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**Ajoy Sarkar**

(1) Entomology Laboratory,  
Department of Zoology,  
University of Gour Banga,  
Malda, West Bengal, India  
(2) Department of Zoology,  
Balurghat Mahila  
Mahavidyalaya, Balurghat,  
West Bengal, India

**Mayukh Hore**

Immunopharmacology and  
Molecular Cell Biology  
Laboratory, Department of  
Zoology, University of Gour  
Banga, Malda, West Bengal,  
India

**Kaushik Chakraborty**

Entomology Laboratory,  
Department of Zoology,  
University of Gour Banga,  
Malda, West Bengal, India

**Subhrajyoti Roy**

Immunopharmacology and  
Molecular Cell Biology  
Laboratory, Department of  
Zoology, University of Gour  
Banga, Malda, West Bengal,  
India

**Correspondence**

**Subhrajyoti Roy**

Immunopharmacology and  
Molecular Cell Biology  
Laboratory, Department of  
Zoology, University of Gour  
Banga, Malda, West Bengal,  
India

## Phytochemical screening and determination of antioxidant activity of Lakhanbhog and Langra mango of Malda district, West Bengal

Ajoy Sarkar, Mayukh Hore, Kaushik Chakraborty and Subhrajyoti Roy

### Abstract

The study was carried out to investigate the presence of different phytochemicals as well as to determine the antioxidant activity of Lakhanbhog and Langra mango (pulp & leaf) of Malda district, West Bengal. Phytochemical screening of the mango pulp and leaf was carried out following standard protocols. Antioxidant activity was determined by DPPH free radical scavenging assay. Tannin, phlobatannin, terpenoid, glycoside, phenol, flavonoid, carbohydrate, and protein were present in Lakhanbhog and Langra mango (pulp & leaf), whereas saponin, anthraquinone, cholesterol were absent. Lakhanbhog & Langra mango pulp and leaf also showed significant DPPH radical scavenging activity. Lakhanbhog and Langra mango (pulp & leaf) are the good sources of different pharmaceutically important phytochemicals that exhibit potent antioxidant activity and thereby indicating their importance from the medicinal point of view.

**Keywords:** Phytochemicals, Lakhanbhog, Langra, DPPH scavenging

### Introduction

Phytochemicals, mostly referred to the secondary metabolites, are regarded as the important compound from medicinal point of view <sup>[1]</sup>. Phytochemicals perform a vital role in providing nutrition and healthcare <sup>[2]</sup>. Phytochemicals act as antioxidant and scavenge free radicals that are produced endogenously inside the cell <sup>[3-5]</sup>. Antioxidant activity, provided by different fruit and vegetable, is due to presence of secondary metabolites like tocopherol, carotenoids, phenolic compounds, anthocyanins, etc. <sup>[6]</sup>. Ischemia, lung damage, inflammation etc. are found to be associated with excess amount of free radicals present in the body <sup>[7]</sup>. Phytochemicals extracted from plants play an important role in combating inflammation, cancer, microbial infection, cellular damage, immunomodulation, etc. <sup>[8, 9]</sup>. For example, terpenoids help to fight against cancer, inflammation, malaria, cholesterol synthesis and protects our body against bacterial and viral infection.

It has been reported that fruit peels are capable of showing antioxidant activity <sup>[10]</sup>. Mango (*Mangifera indica*), one of the most popular fruits of the world, belonging to the family Anacardiaceae, is known for its strong aroma and delicious taste. The pulp of mango (unripe & ripe mesocarp), mango leaf and mango bark show antioxidant and free radical scavenging activities <sup>[11]</sup>. In a recent study, it has been found that mango peel showed higher free radical scavenging activity than that of mango pulp <sup>[12]</sup>. Phytochemicals present in mango protect our body against fungal- and viral infection, inflammation, diarrhea and other diseases. The kernels of mango also found to possess immunomodulatory properties in fish <sup>[13]</sup>. Mango is a good source of vitamins and minerals <sup>[14, 15]</sup>. Vitamins like vitamin A, E, B6, C and Vitamin K, along with potassium, phosphorus, magnesium, copper, iron, zinc, calcium, fiber are the some of the major components of mango <sup>[16, 17]</sup>. Different parts of mango can be used as folk medicine <sup>[18, 19]</sup>.

Mango has been cultivated in India from time immemorial and Malda, Murshidabad and Nadia districts of West Bengal, India are famous mango growing areas where local varieties like Langra, Mallika, Lakhanbhog and Himsagar are cultivated profoundly <sup>[20]</sup>. Till date, a lot of phytochemical studies have been performed so far on different varieties of mango all over the world <sup>[21-23]</sup>. But, very little work has been done so far on the local varieties, especially on the varieties like Langra, Mallika, Lakhanbhog and Himsagar. Therefore, the aim of the present study was to investigate the antioxidant activity and the qualitative determination of phytochemicals such as tannin, phlobatannin, terpenoid, glycosides, phenol, flavonoid, steroid,

anthraquinone, saponin, alkaloid, cholesterol, carbohydrate and protein of Lakhanbhog and Langra varieties.

## Material and methods

### Sample collection

Leaf and fruit of Lakhanbhog and Langra mango were collected from the villages of Sattari, Kajigram, Gokul nagar and Kamat of Malda district, West Bengal during the month of April-June of 2016-2017.

### Sample preparation

Leaves were collected and washed with tap water to remove the dirt, and then cut in to small pieces discarding the mid-rib region. The small pieces of leaves were then kept under shade until completely dried and powdered by using a grinder. The powdered sample was then kept at refrigerator for future use.

Fruits (mangoes) were washed in tap water to remove the dirt. The mango pulps were cut into thin slices, kept under shade until completely dried and then grounded into powdered form which was then kept at refrigerator for future use. Phytochemical analysis of the powdered sample of mango pulp and mango leaf were performed following the standard procedures with little modifications<sup>[24-26]</sup>.

The powder (50 g) was mixed with a 500 ml methanol-water solution (7:3) using a shaker for 15 h. Then the mixture was centrifuged at 2000 rpm in a centrifuge and the supernatant was decanted. The pellet was mixed again with a 500 ml methanol-water solution, and the entire process was repeated once again, i.e., the extraction procedure was performed twice. The supernatants collected from the two phases were mixed in a round-bottom flask and concentrated under reduced pressure in a rotary evaporator. The residue (extract) was kept at -20 °C for future use. This extract was used to evaluate the DPPH free radical scavenging activity.

### Qualitative analysis

10 gm of the crude sample was taken in a 250 ml conical flask and 100 ml of distilled water was added in to it. The sample was then mixed using a magnetic stirrer for 10 hr and the filtrate was separated by Whatman filter paper No. 1. The supernatant (aqueous extract) was used in the following qualitative tests.

### Tannin

0.1% ferric chloride solution was added with 10 ml of the aqueous extract. Appearance of black precipitation indicated the presence of tannin.

### Phlobatannin

10 ml of the aqueous extract and 2 ml of concentrated HCl were taken in a test tube and boiled for one minute. Deposition of red precipitate indicated the presence of phlobatannin.

### Carbohydrate

2 ml of aqueous extract and 2 ml of Molish's reagent (5%  $\alpha$ -naphthol in absolute ethanol) were shaken properly. 2 ml of concentrated sulphuric acid was added carefully along the wall of the test tube into the solution. Formation of reddish-violet ring at the junction of two liquid indicated the presence of carbohydrate.

### Protein

2 ml of aqueous extract and 1 ml of 40% sodium hydroxide

solution were mixed properly and 1-2 drops of copper sulphate solution was added to the mixture. Change in the color of the solution to violet indicated the presence of protein.

10 gm of crude sample and 100 ml of 70% methanol were taken in a conical flask and shaken in a magnetic stirrer for 10 hr. The mixture was filtered by Whatman filter paper No. 1 and the filtrate was used to perform the following qualitative tests.

### Terpenoid

5 ml of methanolic extract was mixed with 2 ml of chloroform. Then 3 ml of concentrated sulphuric acid was added carefully into the solution along the wall of the test tube. Appearance of reddish-brown coloration at the junction of two liquid phases indicated the presence of terpenoid.

### Glycoside

5ml of methanolic extract and 2 ml of glacial acetic acid (containing 2% ferric chloride solution) were taken in a test tube. Then, 1ml of concentrated sulphuric acid was added carefully into the solution along the wall of the test tube. Appearance of a brown ring at the interphase of two liquid phases indicated the presence of glycoside.

### Steroid

5 ml of the methanolic extract and 0.5 ml of anhydrous acetic acid were mixed in a test tube and kept in ice bath for 15 min. After incubation, 0.5ml of chloroform and 1 ml of concentrated sulphuric acid were added carefully along the wall of the test tube. Formation of reddish-brown ring at the separation level of two liquids indicated the presence of steroid.

### Cholesterol

2 ml of methanolic extract was mixed with 2 ml of chloroform. After that, 10-12 drops of acetic acid anhydride was added to the mixture and shaken well. Then, 2 drops of concentrated sulphuric acid was added carefully to the solution. Change in reddish brown color to blue green indicated the presence of cholesterol.

### Alkaloid

2 ml of methanolic extract solution and 2 ml of 2N HCl were taken in a test tube, shaken vigorously to mix and allowed to stand for 5 min at room temperature. After incubation, the aqueous phase was separated and 1-2 drops of Mayer's reagent ( $\text{HgCl}_2 + \text{KI}$  in water) was added to it. Appearance of creamy colored precipitation indicated the presence of alkaloid.

### Phenol

10 ml of methanolic extract was mixed with 4-5 drops of 2% ferric chloride solution. Change in the color of the solution indicated the presence of phenol.

### Flavonoid

2 gm of crude sample and 10 ml of ethyl acetate were taken in a conical flask and heated over water bath for 5-6 min. The solution was filtered through Whatman filter paper No. 1 and 4 ml of filtrate was mixed with equal amount of dilute ammonia solution (10%) and shaken vigorously. Appearance of yellow color indicated the presence of flavonoid.

### Anthraquinone

0.5 gm of crude sample was taken in a conical flask and 20 ml of benzene was added in to it. The mixture was shaken in a magnetic stirrer for 4 hr. After that, the solution was filtered and 10ml of the filtrate was mixed with 0.5 ml of ammonia solution. Appearance of violet color indicated the presence of anthraquinone.

### Saponin

0.5 gm of crude sample was boiled with 15 ml of distilled water. Formation of intensive froth indicated the presence of saponin.

### DPPH radical scavenging activity

The ability of the methanolic extract to scavenge the DPPH radical was determined according to a previously described protocol with little modification [27]. Briefly, 0.1 mM solution of DPPH (1, 1-diphenyl 2-picrylhydrazyl) in ethanol was prepared. Then 3 ml of this solution was added with 1 ml of sample solution at different concentrations (0–200 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. The lower absorbance of the reaction mixture determined the higher free radical scavenging activity.  $\alpha$ -tocopherol was taken as a

positive control.

### Statistical analysis

Data are taken as mean  $\pm$  SD of three observation. KyPlot version 2.0 beta 15 (32 bit) software was used for statistical analysis. Differences in mean  $\pm$  SD among different groups were statistically calculated using ANOVA followed by Dunnett's test.  $p < 0.05$  was taken as significant.

### Result and Discussion

Phytochemicals are responsible for several pharmacological activities and therefore, requires much attention in terms of their proper identification in crude plant materials. In the present study, the phytochemical analysis of the Lakhanbhog and Langra mango pulp and leaf was carried out following standard routine chemical tests and the results indicated the presence of phytochemicals like tannin, phlobatannin, terpenoid, glycoside, alkaloid, phenol, flavonoid, carbohydrate and protein. All of these phytochemicals are the essential constituents of herbal medicine and are directly related to different health promoting activities, viz. anticancer, antifungal, anti-inflammatory, antihyperglycemic, antiviral, antispasmodic, anti-appetite, insecticidal, immunomodulatory, neuromodulatory and wound healing properties [28-32].

**Table 1:** Phytochemical screening of Lakhanbhog & Langra (leaf & pulp)

Phytochemicals	Lakhanbhog leaf	Lakhanbhog pulp	Langra leaf	Langra pulp
Tannin	+	+	+	+
Phlobatannin	+	+	+	+
Carbohydrate	+	+	+	+
Protein	+	+	+	+
Terpenoid	+	+	+	+
Glycoside	+	+	+	+
Steroid	–	+	–	+
Cholesterol	–	–	–	–
Alkaloid	+	+	+	+
Phenol	+	+	+	+
Flavonoid	+	+	+	+
Anthraquinone	–	–	–	–
Saponin	–	–	–	–

(+) denotes the present, (-) denotes the absent

We have investigated the DPPH radical scavenging property of mango pulp and leaf extracts to support our findings on the presence of different secondary metabolites. The use of DPPH provides an easy and rapid way to evaluate antioxidant activity. The mechanism involved in the reduction of DPPH free radicals is based on the capacity of some compounds to donate hydrogen. Some plants are rich in secondary metabolites, such as, flavonoids, phenolic acids, and tannins. These phenolic compounds are able to donate hydrogen,

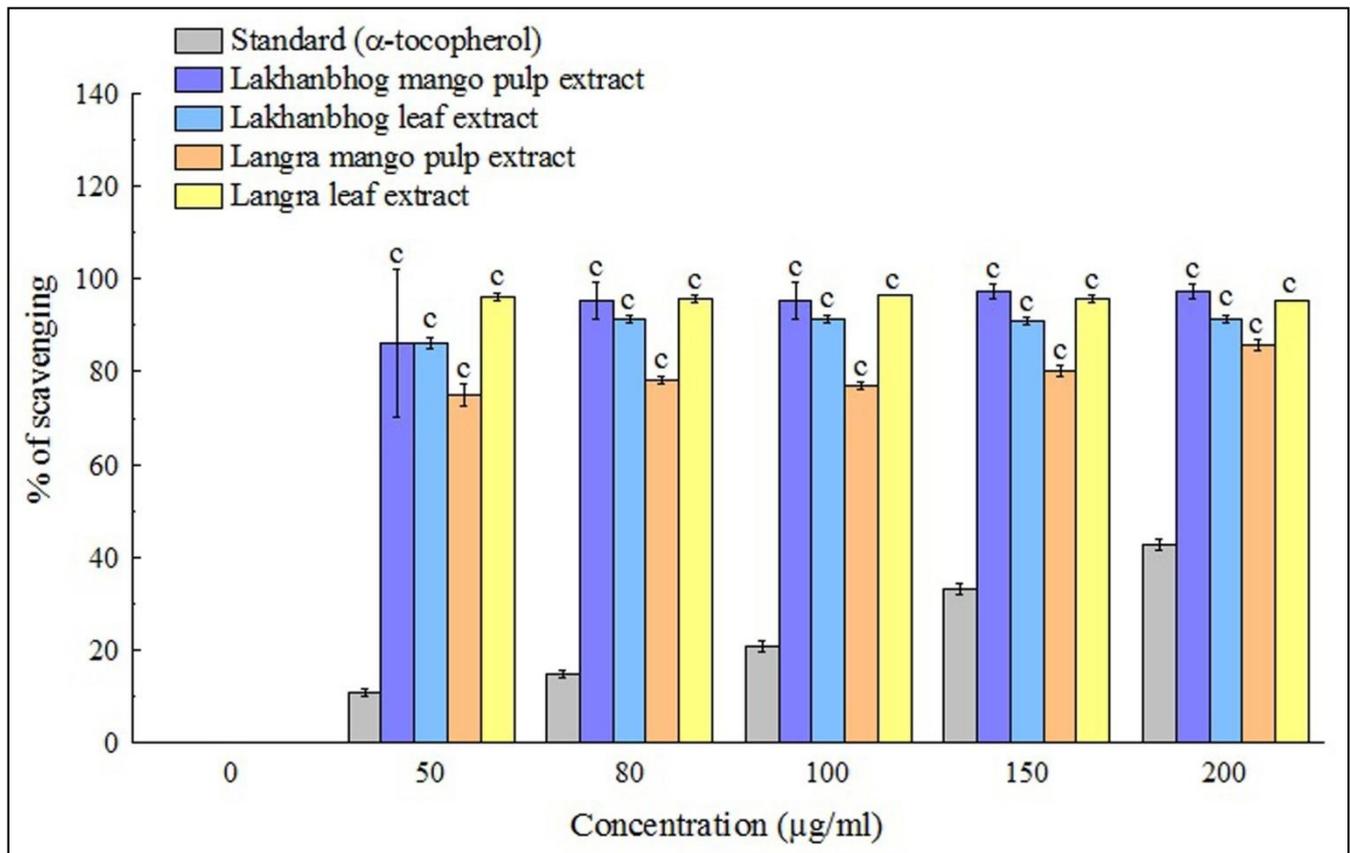
presenting antiradical activity. It measures the capacity of the extract to scavenge free radicals in the solution. In the present study, the DPPH scavenging potential of Lakhanbhog and Langra mango pulp and leaf extracts was evaluated. In each of the cases, the sample extract was found to be more potent than the standard  $\alpha$ -tocopherol, as shown in their respective IC<sub>50</sub> values, and therefore, indicating the mango extracts to be an excellent free radical scavenger (Figure 1).

**Table 2:** DPPH scavenging activity of Lakhanbhog and Langra (pulp & leaf)

Concentration (µg/ml) of extract	Percentage (%) of DPPH radical scavenging				
	$\alpha$ -tocopherol	Lakhanbhog pulp	Lakhanbhog leaf	Langra pulp	Langra leaf
50	10.95 $\pm$ 0.76	86.27 $\pm$ 15.84	86.35 $\pm$ 1.35	75.04 $\pm$ 2.43	96.10 $\pm$ 0.67
80	14.89 $\pm$ 0.77	95.41 $\pm$ 3.89	91.42 $\pm$ 0.67	78.16 $\pm$ 0.67	95.71 $\pm$ 0.67
100	20.66 $\pm$ 1.35	95.41 $\pm$ 3.89	91.42 $\pm$ 0.67	76.99 $\pm$ 0.67	96.49 $\pm$ 0.00
150	33.29 $\pm$ 1.26	97.31 $\pm$ 1.71	91.03 $\pm$ 0.67	80.11 $\pm$ 1.16	95.71 $\pm$ 0.67
200	42.61 $\pm$ 1.12	97.31 $\pm$ 1.71	91.42 $\pm$ 0.67	85.96 $\pm$ 1.16	95.32 $\pm$ 0.0

**Table 3:** IC<sub>50</sub> values of Lakhanbhog and Langra (pulp & leaf) compared to standard

IC <sub>50</sub> value (µg/ml)	$\alpha$ -tocopherol	Lakhanbhog pulp	Lakhanbhog leaf	Langra pulp	Langra leaf
	327.35 $\pm$ 7.10	11.67 $\pm$ 3.41	9.66 $\pm$ 0.07	23.50 $\pm$ 0.96	10.82 $\pm$ 0.4



**Fig 1:** DPPH radical scavenging activity of Lakhanbhog and Langra mango. Data represents Mean  $\pm$  SD of three observations. 'c' represents significant difference compared to standard at  $p < 0.001$  level.

## Conclusion

Phytochemical screening and study of antioxidant activity of Lakhanbhog and Langra mango revealed that these mango varieties are not restricted to nutrition but also have medicinal values. Lakhanbhog and Langra mango (pulp & leaf) possess different important phytochemicals such as tannin, phlobatannin, terpenoid, glycoside, alkaloid, phenol, flavonoid, carbohydrate and protein. The presence of these bioactive compounds in these mango varieties establishes themselves as the sources of natural therapeutic agents that can act as potent free radical scavengers and therefore, can be used against different infectious and other diseases.

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