ABSTRACT

Medicinal plants have bioactive compounds which are used for curing various human diseases and also play an important role in healing. Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development. Antioxidants are the compounds which terminate the attack of reactive species and reduce the risk of diseases. The study was conducted to determine phytochemical screening and total antioxidant activity of Averrhoa carambola fruit. Extraction of Averrhoa carambola fruit was done in different solvents. The results of the Averrhoa carambola analysis of these medicinal plants showed the presence of alkaloids, phenols, flavonoids, protein, tannins, steroids and glycosides. The hydroethanolic extract showed better result of phytochemical analysis than other solvents. The total antioxidant activity in fruit of Averrhoa carambola determined by ferric thiocyanate and thiobarbituric acid method showed low absorbance value, which indicated the high level of antioxidant activity. The present study reveals that the selected fruit would exert several beneficial effects by virtue of its phytochemical constituents and antioxidant activity which could be harnessed as drug formulation.

KEYWORDS: Averrhoa carambola, phytochemicals, antioxidant, FTC, TBA.

1.0 INTRODUCTION

Traditional use of herbal medicine is usually an integral part of culture around the world, which has been used in medical practice for thousands of years and has made a great
contribution for maintaining human health before spread of modern science (Verma and Singh, 2008). The emerging importance of biologically active medicinal plants and their constituents as possible therapeutic measures has become a subject of active scientific investigation. The plant sources are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Nikhal, 2010). Reactive Oxygen Species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide play a crucial role in the development of various ailments. The free radicals in the human body are generated through aerobic respiration or from exogenous sources. Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases. There is a growing interest all over the world for discovering the untapped reservoir of medicinal plants (Balakrishnan et al., 2011). Correlation between the phytoconstituents, antioxidant activity and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well (Manjulika Yadav et al., 2014).

Averrhoa carambola belongs to the Oxalidaceae is commonly known as star fruit, carambola is known as Kamrakh (hindi), Thambaratham (Tamil). Its nutritive values are also useful to the human health. Various parts of tree has been used in traditional folkloric medicine (Singhal et al., 2012).

Hence, the present study was aimed to screen the fruit extract for different phytochemicals and to determine the total antioxidant activity.

2.0 MATERIALS AND METHODS

2.1 COLLECTION AND PREPARATION OF PLANTS POWDER MATERIAL
Fresh Averrhoa carambola fruits were collected in and around Ooty and were shade dried over a polythene cover at 21°C and pulverized using a mixture grinder. The coarse powder of the fruit was used for the preparation of the extract.

2.2 PREPARATION OF FRUIT EXTRACTS
The powdered fruit was extracted in various solvents, viz hydroethanol, ethanol, water, chloroform and petroleum ether. One part of the powdered fruit was macerated in ten parts of the solvents separately. It was taken in a round –bottomed glass flask and evaporated to separate the fraction. Then the liquid portion of this sample was evaporated to dryness at a
low temperature (>40°C) under reduced pressure in a rotatory evaporator. Finally dark brown colored crystals were obtained.

2.3 PHYTOCHEMICAL SCREENING OF EXTRACT
The methods described by Trease and Evans, 1989 and Abalaka et al., 2011 were used for screening of phytochemicals like tannin, saponin, flavonoids, phenols, glycosides, steroids, alkaloids and proteins in five different solvents.

2.4 QUANTITATIVE ESTIMATION OF SECONDARY METABOLITES

2.4.1 ESTIMATION OF FLAVONOIDS (Narendra Devanaboyina et al., 2013)
Added 0.5ml of the sample into a test tube containing 1.25ml of distilled water. Then added 0.075ml of 5% sodium nitrite solution and allowed to stand for 5 minutes. Added 0.15ml of 10% aluminum chloride, after 6 minutes 0.5ml of 1M NaOH were added and the mixture were diluted with another 0.275ml of distilled water. The absorbance of the mixture at 510nm was measured immediately. The flavonoid content was expressed as milligram catechin equivalents/g sample.

2.4.2 ESTIMATION OF PHENOLS (Singleton and Rossi., 1965).
Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce a blue-coloured complex (molybdenum blue) that can be estimated calorimetrically at 650nm. 1.0ml of the sample was mixed with 1.0ml of folin’s ciocalteau’s phenol reagent. After 3 minutes 1.0ml of saturated 35% sodium carbonate was added to the mixture and made upto 10.0ml by adding distilled water. The reaction was kept in the dark for 90 minutes, after which its absorbance were at 725nm. A calibration curve was constructed with different concentrations of gallic acid (0.1-0.5ml) as standard. The results were expressed as mg as gallic acid equivalents per gram of extract.

2.4.3 ESTIMATION OF TANNINS (Schanderl, 1970)
The vanillin reagent will react with any phenol that has an unsubstituted resorcinol or phloroglucinol nucleus and forms a coloured 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.

2.4.4 ESTIMATION OF ALKALOIDS (Narendra Devanaboyina et al., 2013)
A part of extract residue was dissolved in 2N HCL and then filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and complex extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform.

2.5 DETERMINATION OF TOTAL ANTIOXIDANT
2.5.1 FERRIC THIOCYANATE METHOD (FTC) (Kikuzaki and Nakatani, 1993)
A mixture of 4.0mg of various extracts of plant sample in 4.0ml of absolute ethanol, 4.0ml of 2.52% linolenic acid in absolute ethanol, 8.0ml of 0.05M phosphate buffer (pH7.0) and 3.9ml of water placed in a vial with a screw cap and then placed in dark over at 40°C. To 0.1ml of this solution were added 9.7ml of 75% ethanol and 0.1ml of 30% ammonium thiocyanate. Precisely 3 minutes after the addition of 0.1ml of 0.02M ferrous chloride in 3.5% HCl to the reaction mixture. The absorbance of red colour was measured at 500nm every 24 hour until one day after the absorbance of control reached its maximum. Ascorbic acid was used as positive controls, while a mixture without a plant sample was used as the negative control.

2.5.2 THIOBARBITURIC ACID (TBA) ASSAY (Ottolenghi, 1959)
2ml of 20% trichloroacetic acid and 2ml of 0.67% of 2-thiobarbituric acid were added to 1ml of sample solution, prepared with the FTC method. The mixture was placed in a boiling water bath and after cooling was centrifuged at 3000rpm for 20 minutes. Absorbance of supernatant was measured at 552nm. Antioxidant activity was based on the absorbance on the final day of the FTC method.
3.0 RESULTS AND DISCUSSIONS

3.1 QUALITATIVE PHYTOCHEMICAL ANALYSIS

TABLE: 1 QUALITATIVE PHYTOCHEMICAL ANALYSIS OF AVERRHOA CARAMBOLA FRUIT IN DIFFERENT SOLVENT EXTRACT

<table>
<thead>
<tr>
<th>Test</th>
<th>50% Ethanolic Extract</th>
<th>Water Extract</th>
<th>Ethanol Extract</th>
<th>Acetone Extract</th>
<th>CHCl₃ extract</th>
<th>Pet. Ether extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Phenols</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteins Glycosides</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

++ = High       ++ = Moderate     + = Low          = Absence.

Phytochemical analysis is of paramount importance in identifying new source of therapeutically and industrially valuable compounds in medicinal plants.

Flavonoids, alkaloids and phenolic compounds are the most important groups of secondary metabolites and bioactive compounds in the plants and good sources of natural antioxidants in human diets. Phytochemicals have been reported to directly influence the antioxidant properties of the plant extract (Kim et al., 2003).

The result of qualitative phytochemical screening of the plant extracts are shown in table 1. The present study reveals that the extracts of Averrhoa carambola fruit were found to have alkaloids, phenols, flavonoids, protein, tannins, steroids and glycosides. The hydroethanolic extract showed better result of phytochemical analysis than other solvents and hence it was used for further analysis.

The results of similar studies indicate that Morinda citrifolia fruit is a good source of phytochemicals and antioxidant and the solvents have the ability to dissolve many useful organic molecules found in plant (Jung, 2014).

3.2 QUANTITATIVE ESTIMATION OF SECONDARY METABOLITES IN HYDROETHANOLIC FRUIT EXTRACT OF AVERRHOA CARAMBOLA

Medicinal plants are being used traditionally around the globe. Therefore such plants should be explored to understand and identify their properties, safety and efficacy. Plants produce a
diverse range of bioactive molecules which make them a rich source of medicinal compounds that play a dominant role in the maintenance of human health. Complex antioxidant systems play decisive role in protecting cellular membranes and organelles from the damage caused by reactive oxygen species (Mukesh et al., 2012).

Quantitative assay of secondary metabolites were carried out for the estimation of phenols, flavanoids and tannins and Alkaloids the result obtained in the estimation of secondary metabolites were shown in figure 1. *Averrhoa carambola* fruit sample contains 29 ± 0.32 mg/g phenols, 85.45 ± 0.87 mg/g flavanoids, 51.8 ± 0.12 mg/g tannins and 115 ± 0.65 mg/g alkaloids.

The presence and quantification of various secondary metabolites such as alkaloids, flavonoids, phenols etc were reported by Ashafa et al. (2010) from *Felicia Muricata* leaves.

![Quantitative Analysis Graph](image)

**FIGURE: 1 QUANTITATIVE ESTIMATION OF SECONDARY METABOLITES**

### 3.3 DETERMINATION OF TOTAL ANTIOXIDANT IN HYDROETHANOLIC FRUIT EXTRACT OF *AVERRHOA CARAMBOLA*

#### 3.3.1. FERRIC THIOCYANATE METHOD

The total antioxidant assay of the hydroethanolic fruit extract of *Averrhoa carambola* by FTC was shown in Figure:2.
The FTC assay was carried out as described by Kikuzaki and Nakatani, 1993. The FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation in which peroxide react with ferrous chloride and form ferric ion. The ferric ion then combines with ammonium thiocyanate and produce ferric thiocyanate. In FTC method, the extract exhibits strong antioxidants potential compared with vitamin E (positive control).

The Figure-2 illustrated the absorbance of various incubation periods showing the activity of extract compared with standard for seven consecutive days. The plant showed low absorbance value, which indicated the high level of antioxidant activity. The sample showed absorbance values greater than negative control (without plant extract) and less then positive control at the end point of the method indicating the presence of antioxidant activity.

Sharipah Ruzaina Syed Aris (2009) also demonstrated the antioxidant activity in fruit of ficus Deltrideavaru Angustifolia sp by FTC method and the plant showed low absorbance value, which indicated the high level of antioxidant activity.

### 3.3.2 THIO BARBITURIC ACID METHOD (TBA)

The total antioxidant assays of the hydroethanolic leaf extract of Averrhoa carambola by TBA method was shown in the Figure 3.
The TBA method was used to determine the amount of at a later stage of lipid oxidation when peroxide decomposes to form carbonyl compounds. The test was conducted according to the method of Ottolenghi, 1959.

The Figure-3 illustrated the absorbance of various incubation periods showing the activities of crude extract compared with standard for seven consecutive days. In TBA method the extract exhibit strong antioxidant potential compared with vitamin E (positive control). The plant sample showed low absorbance value, which indicates the high level of antioxidant activity. The sample showed absorbance values were greater than negative control (without plant extract) and less then positive control at the end point of the method indicating the presence of antioxidants activity. The present study revealed that the antioxidant activity was present in 50% ethanolic fruit extract of *Averrhoa carambola*.

In a similar study demonstrated by Sharipah Ruzaina Syed Aris (2009) it was confirmed that Fruit of *ficus Deltrideavar Angustifolia* by TBA method and the plant showed low absorbance value, which indicated the high level of antioxidant activity.

**4.0 CONCLUSION**

Screening of *Averrhoa carambola* fruit clearly reveals that the maximum classes of phytoconstituents are present in the hydroethanolic fruit extract. The antioxidant activity of *Averrhoa carambola* hydroethanolic fruit extract may be due to the presence of secondary metabolites, containing the hydroxyl group that confers the hydrogen donating ability. Hence,
the above fruit extract could be explored for its highest therapeutic efficacy by pharmaceutical companies in order to develop safe drugs for various ailments.

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5.0 REFERENCE


