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Research Article

IN VITRO ANTIOXIDANT ACTIVITY IN LEAF GALLS OF *MANGIFERA INDICA* L.

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ABSTRACT

Objective: The *in vitro* antioxidant activity was studied in the aqueous extract of leaf galls in *Mangifera indica*.

Methods: Aqueous gall extract was initially estimated for its phytochemical content, and antioxidant assays were carried out.

Results: It was evident that the total tannin, flavonoids, lycopene, and β-carotenoid content were 20%, 28%, 8%, and 5% higher than the healthy leaf tissue at a higher concentration of 1000 µg/ml. The iron chelating, superoxide anion scavenging, hydroxyl radical scavenging, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) scavenging, and total antioxidant activity were 13%, 10%, 6%, 48%, and 14%, respectively, from that of healthy tissue at 1000 µg/ml concentration.

Conclusion: The antioxidant activity was directly proportional to the concentration of the extract used in the study and the phytochemical constituents had a positive correlation with the antioxidant activity.

Keywords: Antioxidant activity, Leaf galls, *Mangifera indica,* Tannin, Lycopene, Flavonoid, β-carotene.

INTRODUCTION

Mangifera indica a member of Anacardiaceae has numerous medicinal properties. The leaves are astringent, refrigerant styptic, vulnerary, and constipating. They are used in hyperdipsia, hemorrhoids, hemoptysis, wounds, ulcers, diarrhea, dysentery, diabetes [1], pharyngopathy, scorpion sting, and stomachopathy. The smoke from burning leaves is inhaled for relief of hiccup and throat diseases. The leaves have enormous pharmaceutical applications such as analgesic, anti-inflammatory [2], antiplasmodial [3], antiallergic properties [4], antibacterial activity [5], and antioxidant properties [6]. They are also used in food [7] and cosmetics industries [8].

Leaf galls are the bizarre lumps, bumps, and outgrowths that develop on different parts of plants due to invasion by very unique organisms [9]. Mango leaf galls are caused due to the interaction with the gall midge (*Procontarinia matteiana*) which triggers a hypertrophic activity that results in gall formation. In general, galls are rich in protease, invertase, chitinase, cellulase [10], carbohydrates, amino acids, and phenolic compounds. Oxidative stress can be considered to be the cause and the consequence of several diseases such as cancer, Alzheimer's disease, atherosclerosis, heart failure, Parkinson's disease, sickle cell disease, autism, and chronic fatigue syndrome. Antioxidants can be used to cure diabetes and cancer causing tumors [11]. Since leaf galls are rich in phytochemical, the antioxidant activity is expected to be higher. The therapeutic value of these galls has not been investigated earlier. Hence, the objective of the work is to determine the antioxidant activity in the aqueous extract of *M. indica* leaf galls.

METHODS

Preparation of gall extract and estimation of phytochemicals

The leaf galls were detached from the *M. indica* leaves, washed in running tap water and blotted dry. An aqueous extract of the selected galls was obtained by extracting with distilled water. The tannin content was estimated by the Folin phenol method with gallic acid as standard.

The total flavonoid content was determined as follows briefly to the 0.1 ml of the diluted extract of leaf gall 0.1 ml of aluminum chloride was added and made up to 3 ml. The absorbance was read at 415 nm. The experiment was performed in triplicates with Rutin as standard**.** Total β-carotene and lycopene were determined according to Nagata and Yamashita method [12]**.** The process, in brief, was 10 mg of the extract was vigorously shaken with 10 ml of acetone-hexane mixture (4:6) for 1 minute, and the absorbance of the mixture was measured at 453, 505, and 630 nm. β-carotene and lycopene contents were calculated according to the following equations:

Lycopene (mg/10 ml)=0.0458 A630+0.372 A505−0.0806 A453

β-carotene (mg/10 ml)=0.216 A630−0.304 A505+0.452 A453.

The iron chelating assay

The iron chelating activity was determined by adding 1 ml of 0.05% of phenanthroline and 2 ml of ferric chloride solution. Ethylenediaminetetraacetic acid served as standard. The absorbance was read at 510 nm. The experiment was performed in triplicates**.**

Superoxide anion scavenging assay

Leaf gall extract was diluted 10 times and 0.3 ml of the extract as taken and added 0.2 ml of 0.03 g of pyrogallol dissolved in 10 ml of hydrochloric acid. The absorbance was read at 420 nm about every 30 seconds interval for 3 minutes. The experiment was repeated thrice. The percentage inhibition can be calculated by the formula:

A '3' minutes (420 nm)−A '0' minute (420 nm)−A '3' minutes (420 nm)×100.

Hydroxyl radical scavenging (HRS) assay

HRS activity was assayed as follows 1.0 mm ferric chloride, 1 mm 1.10 - phenanthroline, 0.2 m phosphate buffer (pH 7.8), 0.17 m hydrogen peroxide, and test solution at various concentrations are mixed. Hydrogen peroxide was added to initiate the reaction, and the mixture is kept for incubation at room temperature for 5 minutes. After incubation, the absorbance of mixture was read at 560 nm using a spectrophotometer, and the HRS activity was calculated.

The 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging assay

The ABTS scavenging activity was evaluated by allowing ABTS (7 mm) to react overnight with potassium persulfate (2.45 mm) in dark to yield ABTS radical cations. The ABTS radical cation was diluted with 50% methanol for an initial absorbance of about 0.70 ± 0.02 at 745 nm at 30°C. Free radical scavenging activity was determined by mixing 300 µl of the test sample with 3.0 ml of ABTS working standard. The decrease in absorbance was measured immediately after 1 minute till 6 minutes. The inhibition was calculated according to the formula:

% Inhibition = (Absorbance of control−Absorbance of sample)/ Absorbance of control×100.

Total antioxidant assay

In phosphomolybdenum assay (Total antioxidant activity), the test sample was mixed with 3 ml of reagent solution containing 0.6 m sulfuric acid, 28 mm sodium phosphate, and 4 mm ammonium molybdate and incubated at 95°C for 90 minutes. Ascorbic acid was the standard used. The absorbance was measured at 695 nm with methanol blank.

RESULTS AND DISCUSSIONS

Antioxidants inhibit the oxidation reaction in a cell by removing the free radicals or by inhibiting the reaction. Often, an oxidation reaction releases free radicals that cause damage or death to the cells. Antioxidants are reducing agents such as thiols, ascorbic acid, or polyphenol [13]. Phenolic compounds in plants have been reported to have multiple biological effects [14]. Antioxidant activity in plant sources is mainly due to the presence of phenolic components such flavonoids [15], phenolic acids, and diterpenes. Tannin content in the leaf gall extract, as well as healthy extract, was compared; Gall extract from 1 ml concentration seemed to have 20% more tannin content than healthy extract and was concentration dependent (Fig. 1). Phenolic compounds are secondary metabolites in plants, and its antioxidant activity is due to their redox properties of chelating and scavenging free radicals [16]. It was evident that higher tannin content in gall infested leaves had a very strong positive correlation of r=0.99, p=0.001; and r=0.99, p=0.006, respectively, with iron chelating activity and ABTS scavenging activity (Fig. 1).

Similarly, the flavonoid content was 28% more than healthy leaf extract and concentration dependent (Fig. 2). Flavonoids are compounds with hydroxyl groups which are responsible for the antioxidant effects of plants [17]. Amount of flavonoid contents of leaf galls extracts of *M. indica* suggests that it possessed high antioxidant activity since a very strong positive correction of r=0.98, p=0.003; and r=0.97, p=0.006 with iron chelating activity and ABTS scavenging activity.

The result showed that iron chelating activity was concentration depend. The activity at 1000 µg/ml was 15% more than the lower concentration such as 100 µg/ml. When compared to the healthy, the galled leaves had 10% more iron chelating activity (Fig. 2). Iron is essential for oxygen transport, respiration, and activity of many enzymes, but it is proved that iron reacts and catalyze those compounds with high antioxidant properties. This effect may be due to the presence of polyphenols which has potent iron chelating capacity [18] which is very clearly evident in the study.

Gall extract had significant ABTS radical scavenging activity which was also concentration dependent. The gall extracts exhibited 5% more activity than the normal leaf tissue (Fig. 2). Lycopene and β-carotene content in the gall extract increased with concentration. The healthy leaf extract had 8% less lycopene and β-carotene content when compared to gall extract (Fig. 3). The lycopene is reported to offers protection against gamma-radiation by scavenging the free radicals. Carotenoids such as β-carotene and lycopene are excellent antioxidants. They act by quenching singlet oxygen molecules [19]. The experimental results show a very strong positive correlation of lycopene content to the superoxide anion scavenging activity (r=0.97, p=0.006) and hydroxyl ion scavenging activity (r=0.95, p=0.013).

The superoxide anion scavenging activity was also found to be directly proportional to the concentration of galls extract. Here, the higher concentration of 1000 µg/ml had 30% activity than that of 100 µg/ml (Fig. 3). At 1000 µg/ml, the gall extract had 10% more activity than the healthy leaf extract. Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals which get converted to hydrogen peroxide and hydroxyl radical, which damage biomolecules and results in chronic diseases [20]. The result revealed that leaf gall extract had marked increase in activity with increasing concentration.

The leaf gall extract of *M. indica* has good HRS activity, and it was concentration dependent. It was evident that 1 ml concentration had 27% more activity than 0.1 ml (Fig. 3). Scavenging of hydroxyl ions

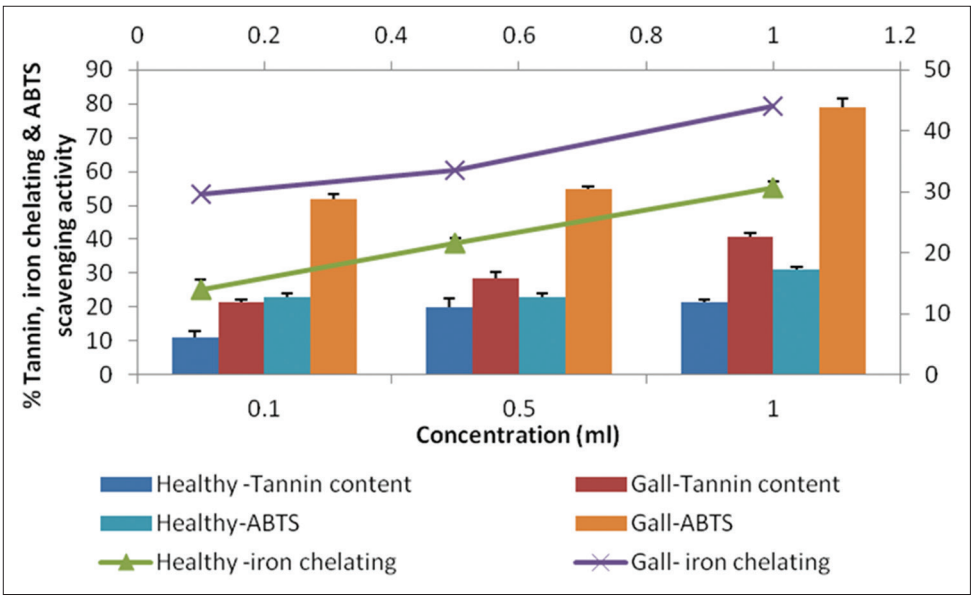

Fig. 1: Total tannin content, iron chelating activity, and 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid scavenging activity in leaf gall extract. All values in triplicate. Mean±standard deviation

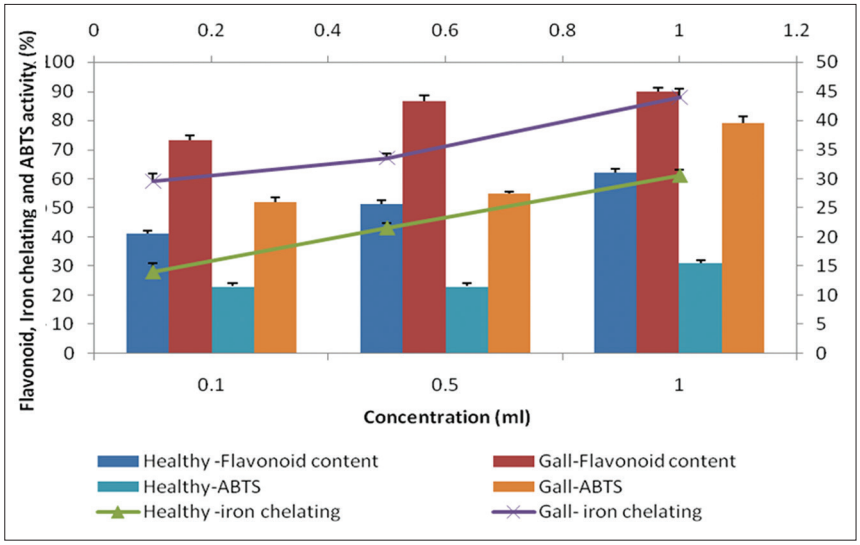

Fig. 2: Total flavonoid content, iron chelating activity, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid scavenging activity in leaf galls extract. All values in triplicate. Mean±standard deviation

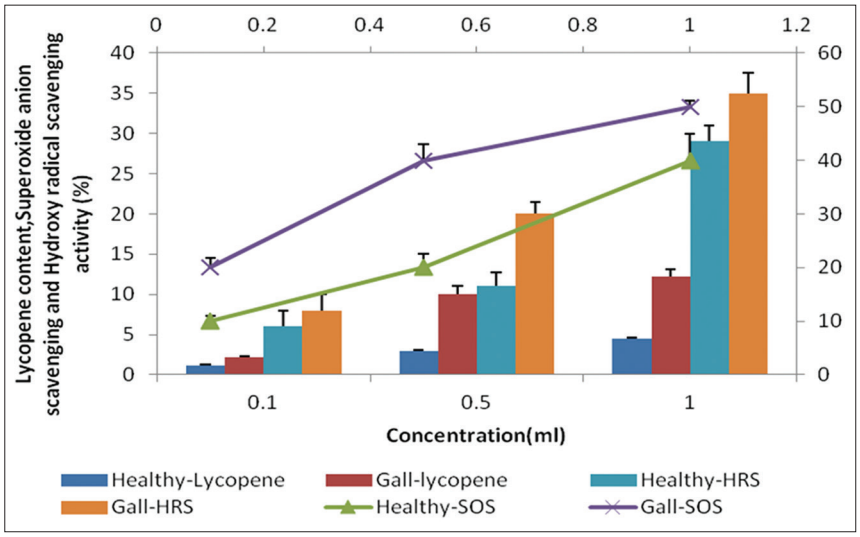

Fig. 3: Total lycopene content, superoxide anion radical scavenging activity, and hydroxy radical scavenging activity in leaf gall extract. All values in triplicate. Mean±standard deviation

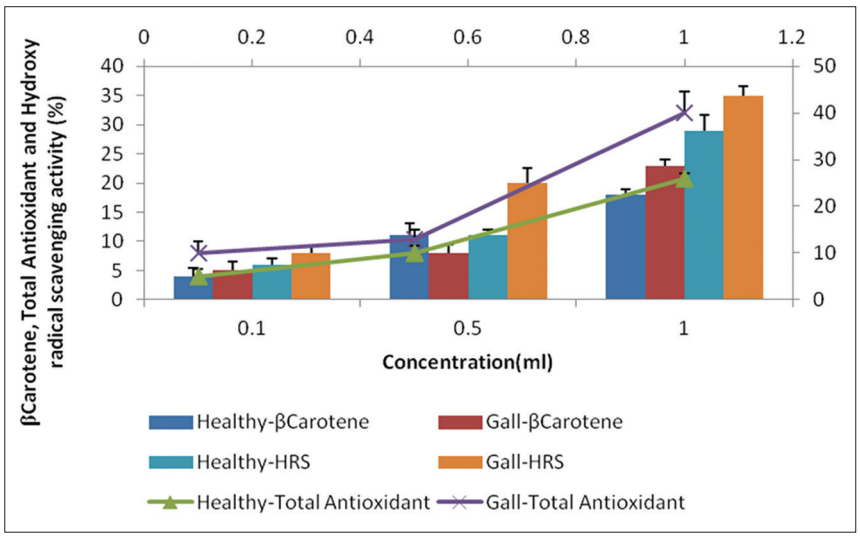

Fig. 4: β-carotene content, total antioxidant activity, and hydroxyl radical scavenging activity in leaf gall extract. All values in triplicate. Mean±standard deviation

is an important antioxidant activity since these hydroxyl ions are highly reactive. It can react with polyunsaturated fatty acid moieties of phospholipids in the cell membrane and causes damage to the cell. Similarly β-carotene had strong correlation with total antioxidant activity (r=0.99, p=0.001) and hydroxyl ion scavenging activity (r=0.97, p=0.006) (Fig. 4). The total antioxidant capacity was 15% higher activity than healthy tissue.

Today, diseases caused due oxidative stress are increasing alarmingly, and *M. indica* leaves are a natural source of antioxidant. Gall infested leaves have elevated levels of phytochemicals which make them potent antioxidant agents. Hence, from the study, it can be concluded that *M. indica* gall infested leaves can be a rich source of antioxidant which can be considered as a food supplement in future. However, an *in vivo* study will help in determining the efficacy before they can be used in food and beverages.

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