

THE QUALITATIVE AND QUANTITATIVE ANALYSIS OF PHYTOCONSTITUENTS OF FRUIT EXTRACTS OF OXALIDACEAE FAMILY

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Abstract

The Oxalidaceae family consists of more than 200 species and is widely distributed in tropical and sub-tropical countries and the genus *Averrhoa* has two species, *Averrhoacarambola* and *Averrhoabilimbi*. The study was conducted to determine the phytochemical screening and total antioxidant activity of *A.carambola* and *A.bilimbi* of Oxalidaceae family.

Introduction

Nature has been a source of many medicinal and non-medicinal plants. Liu (2013) has defined phytochemicals as bioactive non-nutrient compounds in fruits, vegetables, grains and other plant foods that have been linked to reductions in the risk of major non-communicable chronic diseases. Antioxidant is a substance that reduces damage due to oxygen, such as that caused by free radicals. These phytochemical and antioxidant properties provide the plant with many pharmaceutical values. The Oxalidaceae family consists of more than 200 species and is widely distributed in tropical and sub-tropical countries and the genus *Averrhoa* has two species, *Averrhoacarambola* and *Averrhoabilimbi*. The study was conducted to determine the phytochemical screening and total antioxidant activity of *A.carambola* and *A.bilimbi* of Oxalidaceae family. Plants and their secondary metabolites are very useful for the preparation of medicines. According to Sayali, 2010 *Averrhoabilimbi* can be used for many pharmaceutical values. *A.carambola* and *A.bilimbi* have antioxidant, anti-bacterial, anti-fungal properties and can be used to treat many diseases. *A.carambola* and *A.bilimbi* has many phytochemical constituents like flavonoids, alkaloids phenols, saponins, glycosides etc. Both have antioxidant capacity also. The phyto-constituents present in *Averrhoa bilimbi* and *A.carambola* revealed that they are very effective in treating various diseases.

Materials and Methods

Fresh *Averrhoa carambola* and *Averrhoa bilimbi* fruits were collected locally and shade dried over a polythene cover at 21 and pulverized using a -



Figure 1 Habit of *Averrhoa bilimbi*



Figure 2. Habit of *Averrhoa carambola*

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Division	Spermatophyta
Class	Magnolipsida
Order	Geranial's
Family	Oxalidaceae
Genus	<i>Averrhoa</i>
Species	<i>A.bilimbi</i>

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mixture grinder. Methanol was used for extraction. One part of each powdered fruit was macerated in ten parts of solvents separately by using soxhlet extraction method. Finally dark brown colored crystals were obtained.

Kingdom	Plantae
Sub-kingdom	Tracheobionta
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Species	<i>A.bilimbi</i>

Kingdom	Plantae
Sub-kingdom	Viridiplantae
Division	Tracheophyta
Class	Magnolipsida
Order	Oxalidales
Family	Oxalidaceae
Genus	Averrhoa
Species	<i>A.carambola</i>

Qualitative screening of phytochemicals in both fruits

Alkaloids

1ml of the extract was measured into a test tube and adds a little amount of dil. Hydrochloric acid and Mayer's reagent were added to the solution; the formation of a white precipitate indicated the presence indicated the presence of alkaloids.

Flavonoids

1.3 ML of extract was mixed with 0.5g of magnesium turnings; the mixture was boiled for 5 minutes, the appearance of orange to red color indicated the presence of flavonoid.

Phenols

To 1ml of fruit extract add neutral ferric chloride; formation of bluish green color showed the presence of phenol.

Tannins

To 1ml of the fruit extract, add a little amount of lead acetate solution is added; the formation of foamy white precipitate showed the presence of tannin.

Saponins

2.5 ml of extract was added to few drops of distilled water and the mixture was shaken vigorously, a copious lather formation was noticed which indicated the presence of saponin, and the absence of copious lather meant the absence of saponin.

Glycosides

2.5 ml of the extract was mixed with a little quantity of antrone on a watch glass, one drop of concentrated sulphuric acid was added and made into a paste, and heated gently over a water bath; a dark green coloration indicated the presence of glycoside.

Antrquinones

Few drops of magnesium acetate solution were added to 1ml of extract; the formation of pink color showed the presence of anthraquinone.

Quinones

1ml of the extract was mixed was mixed with concentrated sulphuric acid. The appearance of the color formation signified that Quinone was present.

Terpenoids

About 0.5g of plant extract in separate test tube was taken with 2ml of chloroform; 5ml of concentrated sulphuric acid was carefully added to form a layer and observed for presence of reddish-brown color interface to show positive results for the presence results for the presence of terpenoid.

Steroids

2ml of acetic anhydrides was added to 0.5g extract with 2ml of sulphuric acid and observed for the color change from violet to blue or green in samples indicating the presence of steroid.

Amino acids

About 0.2g of plant extract was weighed and treated with ninhydrin solution and observed for a characteristic purple color which indicates the presence of amino acid.

Lignins

0.5ml of aqueous solution of extract was added to 2ml of 2% (v/v) furfuraldehyde in a test tube- Red color indicates the presence of lignins.

Quantitative determination of chemical constituents

Estimation of total flavonoids content (TFC)

0.5 ML of sample, 1.5 ML methanol, 0.1 ML aluminium chloride, 0.1ML potassium acetate solution and 2.8 ML distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminum chloride with distilled water. The absorbance was measured at 415 nm using UV-VISIBLE spectrophotometer (Agilent, Cary 60) against the blank and the values obtained were interpreted using the standard graph of quercetin to get the milligram equivalents of quercetin.

Estimation of total phenolic content (TPC)

5ml of FolinCio-calteau reagent was added to 0.2ml of sample. After 5 minutes of incubation, 4ml of 7.5% sodium carbonate solution was added to it. It was stirred and incubated at room temperature for 2 hours. After 2hours, the absorbance was measured at 750nm using UV-VISIBLE spectrophotometer (Agilent, Cary 60), and the values obtained were interpreted in the standard graph of Gallic acid to get the milligram equivalent of Gallic acid.

Estimation of total tannin content (TTC)

The vanillin reagent with any phenol that has an unsubstituted resorcinol or phloroglucinol nucleus and forms a colored substituted product which is measured at 500nm. Pipetted out 1.0ml of the supernatant. Quickly added 5ml of vanillin hydrochloride reagent. The absorbance was read in a spectrophotometer at 500nm after 20 minutes. Prepared a blank with vanillin hydrochloride reagent alone. A standard graph was prepared with 20-100 µg catechins using the diluted stock solution. From the standard graph, calculated the amount of catechin, i.e., tannin in the sample as per the absorbance values and expressed the results as catechin equivalents.

Estimation of Total Saponin Content (TSC)

The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55°C. The residue of the mixture was re-extracted with another 100 cm³ of 20% aqueous ethanol after filtration and heated for 4 hours at a constant temperature of 55°C with constant stirring. The combined extract was evaporated to 40 cm³ over water bath at 90°C. 20 cm³ of diethyl ether was added to the concentrate in a 250 cm³ separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded.

This purification process was repeated twice. 60 cm³ of n-butanol was added and extracted twice with 10 cm³ of 5% sodium chloride. After discarding the sodium chloride layer the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated.

Estimation of Total Glycosides Content (TGC)

Glycoside content in the methanolic fruit extract of *A.bilimbi* and *A.carambola* was evaluated by using Buljet's reagent. 1ml of the fine powder of sample extract is filtered and the extract obtained was then purified using lead acetate and Na₂HPO₄ solution before the addition of freshly prepared Buljet's reagent (containing 95ml aqueous picric acid + 5ml 10% aqueous NaOH). The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

Determination of Total Antioxidant**DPPH Radical Scavenging Assay**

Different volumes of sample such as 1.25µL from the stock solution were made up to a final volume of 20µl with DMSO and 1.48ml DPPH (0.1mm) solution was added. A control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control. The antioxidant capacity was expressed in mean±standard deviation. Data were analysed by using two-way ANOVA.

Results and Discussion**Qualitative Analysis of the sample extracts**

From the result, it was proved that *A.carambola* contains phytoconstituents like phenols, flavonoids, steroids, glycosides, alkaloids, tannins and saponins and in the case of *A.bilimbi*, phenols, flavonoids, saponins and glycosides, tannin were present and alkaloids were absent (Table 1.1). Abraham (2014) in his study revealed the same that *A.bilimbi* has a negative result on alkaloid tests. Among these fruit extracts, *A.carambola* has more phytochemical constituents than *A.bilimbi*.

Table 1.1. Qualitative phytochemical analysis of sample extracts

Phytochemicals	<i>A.carambola</i>	<i>A.bilimbi</i>
Terpenoids	–	–
Phenols	+++	++
Flavonoids	++	+++
Steroids	+	–
Quinones	–	–
Antraquinones	–	–
Glycosides	++	+
Alkaloids	+	–
Tannins	++	+
Lignin	–	–
Saponins	++	++
Amino acids	–	–

Quantitative Analysis

Total Phenolic Content (TPC)

The total phenolic content of *A.carambola* was more than that of *A.bilimbi*. The methanolic extract of *A.carambola* had a total phenolic content of 0.890 mg/ml and while considering *A.bilimbi* it has phenolic content of 0.676 mg/ml (Table 1.2). *Averrhoacarambola* has more phenolic content than *Averrhoabilimbi*.

Table 1.2. Quantitative estimation of phenols in the sample extracts

Sample	Absorbance	TPC (mg/ml)
<i>Averrhoa carambola</i> (AC)	0.679	0.890
<i>Averrhoa bilimbi</i> (AB)	0.515	0.676

Total Flavonoid Content (TFC)

A.carambola and *A.bilimbi* has flavonoid content. The methanolic extract of *A.carambola* had a flavonoid content of 0.349mg/ml and *A.bilimbi* is 0.671mg/ml (Table 1.3). The *A.bilimbi* has high amount of flavonoid content than *A.carambola*.

Table 1.3. Quantitative estimation of flavonoids in sample extracts

Sample	Absorbance	TFC in (mg/ml)
<i>Averrhoa carambola</i> (AC)	0.423	0.349
<i>Averrhoa bilimbi</i> (AB)	0.813	0.671

Total Tannin Content (TTC)

Tannin was present in fruit methanolic extract of

A.bilimbi and *A.carambola*. Tannin content in *A.carambola*, is 0.791mg/ml and for *A.bilimbi* it is 0.420mg/ml (Table 1.4) and *A.carambola* has more tannin content than *A.bilimbi*.

Table 1.4. Quantitative estimation of tannin in sample extracts

Sample code	Absorbance	TTC (mg/ml)
<i>Averrhoa carambola</i> (AC)	0.438	0.791
<i>Averrhoa bilimbi</i> (AB)	0.531	0.420

Total Saponin Content (TSC)

Saponin is a very important phytochemical and present in *A.bilimbi* and *A.carambola*. *A.carambola* has 0.720 mg/ml saponin and *A.bilimbi* has Saponin in a quantity of 0.511 mg/ml (Table 1.5) and from this it is clear that *A.carambola* has more tannin content than *A.bilimbi*.

Table 1.5. Quantitative estimation of saponin in sample extracts

Sample code	Absorbance	TSC(mg/ml)
<i>Averrhoa carambola</i> (AC)	0.751	0.720
<i>Averrhoa bilimbi</i> (AB)	0.822	0.511

Total Glycoside Content (TGC)

A.carambola and *A.bilimbi* has a small amount of glycoside content. The fruit methanolic extract of *A. carambola* contains 0.603 mg/ml glycosides and *A. bilimbi* contains 0.479 mg/ml glycoside (Table 1.6). From the analysis it is clear that the *A. carambola* has more glycosidal content than *A. bilimbi*.

Table 1.6. Quantitative estimation of glycosides in sample extracts

Sample code	Absorbance	TGC (mg/ml)
<i>Averrhoa carambola</i> (Ac)	0.538	0.603
<i>Averrhoa bilimbi</i> (AB)	0.821	0.479

Antioxidant Capacity

Average mean variation of percentage inhibition between the different concentrations (1.5µl to 20 µl) varied from 22.52 ± 0.244 to 64.71 ± 0.124 (Table 1.7). From this, the high percentage of inhibition is observed at the concentration of 20 (64.71 ± 0.124). Here, *A.bilimbi* showed lowest percentage of inhibition on lowest concentration and showed highest percentage of inhibition on higher concentration. Percentage of inhibition increased

when concentration increased.

Table 1.7. Antioxidant potential of *Averrhoa bilimbi*

Concentration	Percentage of inhibition	Mean \pm SD
1.5	22.52	22.52 \pm 0.244
2.5	33.38	33.38 \pm 0.163
5	52.56	52.56 \pm 0.169
10	55.40	55.40 \pm 0.094
20	64.71	64.71 \pm 0.124

Table 1.8. Antioxidant potential of *Averrhoa carambola*

Concentration	Percentage of inhibition	Mean \pm SD
1.5	33.69	33.69 \pm 0.169
2.5	63.80	63.80 \pm 0.081
5	65.37	65.37 \pm 0.169
10	76.07	76.07 \pm 0.041
20	81.73	81.73 \pm 0.124

Average mean variation of percentage inhibition between different concentrations (1.5 μ L to 20 μ L) varied from 33.69 \pm 0.169 to 81.73 \pm 0.124 (Table 1.8). According to the result showed, *A. carambola* showed highest percentage of inhibition for the highest concentration. That is, when concentration increases percentage of inhibition also increases.

Discussion

Averrhoa carambola and *Averrhoa bilimbi* contains many phytoconstituents and the investigations in *A. bilimbi* and *A. carambola* revealed that they are useful to treat many health issues. To explore the phytochemical potential, preliminary qualitative and quantitative analysis were followed. *A. bilimbi* showed negative result to alkaloids but *A. carambola* showed positive result.

A. bilimbi and *A. carambola* showed the presence of flavonoids in the preliminary phytochemical analysis. Kim *et al.*, 1997 in his study revealed the data that flavonoids are the most important phytochemical among all other constituents and also conducted the quantitative estimation of flavonoid. In the present quantitative estimation showed that, *A. bilimbi* has high amount of flavonoid content than *A. carambola* (Fig 1.1). Jagadish kumar 2017 estimated high flavonoid content in *A. bilimbi* fruits

methanolic extract. Moresco *et al.*, 2011 identified high flavonoid content in *A. bilimbi* in his study.

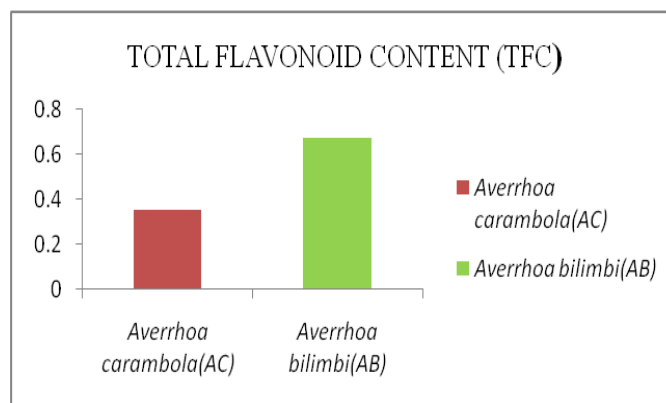


Fig 1.1. Variation of Flavonoid content in sample extracts

A. bilimbi and *A. carambola* showed the presence of phenols and in the quantitative estimation *A. carambola* showed more phenolic content than *A. bilimbi*. Yan *et al.*, 2013 revealed that on comparing both *A. bilimbi* and *A. carambola*, *A. carambola* has high TPC than *A. bilimbi* (Fig 1.2). Azeem *et al.*, 2012 represented the same conclusion that *A. carambola* has high phenolic content than *A. bilimbi*. It gives the phytochemical and pharmacological properties to this fruits. Zama, 2015 in his study also revealed the presence of high amount of phenol in *A. carambola* leaf methanolic extract. In the qualitative estimation of *A. bilimbi* and *A. carambola*, *A. carambola* has more phenolic content (Asna and Noriham, 2018).

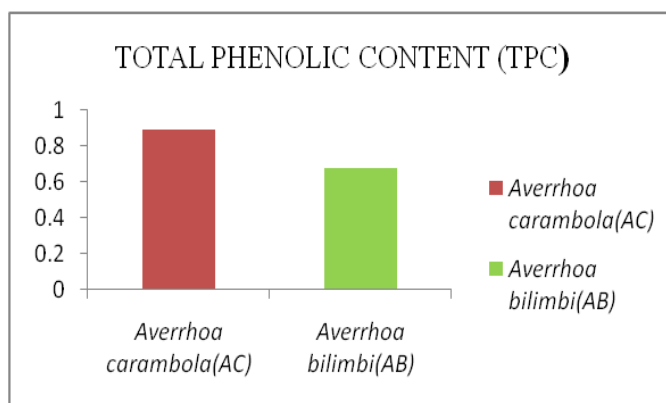


Fig 1.2. Variation of phenolic content in sample extracts

Glycosides are the condensation products of sugar. In the present investigation *A. bilimbi* and *A. carambola* showed positive result to glycosides (Fig 1.3). Ferrara¹⁰ *et al.*, 2008, in his study proved the presence of glycosides in *A. carambola*. Preliminary phytochemical estimation of *A. carambola* by

Pakti¹¹ *et al*, 2012 also defines the same result. The quantitative estimation showed that *A.carambola* has more glycoside content than *A.bilimbi*. Study conducted by Nair¹² *et al*, 2016 also revealed the glycosidal content of methanolic fruit extract of *A.bilimbi*.

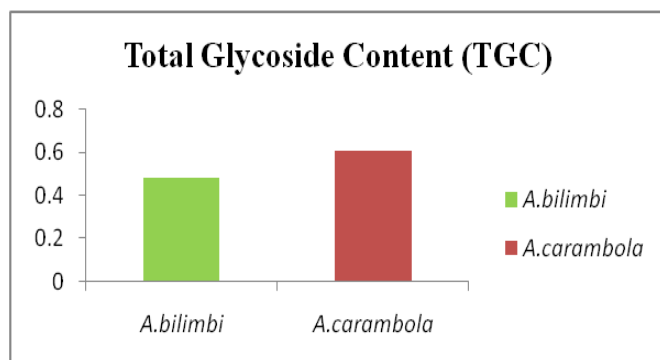


Fig 1.3, Variation of glycosides in sample extracts

Saponins are another type of phytochemicals present in medicinal plants. *A.carambola* and *A. bilimbi* showed the presence of saponins (Fig 1.4). As compared to *A.bilimbi*, *A.carambola* showed more positive results to saponin test. Wahab *et al.*, 2014 revealed the presence of high amount of saponin in *A.carambola*. Ali *et al.*, 2013 discussed the similar results in their studies that *A. carambola* has more saponin content and has more phytochemical properties.

Tannins can widely be seen in almost all plants like *A.bilimbi* and *A.carambola*. Tannins have high molecular weight. *A. bilimbi* and *A. carambola* showed the presence of tannin and among them *A.carambola* has more tannin content than in *A. bilimbi* (Fig 1.5). Rahman *et al.*, 2014 agreed with the same results in his study that both fruit extract showed the presence of tannin. Suluvoy and Grace, 2017 mentioned that *A.bilimbi* has tannin content.

In the whole quantitative phytochemical investigation *A.bilimbi* has phytochemical content lesser than that of *A.carambola* (Fig 1.6). As a result *A. carambola* has high phytochemical property than that of *A. bilimbi*. As a result of the study by Bhaskar and Shantaram, 2013 also founded the similar results that *A. carambola* has more phytochemicals.

Another group of phytochemicals present in oxalidaceae family are steroids. Steroids are known as cardiac glycosides. They are also a major phytoconstit-

uent. The methanolic fruit extract of *A.carambola* showed a slight positive result to steroid test but were absent in *A.bilimbi*.

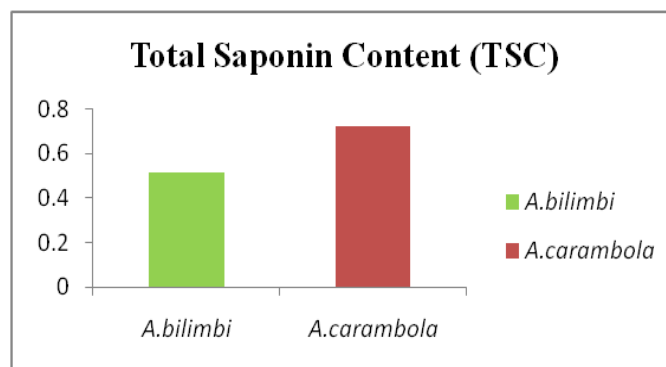


Fig 1.4. Variation of saponin in sample extracts

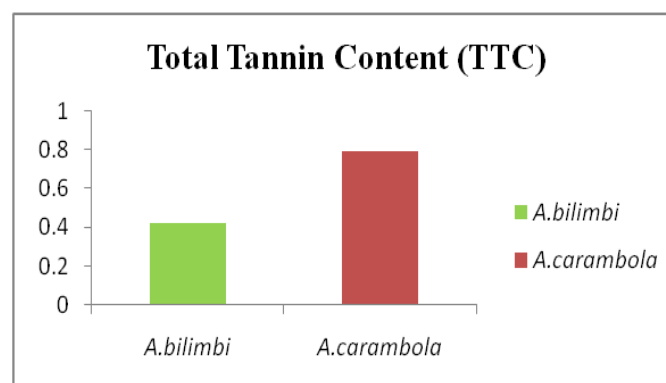


Fig: 1.5. Variation of tannin content in sample extracts

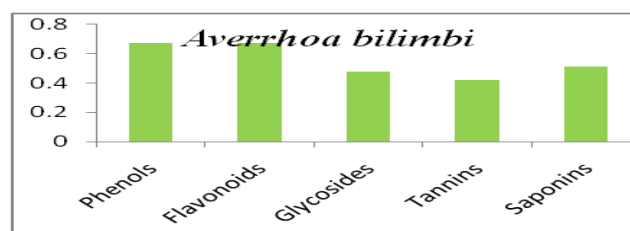


Fig 1.6 a

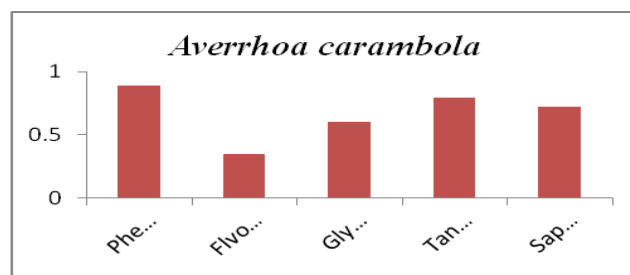


Fig 1.6 . b

Fig 1.6 a and Fig 1.6 . b Comparison of phytochemical quantity in both sample extracts

Siddique *et al.*, 2013 discussed about the absence of steroids in *A.bilimbi*. Steroids can have the ability to promote nitrogen retention. The excessive dose may cause death. Rahman *et al.*, 2015 estimated the phytochemical content in *A.bilimbi* and came to the same conclusion that *A.bilimbi* fruit extract does not contain steroid content.

Preliminary phytochemical analyses were also done for estimation of terpenoids, quinones, antraquinones, lignins, and amino acids. Nurul *et al.*, 2009 noted that in methanolic fruit extracts of averrhoa species terpenoids and quinones were absent. The study revealed only the presence of tannins, phenols and flavonoids. But in the present investigation all these showed negative results and are also very important phytochemicals in medicinal plants but were absent in *A. bilimbi* and *A. carambola*. The absence of these phytochemicals can be seen in almost all studies in *A.carambola* and *A.bilimbi*. Similar studies were conducted by Asna and Noriham, 2014 revealed the absence of terpenoids, quinones, antraquinones, lignins and aminoacids.

In the present study antioxidant analysis were conducted by DPPH radical scavenging assay. Different concentration showed different percentage of inhibition. This showed that *A. carambola* has more antioxidant capacity than *A. bilimbi*. In an updated review by Dasgupta *et al.*, 2013 revealed the same result. While taking different concentration, percentage of inhibition also changes. The percentage of inhibition increases when concentration of sample extracts increases. Saghir *et al.*, 2013, identified similar antioxidant values in *A. carambola* by using DPPH Assay.

The main focus of the present study was to evaluate the phytochemical and antioxidants potential of two fruit methanolic extract samples of oxalidaceae family. It can be concluded that *A.bilimbi* and *A.carambola* has many phytochemical constituents and high antioxidant capacity. So that they can be used to manufacture certain type of drugs to cure many diseases. In this it was evident that due to the presence of certain important phytochemicals, the fruits of *A. carambola* and *A. bilimbi* can be potent to cure many health effects. Among them *A.carambola* has more pharmacological importance than *A.bilimbi* and thereby the species variation can affect the constituents of phytochemicals and antiox-

idant capacity.

During the preliminary screening, it was noticed that alkaloids were absent in *A.bilimbi* but present in *A.carambola*. On comparing both, *A.carambola* has number phytoconstituents than *A.bilimbi*. In the quantitative analysis, *A.carambola* showed more phytochemical content than *A.bilimbi*. In antioxidant analysis also *A.carambola* shows high antioxidant potential than *A.bilimbi*. The analysis revealed that *A.carambola* has more phytoconstituents and antioxidant capacity than *A.bilimbi*. So that the fruit can be used for many pharmaceutical studies.

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