinase K and vortex. Incubate overnight at 55^{o}C. Centrifuge at 7000 rpm and take supernatant (700µl). Add an equal volume of phenol: chloroform: isoamyl alcohol i.e. 25: 24: 1 ratio respectively and mix by shaking. Incubate at room temperature for 30 min and centrifuge at 1400 rpm for 5 min. Take supernatant and add an equal volume of chilled isopropanol (600-700µl) and centrifuge at 800 rpm for 15 min and wash with 70% ethanol, dry and add 60µl distilled H_2O take suspend DNA in water (Hofkar and Deursen, 2011).

2.2.4. DNA Extraction (TNES Method)

Take 20mg of tissue and homogenize in 800µl buffer (extraction TNES Buffer). Add 10µlRNase and homogenize and incubate for 1hour at 42^{o}C. Add 10µl Proteinase K and maintain at 42^{o}C for overnight centrifuge at 3000 rpm and take supernatant (700µl). Add 700µl of phenol: chloroform: isoamyl alcohol i.e. 25: 24: 1 ratio. Centrifuge at 1000 rpm for 15 min. Take top aqueous layer (500µl). Add 1M NaCl (50µl) and two-volume of ethanol

100% and centrifuge at 10000 rpm for 15 min. Discard supernatant, take ppt DNA then wash DNA with 70% ethanol at the end add 60 µl d. H_2O (Zhong et al., 2012).

2.2.5. DNA Extraction (Rapid-MT Method)

Take 20mg tissue and homogenize in 660µl buffer and add 20µl PK (vortex, homogenize). Incubate overnight at 55ºC and centrifuge at 12000 rpm for 15 min. Add 400µl isopropanol and mix by inverting the tube 25 times and centrifuge at 12000 rpm for 10 min. Wash with 70% ethanol. At the end add 60µl water (Mukhopadhyay and Bhattachatjee, 2014).

2.2.6. DNA Extraction (Phenol Chloroform Method)

Take a 20 mg sample. Homogenize with 600-800 µl of extraction buffer (by pestle and mortar) and Take 1.5ml paste in tube. Add 12µl Proteinase K in paste. Mix with vortex mixer. Incubate at 37ºC for 1 hour (in water bath) and incubate at 55ºC for 1 hour. Centrifuge at 500 rpm for 10 min and collect supernatant in new eppendorf tube. Add phenol: chloroform: isoamyl alcohol (25: 24: 1), centrifuge at 12000 rpm for 10min and collect the upper top layer. Add chloroform: isoamyl alcohol (24: 1), centrifuge at 12000 rpm for 10min and collect upper top layer, add 0.1 volume of 3M sodium acetate and an equal volume of ice-cold ethanol 100%. Mix the solution, incubate at -20ºC for 2 hours, and centrifuge at 1000 rpm for 10 min. Remove supernatant (DNA in the palette at lower bottom) and add 100µl of 70% ethanol. Centrifuge at 1000 rpm for 10 min and remove supernatant (ethanol) and dry it (Chowdhury et al., 2016).

2.2.7. DNA Extraction (High Salt Method)

Take 20 mg of the sample, add 600µl TNES buffer and 35µl of proteinase K and incubate samples overnight for5-24 hours at 50ºC (add more PK to speedup reaction and incubate 2-4 hours). Add 166.7 µl of 6M NaCl (or 600µl of 5M NaCl), shake sample vigorously for 20 sec, and centrifuge at 12000-14000 rpm for 5-10 min. Take supernatant, add an equal volume of cold 100% ethanol gently mix by inverting a couple of times (you can see white DNA ppt). More DNA can be obtained if sample is left at -20ºC for a few hours overnight then remove the supernatant carefully (DNA pellet at bottom). Add 200-700µl of 100% ethanol (gently mix) and remove ethanol. Add 70% ethanol (gently mix) and at last remove ethanol (Hofkar and Deursen, 2011).

2.3. Quantification, Visualization and PCR Amplification of Extracted DNA

Analysis of isolated concentration and purity was examined using Nano Drop quantification and PCR amplification with mitochondrial COI genetic marker. The DNA concentration and purity derived from the NanoDrop quantification absorbance (OD) A_{260}/A_{280}. Primes Fish F1 and Fish R1 successfully used for PCR amplification. Fish F1 CO1 5′TCAACCAACCACAA

AGACATTGGCAC-3′ and Fish R1 CO1 5′TAGACTTCTGGGTGGCCAAAGAATCA-3′ were the sequences of the primers. PCR reaction volume 25µl as prepared using 1.5µl DNA template, 12.5µl PCR Master Mix (BLIRT S.A. Taq Nova-Red), forward primer 0.1µl, reverse primer 0.1µl and nuclease-free water 10.8µl. PCR thermal cycler condition was set as initial denaturation at 95°C for 2 minutes, further 30 complete rotations with denaturation at 95°C for 30 seconds, annealing at 54°C for 40seconds, and extension at 72°C for 1 minute. The final extension at 72°C was set for 7 minutes. The success of PCR amplification checked on 2 percent (w/v) agarose gel by running the PCR products.

2.4. Statistical Analysis

The significant level of Isolated DNA concentration evaluated by using ANOVA (one-way analysis of variance) with LSD post hoc test. Statistical analysis of seven different DNA isolation techniques was analyzed using SPSS 17.0 software. The statistically significant levels were evaluated at 5% ($P < 0.05$) of DNA concentration and purity of seven different DNA isolation methods. Minimum concentration, maximum concentration, maximum purity, minimum purity values and Standard Deviation calculated with MS Excel 2016.

3. RESULTS

3.1. Analysis of Isolated DNA Concentrations and Purity

The DNA concentration and purity were evaluated with NanoDrop at optimal absorbance values A_{260}/A_{280}. The concentration of isolated DNA with the Phenol chloroform method was found higher among all fins among all investigated DNA isolation methods. The range purity of isolated DNA with GeneJET Genomic DNA Purification Kit was found higher among all fins as compared to investigated six traditional DNA isolation methods.

3.1.1. Pectoral Fin

In pectoral fin, DNA concentration was found to a maximum of 944 $ng.ul^{-1}$ with Phenol chloroform method as compared with all investigated methods while minimum 304 $ng.ul^{-1}$ DNA concentration was found with SNET Method as compared to investigated other DNA extraction methods (Table 1).

A comparison of mean DNA concentration of seven DNA extraction methods from pectoral fin of *C. marulius* is shown in figure 1A. DNA purity in pectoral fin was found highly pure with an absorbance of 1.75 $ng.ul^{-1}$ with GeneJET Genomic DNA Purification Kit while minimum purity of 1.12 $ng.ul^{-1}$ was found with SNET Method (Table 1). A comparison of mean DNA purity of seven DNA extraction methods from pectoral fin of *C. marulius* is shown in figure 2A.

Table 1. A comparison of DNA concentration and purity obtained with seven DNA extraction methods from pectoral fin of Channa marulius.

Urea SDS Method ± SD	***Abs. A260/ A280 ± SD***	***Phenol Chl. Method ± SD***	***Abs. A260/ A280 ± SD***	***SNET Method ± SD***	***Abs. A260/ A280 ± SD***	***TNES Method ± SD***	***Abs. A260/ A280 ± SD***	***Salt out Method ± SD***	***Abs. A260/ A280 ± SD***	***Rapid MT Method ± SD***	***Abs. A260/ A280 ± SD***	***GeneJET Genomic DNA Purification Kit ± SD***	***Abs. A260/ A280 ± SD***
413-435±5.27	1.40-1.69 ±0.17	911-944 ±10.95	1.71-1.88 ±0.05	304-315 ±3.06	1.12-1.45 ±0.03	801-809 ±8.74	1.82-1.91 ±0.09	446-480 ±4.06	1.34-1.45 ±0.10	321-340 ±6.80	1.22-1.44 ±0.07	870-885 ±11.49	1.71-1.75 ±0.03

Table 2. A comparison of DNA concentration and purity obtained with seven DNA extraction methods from pelvic fin of C. marulius.

Urea SDS Method ± SD	***Abs. A260/ A280 ± SD***	***Phenol Chl. Method ± SD***	***Abs. A260/ A280 ± SD***	***SNET Method ± SD***	***Abs. A260/ A280 ± SD***	***TNES Method ± SD***	***Abs. A260/ A280 ± SD***	***Salt out Method ± SD***	***Abs. A260/ A280 ± SD***	***Rapid MT Method ± SD***	***Abs. A260/ A280 ± SD***	***GeneJET Genomic DNA Purification Kit ± SD***	***Abs. A260/ A280 ± SD***
423-450 ±8.36	1.32-1.60 ±0.09	901-924 ±6.45	1.71-1.85 ±0.04	301-330 ±8.74	1.22-1.55 ±0.11	821-849 ±8.74	1.92-2.01 ±0.03	426-440 ±4.86	1.44-1.55 ±0.03	301-345 ±13.51	1.12-1.54 ±0.13	840-865 ±8.51	1.71-1.73 ±0.01

Table 3. A comparison of DNA concentration and purity obtained with seven DNA extraction methods from dorsal fin of C. marulius.

Urea SDS Method ± SD	***Abs. A260/A 280 ± SD***	***Phenol Chl. Method ± SD***	***Abs. A260/ A280 ± SD***	***SNET Method ± SD***	***Abs. A260/ A280 ± SD***	***TNES Method ± SD***	***Abs. A260/ A280 ± SD***	***Salt out Method ± SD***	***Abs. A260/ A280 ± SD***	***Rapid MT Method ± SD***	***Abs. A260/ A280 ± SD***	***GeneJET Genomic DNA Purification Kit ± SD***	***Abs. A260/ A280 ± SD***
403-455 ±17.55	1.10-1.40 ±0.13	990-1001 ±3.86	1.71-1.99 ±0.08	314- 355 ±12.97	1.42-1.60 ±0.06	820-849 ±10.12	2.22-2.41 ±0.06	436-460 ±9.36	1.14-1.65 ±0.17	301-360 ±20.53	1.12-1.54 ±0.14	905-930 ±8.42	1.71-1.72 ±0.01

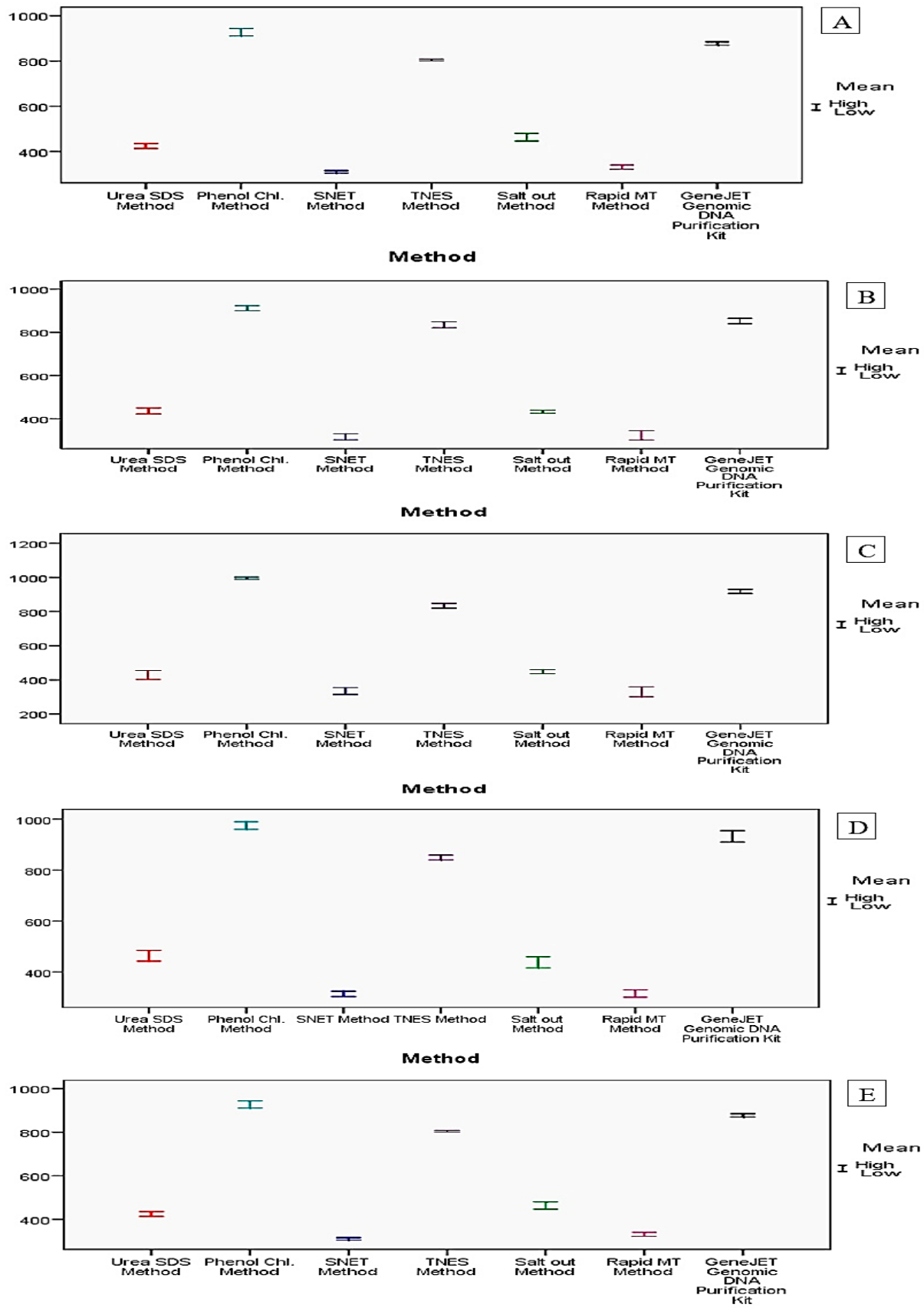

Figure 1. A comparison DNA concentration obtained with seven DNA extraction methods from (A) pectoral fin; (B) pelvic fin; (C) dorsal fin; (D) anal fin; (E) caudal fin of C. marulius. High and low bars indicate the mean concentration values.

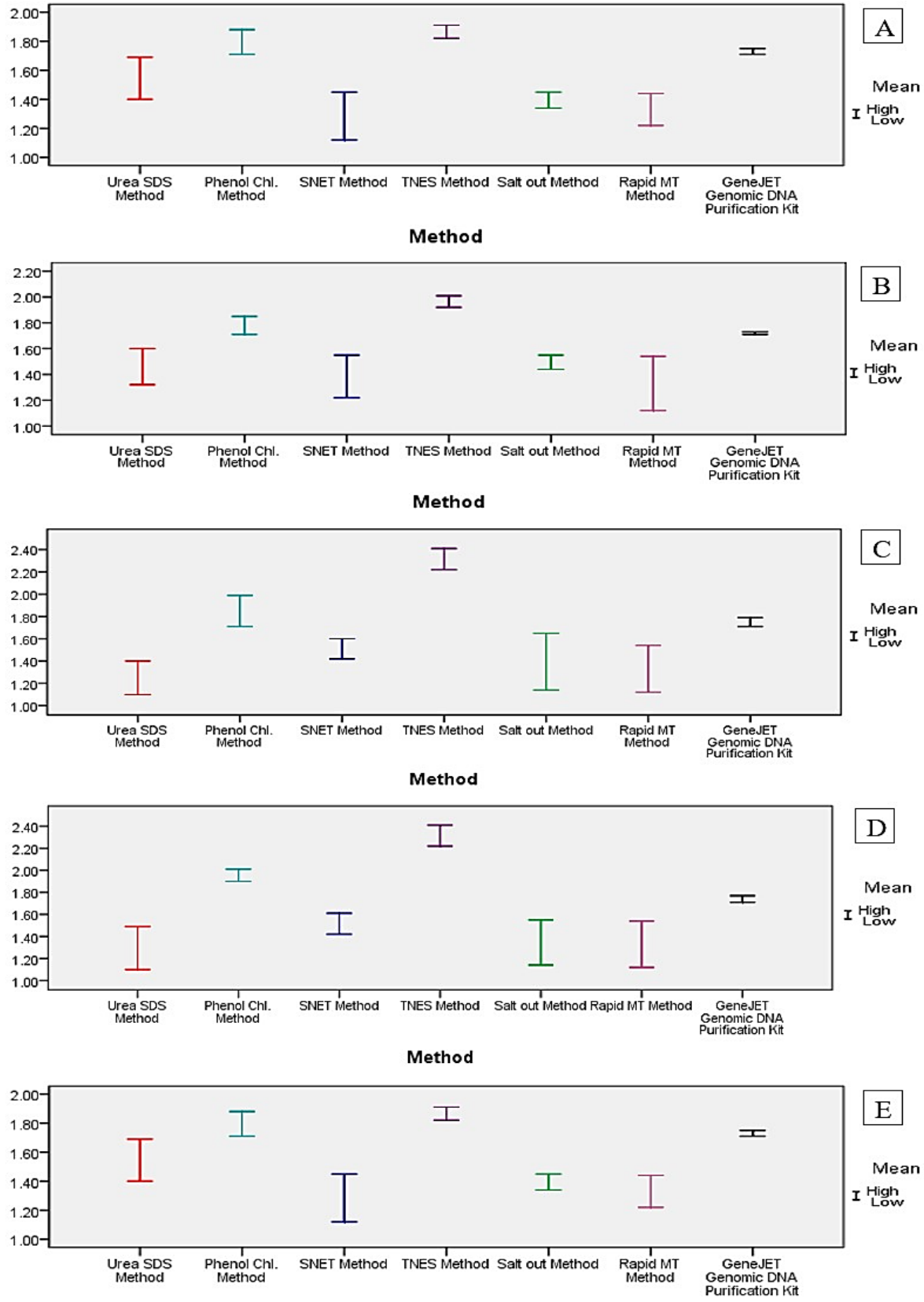

Figure 2. A comparison of DNA purity obtained with seven DNA extraction methods from (A) pectoral fin; (B) pelvic fin; (C) dorsal fin; (D) anal fin; (E) caudal fin of C. marulius. High and low bars indicate the mean concentration values.

3.1.2. Pelvic Fin

In pelvic fin, DNA concentration was found to a maximum of 924 ng.ul^{-1} with Phenol chloroform method as compared with all investigated methods while minimum 301 ng.ul^{-1} DNA concentration was found with both SNET Method and Rapid MT Method (Table 2).

A comparison of mean DNA concentration of seven DNA extraction methods from pelvic fin of *C. marulius* is shown in figure 1B. DNA purity in pelvic fin was found highly pure with an absorbance of 1.73 ng.ul^{-1} with GeneJET Genomic DNA Purification Kit while minimum purity of 1.12 ng.ul^{-1} was found with the Rapid MT Method (Table 2). A comparison of mean DNA purity of seven DNA extraction methods from pectoral fin of *C. marulius* is shown in figure 2B.

3.1.3. Dorsal Fin

In dorsal fin, DNA concentration was found to a maximum of 1001 ng.ul^{-1} with Phenol chloroform method as compared with all investigated methods while minimum 301 ng.ul^{-1} DNA concentration was found with the Rapid MT Method (Table 3).

A comparison of mean DNA concentration of seven DNA extraction methods from pelvic fin of *C. marulius* is shown in figure 1C. DNA purity in dorsal fin was found highly pure with an absorbance of 1.72 ng.ul^{-1} with GeneJET Genomic DNA Purification Kit while minimum purity of 1.12 ng.ul^{-1} was found with the Rapid MT Method (Table 3). A comparison of mean DNA purity of seven DNA extraction methods from pectoral fin of *C. marulius* shown in figure 2C.

3.1.4. Anal Fin

In anal fin, DNA concentration found to a maximum of 990 ng.ul^{-1} with Phenol chloroform method as compared with all investigated methods while minimum 301 ng.ul^{-1} DNA concentration was found with the Rapid MT Method (Table 4).

A comparison of mean DNA concentration of seven DNA extraction methods from pelvic fin of *C. marulius* shown in figure 1D. DNA purity in anal fin was found highly pure with an absorbance of 1.77 ng.ul^{-1} with GeneJET Genomic DNA Purification Kit while minimum purity of 1.12 ng.ul^{-1} was found with the Rapid MT Method (Table 4). A comparison of mean DNA purity of seven DNA extraction methods from pectoral fin of *C. marulius* is shown in figure 2D.

3.1.5. Caudal Fin

In caudal fin, DNA concentration was found to a maximum of 947 ng.ul^{-1} with Phenol chloroform method as compared with all investigated methods while minimum 303 ng.ul^{-1} DNA concentration was found with both SNET Method and Rapid MT Method (Table 5).

Table 4. A comparison of DNA concentration and purity obtained with seven DNA extraction methods from anal fin of C. marulius.

Urea SDS Method ± SD	***Abs. A260/ A280 ± SD***	***Phenol Chl. Method ± SD***	***Abs. A260/ A280 ± SD***	***SNET Method ± SD***	***Abs. A260/ A280 ± SD***	***TNES Method ± SD***	***Abs. A260/ A280 ± SD***	***Salt out Method ± SD***	***Abs. A260/ A280 ± SD***	***Rapid MT Method ± SD***	***Abs. A260/ A280 ± SD***	***GeneJET Genomic DNA Purification Kit ± SD***	***Abs. A260/ A280 ± SD***
443-485 ±14.16	1.10-1.49 ±0.13	960-990 ±9.24	1.90-2.01 ±0.03	304-325 ±6.45	1.42-1.61 ±0.06	840-859 ±6.69	2.20-2.41 ±0.07	416-460 ±14.11	1.14-1.55 ±0.13	301-330 ±9.64	1.12-154 ±48.27	910-955 ±14.66	1.71-1.77 ±0.02

Table 5. DNA concentration and purity analysis in caudal fin of C. marulius.

Urea SDS Method ± SD	***Abs. A260/ A280 ± SD***	***Phenol Chl. Method ± SD***	***Abs. A260/ A280 ± SD***	***SNET Method ± SD***	***Abs. A260/ A280 ± SD***	***TNES Method ± SD***	***Abs. A260/ A280 ± SD***	***Salt out Method ± SD***	***Abs. A260/ A280 ± SD***	***Rapid MT Method ± SD***	***Abs. A260/ A280 ± SD***	***GeneJET Genomic DNA Purification Kit ± SD***	***Abs. A260/ A280 ± SD***
425-452 ±8.36	1.33-1.61 ±0.09	924-947 ±6.45	1.71-1.85 ±0.04	303-332 ±8.74	1.23-1.56 ±0.11	823-851 ±8.74	1.93-2.02 ±0.03	428-442 ±4.86	1.46-1.57 ±0.03	303-347 ±13.51	1.13-1.56 ±0.13	841-866 ±8.51	1.73-1.75 ±0.01

DNA concentration (mean) of seven DNA extraction methods from caudal fin of *C. marulius* shown in figure 1E. DNA purity in caudal fin was found to be highly pure with an absorbance of 1.73 $ng.ul^{-1}$ with GeneJET Genomic DNA Purification Kit while minimum purity of 1.13 $ng.ul^{-1}$ was found with the Rapid MT Method (Table 5). DNA purity (mean) of seven DNA extraction methods from caudal fin of *C. marulius* shown in figure 2E.

The isolated DNA concentration with the Phenol chloroform method, GeneJET Genomic DNA Purification Kit, Urea SDS method, SNET method was found significantly ($P<0.05$) higher, but Salt out method and Rapid MT method concentration were not found non-significantly ($P>0.05$) higher among all fins of *C. marulius*.

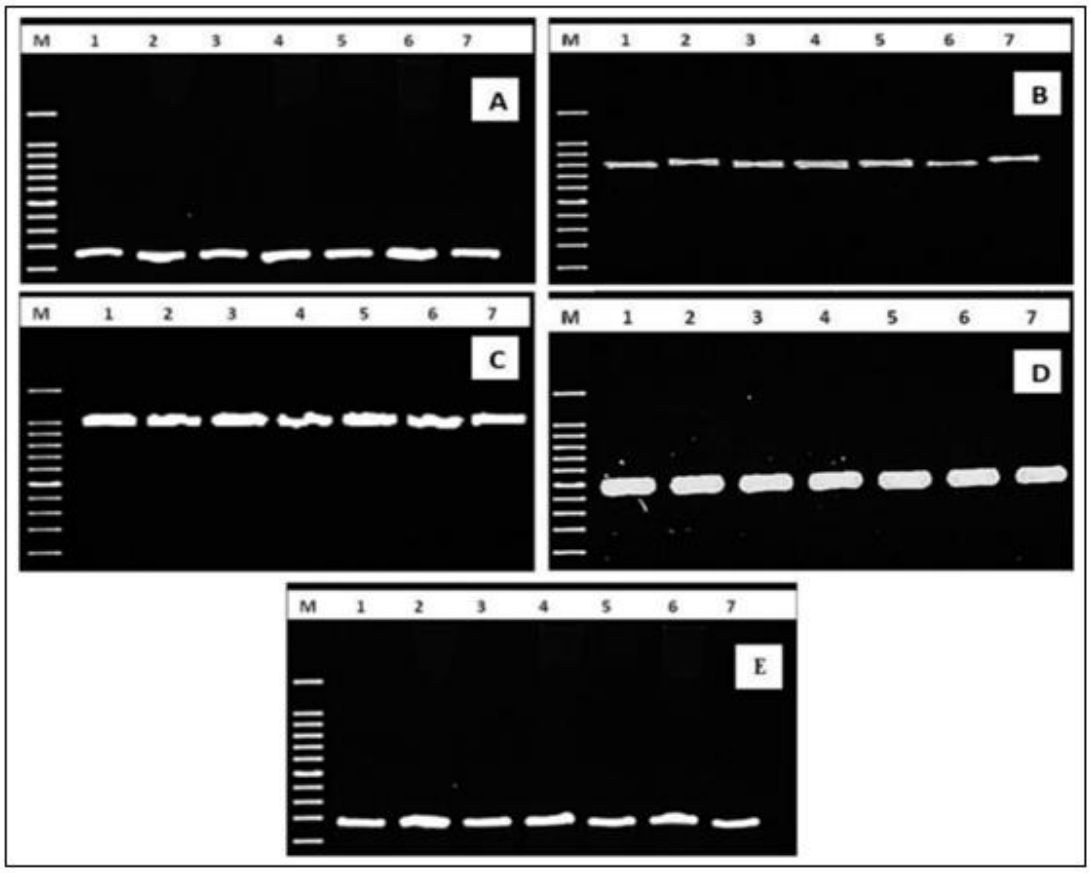

Figure 3. The PCR amplification comparison between seven different DNA isolation techniques. Lane 1; Urea SDS method, Lane2; Phenol chloroform method, Lane3; SNET method, Lane4; TNES method, Lane5; Salt out method, Lane6; Rapid MT method, Lane7; GeneJET Genomic DNA Purification Kit and M; Standard marker in (A) pectoral fin; (B) pelvic fin; (C) dorsal fin;(D) anal fin; (E) caudal fin of C. marulius.

3.1.6. PCR Amplification Success

PCR amplification with isolated DNA in pectoral fins, pelvic fins, dorsal fins, and anal fins were successfully amplified (Fig 3). PCR amplification success rate checked by running the PCR products on 2% (w/v) agarose gel.

3.1.7. Safety Considerations

In terms of safety considerations, the reagents used in all seven different DNA isolation methods evaluated. Among all evaluated methods the GeneJET Genomic DNA Purification Kit was found highly safe while Phenol chloroform method has high safety and health concerns. The Phenol chloroform method considered dangerous because phenol is poisonous to the eyes and burns the skin if accidentally exposed to skin and eyes due to its highly corrosive nature.

3.1.8. Economic Viability

The Phenol chloroform method provides the maximum yield of isolated DNA from a single sample. The cost per microgram calculation of isolated DNA showed that the Phenol chloroform method was estimated economically feasible and cost-effective method in yield and concentration of isolated DNA per microgram, per individual sample.

4. DISCUSSION

Isolation of genomic DNA is a vital step for PCR amplification, DNA barcoding and genetic diversity analysis of species therefore, selected method needs to be efficient and economical (Muhammad et al., 2016; Mezzomoa et al., 2021). Most of the genetic analysis relies on high-quality DNA extraction. In fishes, DNA is extracted mostly from muscle tissues, liver, and blood, which is only possible through animal sacrifice (Estoup et al., 1996; Oosting et al., 2020). In the case of endangered species or individuals from a small population, these tissues are not suitable for DNA-based research. Fish fins appear to be particularly appealing for DNA extraction (Oosting et al., 2020; Mezzomoa et al., 2021). Due to the small size and hard nature of these tissues, only a minimal amount of DNA can be extracted but in present study, we successfully isolate the good quality and high concentration of DNA from fin tissues (Kumar et al., 2007).

In DNA isolation techniques liquid nitrogen use is an effective strategy to dissolve hard tissues to get a high quantity of DNA, which is exactly used in the present study in GeneJET Genomic DNA Purification Kit (Pinto et al., 2000; Mezzomoa et al., 2021). Liquid nitrogen is recommended for digestion and homogenization of tissues to isolate good quality DNA did not give any further improvement in the isolation of DNA in our experiments (Oosting et al., 2020; Mezzomoa et al., 2021).

The data in table 1 revealed that in pectoral fin DNA concentration was found maximum 944 $ng.ul^{-1}$ with Phenol chloroform method as compared with all investigated methods while minimum 304 $ng.ul^{-1}$ DNA concentration was found with SNET Method as

compared to investigated other DNA extraction methods as compared to Ahmad and Naeem (2022) in *Notopterus notopterus*; Mezzomoa et al. (2021); and Parpinelli and Ribeiro (2009). Table 1 also revealed that DNA purity in pectoral fin was found with an absorbance of 1.75 $ng.ul^{-1}$ with GeneJET Genomic DNA Purification Kit while minimum purity of 1.12 $ng.ul^{-1}$ was found with SNET Method as compared to Ahmad and Naeem (2022) in *Notopterus notopterus* and Parpinelli and Ribeiro (2009).

The data in table 2 indicate that in pelvic fin DNA concentration was found to maximum with Phenol chloroform method as compared with all investigated methods while minimum DNA concentration was found in both SNET method and Rapid MT method as compared to Ahmad and Naeem (2022) in *Notopterus notopterus*, Mezzomoa et al. (2021) in *Hypostomus commersoni*, and Parpinelli and Ribeiro (2009) in *Oreochromis niloticus*. Table 2 also indicates that DNA purity in pelvic fin was found high with GeneJET Genomic DNA Purification Kit whereas minimum purity found in the Rapid MT method as compared to Ahmad and Naeem (2022) in *Notopterus notopterus*, Mezzomoa et al. (2021) in *Hypostomus commersoni* and Parpinelli and Ribeiro (2009) in *Oreochromis niloticus*.

The data in table 3 suggest that in dorsal fin DNA concentration was found maximum with Phenol chloroform method as compared with all investigated methods while minimum DNA concentration was found in the Rapid MT method as compared to Ahmad and Naeem (2022) in *Notopterus notopterus*, Oosting et al. (2020); Parpinelli and Ribeiro (2009). Table 3 also suggests that DNA purity in dorsal fin was found high with GeneJET Genomic DNA Purification Kit whereas minimum purity was found in the Rapid MT method as compared to Ahmad and Naeem (2022) in *Notopterus notopterus*; Oosting et al. (2020); and Parpinelli and Ribeiro (2009).

The data in table 4 revealed that in anal fin DNA concentration was found maximum with Phenol chloroform method as compared with all investigated methods whereas minimum DNA concentration was found in the Rapid MT Method as compared to Ahmad and Naeem (2022) in *Notopterus notopterus*; Oosting et al. (2020). Table 4 of present study revealed that DNA purity in anal fin was found high with GeneJET Genomic DNA Purification Kit whereas minimum purity was found in the Rapid MT method as compared to Ahmad and Naeem (2022) in *Notopterus notopterus* and Parpinelli and Ribeiro (2009). Table 5 also revealed that in caudal fin DNA concentration was found maximum with Phenol chloroform method as compared with all investigated methods whereas minimum DNA concentration was found in both SNET Method and Rapid MT Method as compared to Ahmad and Naeem (2022) in *Notopterus notopterus*; Oosting et al. (2020).

The data in table 5 revealed that DNA purity in caudal fin was found high with GeneJET Genomic DNA Purification Kit whereas minimum purity was found in the Rapid MT method as compared to Ahmad and Naeem (2022) in *Notopterus notopterus* and Parpinelli and Ribeiro (2009).

Results of present study revealed that the isolated DNA concentration with the Phenol chloroform method, GeneJET Genomic DNA Purification Kit, Urea SDS method, SNET method was found significantly ($P<0.05$) higher whereas, DNA concentrations with Salt out method and Rapid MT method found lowest among dorsal fins, pelvic fins, pectoral fins, anal fins, and caudal fins of *C. marulius* as reported by Ahmad and Naeem (2022) in fins of *Notopterus notopterus*.

Comparison DNA concentration suggests (Fig 1) that the Phenol chloroform method has produced high concentration of DNA compared to Ahmad and Naeem (2022) reported in *Notopterus notopterus*; and Parpinelli and Ribeiro (2009) reported in in *Oreochromis niloticus*. In terms of DNA purity the present study (Fig 2) showed that GeneJET Genomic DNA Purification Kit produced high purity of DNA as reported by Ahmad and Naeem (2022) in *Notopterus notopterus,* Present study showed that PCR amplification (Fig 3) with isolated DNA in pectoral fins, pelvic fins, dorsal fins, anal fins and caudal fin were successfully amplified as reported by Ahmad and Naeem (2022) in *Notopterus notopterus*; and Parpinelli and Ribeiro (2009) reported in *Oreochromis niloticus*.

In the Phenol chloroform method, the use of phenol, chloroform, and isoamyl alcohol is very essential for the separation of proteins from the DNA in the lysed tissue. Following this, a chloroform treatment of the extracted DNA is essential to remove any left-over proteins that may have been introduced during pipetting. The addition of sodium acetate salt to the isopropanol is also crucial for the condensation of DNA threads to a detectable level, which is then eliminated by giving the DNA molecule a double wash with 70% ethanol (Pinto et al., 2000; Parpinelli and Ribeiro, 2009). Comparison of isolated DNA concentration at absorbance A_{260}-A_{280}nm yielded a DNA-proteins relationship of 1.7-2.0, indicating acceptable quality of isolated DNA (Chowdhury et al., 2016). PCR was amplified using mitochondrial COI genetic markers with isolated DNA of all seven investigated methods successfully showed in figure 3 of present study as Ahmad and Naeem (2022) used mitochondrial COI genetic markers for PCR amplification in *Notopterus notopterus*.

DNA concentration was found to be high with Phenol chloroform method in dorsal fin while minimum DNA concentration was found with Rapid MT Method. DNA purity with GeneJET Genomic DNA Purification Kit was found maximum among all investigated

methods. The isolated DNA concentration with the Phenol chloroform method, GeneJET Genomic DNA Purification Kit, Urea SDS method, SNET method was found significantly ($P<0.05$) higher, but Salt out method and Rapid MT method concentration found lowest among dorsal fins, pelvic fins, pectoral fins, anal fins, and caudal fins of *C. marulius*.

5. CONCLUSION

This study concludes that DNA isolated with Phenol chloroform method from fin tissue of freshwater fish *C. marulius* produces high yield and good quality of DNA. In terms of purity GeneJET, Genomic DNA Purification Kit produce highly pure DNA. Moreover, SNET method and Rapid MT method were found least successful and produced the lowest quantity and less quality DNA from fins of *C. marulius* among all methods. So, the Phenol chloroform method and GeneJET Genomic DNA Purification Kit methods can be effectively applied for DNA isolation from the fin of other freshwater fish species.

6. ACKNOWLEDGEMENTS

We would like to thank Waqas Ahmad, Bilal Ahmad, for assisting in sample collection.

7. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

8. REFERENCE

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