



Effect of *Psidium guajava* and *Mangifera indica* Leaves Extracts on Growth, Antioxidant and Metabolic Enzymes Activities of *Labeo rohita* Fingerlings

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ABSTRACT

Leaf extracts of guava and mango were selected for this study after *in vitro* antioxidant screening test, and a feeding trial was conducted to examine their effects on growth, antioxidant and metabolic enzymes activities in *Labeo rohita* (rohu) fingerlings. Six diets were formulated containing either 0 (Control), 0.5%, or 1 % leaf extracts of guava or mango. The growth performance indices increased significantly in the treatment groups compared with the control. Feed conversion ratio (FCR) among the various groups differed significantly, but feed intake and hepatosomatic index showed no differences ($P>0.05$). Significantly higher ($P<0.05$) serum glucose level, antioxidant, and tissue metabolic enzymes activities were recorded in the control compared with the leaf extracts fed groups. The current study revealed that the leaf extracts of guava or mango positively enhanced the growth performance of the fed fish (*L. rohita*) and has the potential to attenuate oxidative-induced stress.

1.0 Introduction

Medicinal plants serve as a reservoir of diverse chemicals with potency to inhibit oxidative damage and serves as free radical scavenger and immuno-modulator in fish, shrimp and livestock animal (Vendemiale et

al., 1999, Makare et al., 2001, Citarasu et al., 2006, Fawole et al., 2016). Reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (HO) are regularly formed inside the animal body from normal cellular metabolic activities (Tocher et al., 2002).

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and their actions are brought under control by a balanced system of endogenous antioxidant enzymes. However, disrupting this balance may cause oxidative stress because the amount of antioxidant present in the body under normal physiological condition may be insufficient to quench the free radicals generated under stress conditions, thereby inflicting damages on cell and macromolecules (Halliwell and Gutteridge, 1999, Amaeze *et al.*, 2011). Studies have shown that one of the best ways to fight against free radical effect is to improve the cellular antioxidant level so as to maintain oxidative stability in the body (Gülçin, 2011). Recently, the use of natural antioxidants of plant origin has considerably increased in fish and animal nutrition because of their enormous potential and magnitude.

Reported findings indicated that certain plant materials rich in polyphenolic compounds can attenuate neuronal cell death induced by oxidative stress (Perez-Perez and Rodriguez-Malaver, 2005, Mancuso *et al.*, 2007), and protect membrane integrity. Having this in mind, guava and mango leaves were selected, tested for their antioxidant capacity (Fawole *et al.*, 2013) and used in this study. Guava (*Psidium guajava*) leaf are very rich in antioxidants (Fawole *et al.*, 2013), and the fruits contain high level of vitamin C (Thaipong *et al.*, 2006). Cheng and Yang, (1983) reported that guava juice (1 g/kg) fed to mice produced hypoglycaemic effects, and several reports attributed the biological effect of guava leaves to the polyphenolic (gallic acid, caffeic acid, quercetin, rutin) compounds present. Similarly, Mango (*Mangifera indica* L.) plants are widely used in traditional medicine for the treatment of several diseases. Their biological effects can be attributed to the active principles such as mangiferin, catechins, quercetin, anthocyanins, gallic acids

(Sanchez *et al.*, 2000, Martin and Qian, 2008). The aqueous extract of the leaves of *M. indica* possess hypoglycaemic activity (Aderibigbe *et al.*, 2001), and the seed kernel was reported to show high antioxidative and tyrosinase inhibitory properties (Maisuthisakul and Gordon, 2009). Several studies have been conducted on different plant extracts in fish such as *Allium sativum* in *Oreochromis niloticus* (Metwally, 2009), *Cinnamomum zeylanicum* in Nile Tilapia (Ahmad *et al.*, 2011), *Mucuna pruriens* in *Labeo rohita* (Ojha *et al.*, 2014), and anthraquinone extracts in *Cirrhinus mrigala* (Sikendra *et al.*, 2015) for their effects on growth and metabolic enzyme activities. Also, ample of *in vitro* studies have been done on the antioxidative capability of guava and mango leaves, but there are dearth of information on their *in vivo* effects on the metabolic and antioxidant enzymes activities in rohu (*Labeo rohita*) fingerlings, one of the most cultured fish in South-east Asia.

Therefore, the present study aimed to investigate the dietary effect of guava and/or mango leaf extracts on the growth, antioxidative and metabolic enzymes activities in rohu fingerlings.

2.0 Material and Methods

2.1. Plant Extracts and Diets Preparation

The leaves of *Psidium guajava* and *Mangifera indica* were collected in and around the campus of ICAR-CIFE, Mumbai. Fresh leaves were cleaned, air dried and ground to powder. The extracts and diets were prepared as described in our previous work (Fawole *et al.*, 2016).

2.2 Experimental Set-up

Labeo rohita fingerlings of similar size (10.36 $\pm$ 0.04 g), were purchased from Hans Aquaculture Pvt Ltd, Mahad, Raigad District,

Maharashtra, India. After acclimatization for 2 weeks under aerated condition, 180 fishes were randomly assigned into 18 plastic rectangular tubs (75 L capacity each), each containing 10 fish, in triplicates. Six different experimental diets were prepared to include, Control (basal diet [BD] + 0% extract); TG-0.5% ([BD] + 0.5% guava leaf extract); TG-1% ([BD] + 1% guava leaf extract); TM-0.5% ([BD] + 0.5% mango leaf extract); TM-1% ([BD] + 1% mango leaf extract); and TGM ([BD] + 0.5% guava leaf extract + 0.5% mango leaf extract). Feeding was done to

satiation, with daily ration divided into 2 equal parts and fed at 10:00 and 18:00. The water quality parameters during the experimental period were within the optimal range for rohu, temperature range between 24 to 28 °C, pH 7.5 to 8.5, dissolved oxygen 6.1 to 7.2 ppm, and ammonia ranged between 0.05 to 0.09 ppm. The experiment was carried out at the Central Wet Laboratory, ICAR-CIFE, Mumbai, and lasted for 5 weeks.

Table 1: Experimental diets and proximate composition of the diets used

Ingredients (g/kg)	Treatments ¹					
	Control	TG-0.5%	TG-1%	TM-0.5%	TM-1%	TGM
Casein (fat free) ²	297	297	297	297	297	297
Gelatin ²	100	100	100	100	100	100
Dextrin ²	183	183	183	183	183	183
Starch soluble ²	215	215	215	215	215	215
Cellulose ²	100	95	90	95	90	90
Cod liver oil ³	30	30	30	30	30	30
Sunflower oil ⁴	30	30	30	30	30	30
Vit-min mix ⁵	20	20	20	20	20	20
CMC ²	15	15	15	15	15	15
Betaine ²	9	9	9	9	9	9
BHT ²	1	1	1	1	1	1
Leaf extract	0	5	10	5	10	10
Total	1000	1000	1000	1000	1000	1000

CMC = Carboxymethyl cellulose; BHT = Butylated hydroxytoluene.¹ Control (0 extract); TG-0.5% (0.5% guava leaf extract); TG-1% (1% guava leaf extract); TM-0.5% (0.5% mango leaf extract); TM-1% (1% mango leaf extract); and TGM (0.5% guava leaf extract + 0.5% mango leaf extract).² Himedia India, Ltd. ³ Universal Medicare PVT. Limited, Mumbai, India.⁴ Purchased from local market, Mumbai, India.⁵ Composition of vitamin-mineral mix (Agrimin) (quantity/2.5kg), Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mcg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 L- lysine, 10 g; DL-Methionine, 10 g; Selenium, 50 ppm.

2.3 Growth Assessment

Fish were starved for 24h at the end of the experiment and batch weighed using

electronic weighing balance. The growth performance was evaluated according to the formula described by Fawole *et al.*, (2016).

2.4 Sample collection and enzyme assays

Sample collections was as described in our previous study (Fawole *et al.*, 2016). Serum glucose was estimated using a diagnostic kit (GOD/POD; INNOLINE MERCK SPECIALITIES, Mumbai, India). Serum total superoxide dismutase (SOD) and catalase (CAT) activities were estimated according to Mishra and Fridovich, (1972), and Takahara *et al.*, (1960), respectively. Protein level in serum was estimated using a commercial kit (Total protein kit; INNOLINE MERCK SPECIALITIES, Mumbai, India), in order to express enzymatic activities of the serum as a function of the protein content. Liver and muscle tissues were collected from the sacrificed fish and supernatant collected as described by Fawole *et al.*, (2016). Aspartate amino transferase (AST) and alanine amino transferase activities were measured by the method described by Wooten, (1964). The hepatic glucose-6-phosphate dehydrogenase (G6PDH) enzyme activity was assayed according to the method of De Moss, (1953). Total soluble protein content of the tissue samples were determined according to

Bradford (1976) and used to express enzyme activities as a function of the protein content.

2.5 Data Analysis

All data were subjected to a one-way analysis of variance (ANOVA-SPSS version 16), and Duncan's multiple range test (DMRT) was used to determine the differences between the means and comparisons were made at the $P < 0.05$. Data expressed as Means $\pm$ SE.

3.0 Results

3.1 Growth and Nutrient Utilization Parameters

At the end of the feeding trial, the weight gain, SGR, MGR, and PER values of the fish fed the control diet was significantly lower than those fed leaf extract-based diets (Table 2). However, there were no significant variations ($P < 0.05$) among the fish fed TG, TM and TGM diets, irrespective of the inclusion levels. The results also revealed that the FCR value decreased significantly ($P < 0.05$) in fish fed TM-0.5% compared with the control, while the feed intake and hepatosomatic index showed no significant differences among the various groups ($P > 0.05$).

Table 2: Growth parameters of *Labeo rohita* fingerlings fed different experimental diets

Variables	Treatments ¹						p-value
	Control	TG-0.5%	TG-1%	TM-0.5%	TM-1%	TGM	
WG (%)	36.37 $\pm$ 0.0 5 ^a	47.64 $\pm$ 1.1 4 ^b	47.99 $\pm$ 2.4 5 ^b	47.45 $\pm$ 0.8 2 ^b	48.41 $\pm$ 1.4 1 ^b	49.43 $\pm$ 0.9 2 ^b	<0.001
MGR	3.61 $\pm$ 0.01 ^a	4.43 $\pm$ 0.07 ^b	4.54 $\pm$ 0.20 ^b	4.51 $\pm$ 0.06 ^b	4.58 $\pm$ 0.12 ^b	4.68 $\pm$ 0.07 ^b	<0.001
SGR	0.97 $\pm$ 0.01 ^a	1.44 $\pm$ 0.16 ^b	1.30 $\pm$ 0.06 ^b	1.29 $\pm$ 0.02 ^b	1.31 $\pm$ 0.03 ^b	1.33 $\pm$ 0.02 ^b	0.01
PER	1.10 $\pm$ 0.05 ^a	1.32 $\pm$ 0.01 ^b	1.29 $\pm$ 0.04 ^b	1.38 $\pm$ 0.06 ^b	1.32 $\pm$ 0.07 ^b	1.31 $\pm$ 0.02 ^b	0.03
FCR	2.58 $\pm$ 0.11 ^b	2.15 $\pm$ 0.01 ^a	2.20 $\pm$ 0.07 ^a	2.06 $\pm$ 0.09 ^a	2.16 $\pm$ 0.12 ^a	2.17 $\pm$ 0.03 ^a	0.02
FI	7.18 $\pm$ 0.35	7.38 $\pm$ 0.16	7.70 $\pm$ 0.08	7.18 $\pm$ 0.24	7.64 $\pm$ 0.24	7.83 $\pm$ 0.07	0.21
HSI	1.18 $\pm$ 0.12	1.29 $\pm$ 0.11	1.16 $\pm$ 0.19	1.26 $\pm$ 0.15	0.99 $\pm$ 0.17	1.05 $\pm$ 0.25	0.80

¹Control (0 extract); TG-0.5% (0.5% guava leaf extract); TG-1% (1% guava leaf extract); TM-0.5% (0.5% mango leaf extract); TM-1% (1% mango leaf extract); and TGM (0.5% guava leaf extract + 0.5% mango leaf extract). WG = Percentage weight gain; FCR = Feed conversion ratio; PER = Protein efficiency ratio; SGR = Specific growth rate; MGR = Metabolic growth rate; FI = Feed intake; HSI = Hepatosomatic index.^a

^b Mean values bearing different superscripts in the same row differ significantly ($P < 0.05$). Data expressed as Mean $\pm$ SE.

3.2 Antioxidant Enzyme Activity and Glucose level

There was a significant ($P<0.05$) difference in the serum SOD and CAT enzyme activities of *L. rohita* fingerlings fed different diets. A significantly higher activity of these enzymes were recorded in the control compared with the leaf extract supplemented groups. The TG-

0.5% and TM-0.5% groups registered the lowest SOD and CAT enzymes activities, respectively (Fig. 1). The serum glucose level among the various groups differed significantly ($P<0.05$) with the highest value recorded in the control compared with the leaf extracts fed groups (Fig. 1).

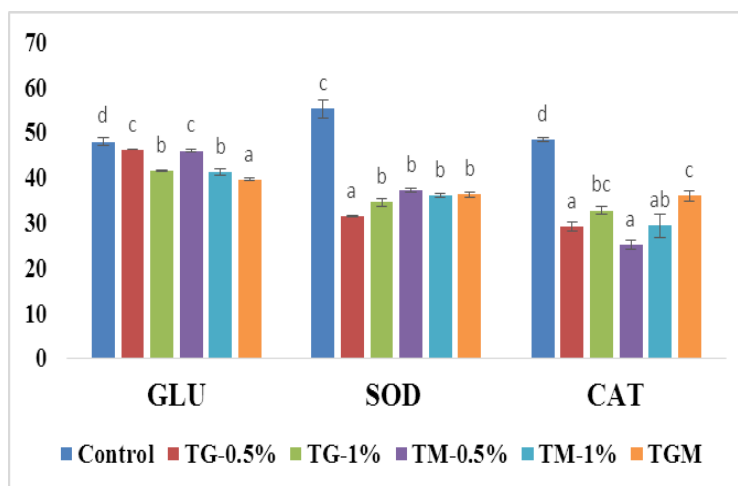


Figure 1: Serum glucose and antioxidant enzyme activities of *Labeo rohita* fingerlings fed different experimental diets. Superoxide dismutase (SOD): $\mu\text{mol}/\text{mg}$ protein/min at 37°C . Catalase (CAT): $\text{mmol H}_2\text{O}_2$ decomposed/min/mg protein at 37°C . Glucose (GLU): mg/dL .^{a, b, c, d} Mean values bearing different superscripts differ significantly ($P<0.05$). Data expressed as Mean $\pm$ SE. Control (0 extract); TG-0.5% (0.5% guava leaf extract); TG-1% (1% guava leaf extract); TM-0.5% (0.5% mango leaf extract); TM-1% (1% mango leaf extract); and TGM (0.5% guava leaf extract + 0.5% mango leaf extract).

3.3 Metabolic Enzyme Activities

A significantly ($P<0.05$) higher AST activity was recorded in the control compared with the treatment groups irrespective of the leaf extracts fed and level of inclusion. The AST activities in the muscle was found to be higher than the liver but differed significantly

($P<0.05$) with the control in both the tissues. A similar trend was also observed for ALT in both the liver and muscle (Table 3). There was a significant difference in the hepatic G6PDH enzyme activity among the various groups ($P < 0.05$) with the highest activity recorded in TG-fed group (Fig. 2).

Table 3: Metabolic enzymes activities of *Labeo rohita* fingerlings fed different diets

Treatments ¹	AST ²		ALT ³	
	Liver	Muscle	Liver	Muscle
Control	10.96±0.24 ^c	14.90±0.03 ^f	8.11±0.02 ^c	19.39±0.38 ^e
TG-0.5%	6.53±0.08 ^a	8.16±0.06 ^b	6.53±0.20 ^b	9.67±0.19 ^c
TG-1%	7.71±0.21 ^b	7.82±0.05 ^a	5.74±0.15 ^{ab}	7.34±0.06 ^a
TM-0.5%	8.08±0.03 ^b	11.27±0.04 ^e	5.57±0.38 ^a	9.76±0.15 ^c
TM-1%	8.08±0.06 ^b	8.48±0.17 ^c	5.73±0.25 ^{ab}	8.46±0.12 ^b
TGM	7.66±0.39 ^b	9.12±0.05 ^d	5.26±0.34 ^a	10.53±0.11 ^d
p-value	<0.001	<0.001	<0.001	<0.001

^{a,b,c} Mean values bearing different superscripts in the same column differ significantly ($P < 0.05$). Data expressed as Mean±SE. ¹ Control (0 extract); TG-0.5% (0.5% guava leaf extract); TG-1% (1% guava leaf extract); TM-0.5% (0.5% mango leaf extract); TM-1% (1% mango leaf extract); and TGM (0.5% guava leaf extract + 0.5% mango leaf extract). ²AST = Aspartate aminotransferase: nano moles oxaloacetate released/mg protein/min at 37°C ³ALT = Alanine aminotransferase: nano moles of sodium pyruvate formed/mg protein/min at 37°C

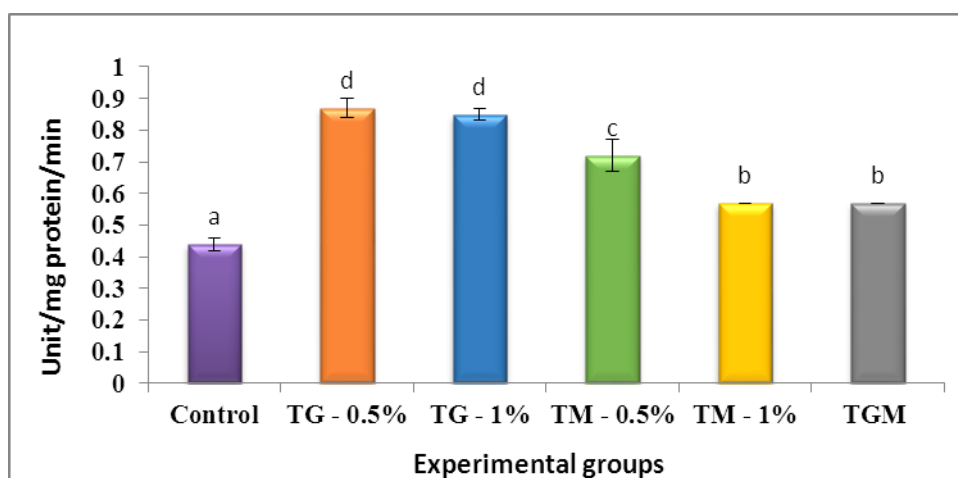


Figure 2: Glucose-6-phosphate dehydrogenase (G6PDH) enzyme activities of *Labeo rohita* fingerlings fed different experimental diets. ^{a, b, c, d} Mean values bearing different superscripts differ significantly ($P < 0.05$). Data expressed as Mean±SE. Control (0 extract); TG-0.5% (0.5% guava leaf extract); TG-1% (1% guava leaf extract); TM-0.5% (0.5% mango leaf extract); TM-1% (1% mango leaf extract); and TGM (0.5% guava leaf extract + 0.5% mango leaf extract).

4.0 Discussion

The growth and immune stimulating potential of plant extracts have been attributed to several bioactive compounds present in it, which often render their effects by modulating enzyme activity, stimulate appetite, act as antioxidant, antistress, and enhance innate immune responses in fish (Chakraborty *et al.*, 2014, Fawole *et al.*, 2016, Wang *et al.*, 2017). In the current study, both guava and mango leaf extracts improved the growth performance and nutrient utilization indices in *L. rohita* fingerlings, which was in accord with previous study in which *Allium sativum* remarkably enhanced the growth performance of *Oreochromis niloticus* and rainbow trout (Nya *et al.*, 2009, Abdel-Hakim *et al.*, 2010). Similarly, Giri *et al.* (2015) reported that dietary supplementation with guava leaves powder at 5 g/kg significantly increased the growth performance and nutrient utilization in *L. rohita* fingerlings. The growth augmenting effects observed in the leaf extracts groups could be attributed to the systemic bioavailability of the bioactive principles present in the leaves extracts, which appears to enhance digestive enzyme function, thereby improving nutrient uptake more efficiently through the wall of the digestive tract. Similar view was expressed by Khalil *et al.*, (2001) that bioactive compound (allicin) present in garlic has the capability to improve the performance of the intestinal flora, and thus, enhanced nutrient digestion in fish. However, the insignificant variation observed among the groups fed leaf extracts might be due to the short duration of the study. Furthermore, the insignificant feed intake recorded among the various groups indicate that the leaf extract had no negative effect on the feed acceptability by the fish, which is similar to the report of Wang *et al.*, (2017) in Japanese seabass fed mixed Chinese herbal medicines.

All vertebrate animals including fish possess a strong immune system to fight against deleterious effects of ROS so as to maintain their health and prevent oxidative induced damage (Jacob, 1995). SOD and CAT are one of the important antioxidant defense systems that help to counteract the harmful effects of this ROS. As observed in this study, the fish fed with various leaf extracts containing diet had a significantly decreased SOD and CAT values compared with the control. This shows that the extracts fed in this study exert antioxidant actions by scavenging ROS, which could result from high blood glucose as reported by Lee and Chung, (1999) that high blood glucose and many biochemical pathways associated with hyperglycemia can lead to increased release of free radicals. Further-more, the lower serum glucose level observed in the treatment groups (TG-1% and TM-1%) compared with the control revealed that the leaf extracts of guava and mango possesses hypoglycaemic effect, and this may be due to their capacity to mimic insulin, thus, improve the cells sensitivity to insulin and enhanced the peripheral utilization of glucose (Eskander and Won, 1995, Reda *et al.*, 2010). Similar observation was made by Ji *et al.*, (2007) in juvenile Japanese flounder fed herbal mixtures.

Transaminase enzymes (AST and ALT) play a major role in the mobilization of amino acids for gluconeogenesis, and act as a link between protein and carbohydrate metabolism during fluctuating energy demands of an organism under different adaptive situation (Metwally, 2009, Gao *et al.*, 2014). The result of the present study revealed that the activities of AST and ALT enzymes in both the organs decreased significantly in the leaf extracts fed groups compared with the control. The higher activities recorded in the control fed fish may be due to the increased deamination or

transamination of amino acids to produce TCA cycle intermediates, thus helping in energy production in the form of ATP to cope with the physiological needs. This situation usually arises when fish requires more energy under altered physiological condition, which is often met through amino acids catabolism. A similar significant decrease in AST and ALT enzymes activities were also reported in *Cirrhinus mrigala* fed anthraquinone extract and *L. rohita* fed ethanolic extract of *Mucuna pruriens* supplemented diet (Ojha *et al.*, 2014, Sikendra *et al.*, 2015).

G6PDH is an enzyme of the pentose phosphate pathway that helps in the conversion of glucose-6-phosphate to 6-phospho-gluconolactone using NADP⁺ as a coenzyme and releases NADPH. The NADPH generated has been related to the internal antioxidant defense of fish through the action of various other related enzyme activities (Bautista *et al.*, 1989), and are utilized by NADPH oxidase for producing ROS for destroying the phagocytosised material. In the current study, G6PDH activity was found to be significantly higher in the treatment groups compared with the control, suggesting that the plant extracts elevated the phagocytic activity and superoxide anion production, thereby enhancing the non-specific immunity of the fed fish. This is complementary to our previous findings where we reported a significantly higher respiratory burst activity of blood leucocyte of *L. rohita* fingerlings fed leaf extracts of guava and mango (Fawole *et al.*, 2016).

5.0 Conclusion

In summary, the results of the current study revealed that the extracts of guava and mango leaves had a marked effect on the serum glucose level, antioxidant and metabolic enzymes activities in *L. rohita* fingerlings. The

leaf extracts positively enhanced the fish performance in terms of growth and could be used as natural feed additive for the protection of cells and vital organs against oxidative insult under culture condition.

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