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# In vitro Antioxidant Activity of Plant Extracts; A Comparative Study

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***Abstract***
*There is increasing recognition that many of today's diseases are due to the "oxidative stress" that results from an imbalance between the formation and neutralization of reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can be removed with antioxidants. The main objective of the present study was to evaluate the antioxidant activity of plants in different solvent systems. The antioxidant activity of the plant has been studied using its reducing power and the total phenolic content was estimated using Folin-Ciocalteau. All the selected plants showed some degree of antioxidant activity. Comparatively high antioxidant value was observed in acetone and aqueous extracts of most of the selected plants. Among the three selected plants Manihot esculenta showed maximum antioxidant activity and the highest phenolic content. Analysis of plant extracts revealed a high amount of phenolic compounds suggesting a possible role of these phytoconstituents in the antioxidant property.*

***Keywords:*** *Reactive oxygen species, antioxidant activity, acetone, petroleum ether, phenoli content, Manihot, Alamanda,*

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## I. INTRODUCTION

Antioxidants play a crucial role in preventing cellular damage, which is linked to various diseases such as cancer, aging, stroke, heart attacks, diabetes, and neurodegenerative disorders like Parkinson's and Alzheimer's[1]. These diseases can result from an excess of free radicals—unstable molecules with unpaired electrons formed when oxygen interacts with other substances. Free radicals can cause damage by reacting with essential cellular components like DNA and cell membranes[2].

Antioxidants neutralize free radicals, stopping them from causing further damage. They achieve this by undergoing oxidation themselves, thereby protecting vital molecules in the body. In addition to safeguarding cells, antioxidants can enhance immune function and lower the risk of cancer, heart disease, age-related conditions, and other health issues. They also support immune defense by boosting the activity of various immune cells and can help prevent cataracts and other age-related disorders[3,4].

*Manihot esculenta*, commonly known as cassava and belonging to the Euphorbiaceae family, contains potentially harmful compounds such as cyanogenic glycosides, including linamarin and lotaustralin. These compounds can release cyanide, posing risks to both human and animal health. The levels of these toxic substances can vary significantly depending on the cassava variety and environmental conditions. Research on cassava leaf extracts has demonstrated that certain varieties possess antioxidant and antimutagenic properties. In particular, studies assessing the total aqueous extracts from cassava leaves have highlighted their notable antioxidant and antimutagenic effects. The total phenol content of these extracts, measured using the Folin-Ciocalteau method, was found to be positively correlated with their antioxidant and antimutagenic activities[5].

The antioxidant activity of cassava leaf extracts was evaluated using several methods, including the DPPH scavenging assay and ferric reducing power assay. The methanolic extracts of *Manihot esculenta* leaves exhibited high levels of total phenolic compounds and ascorbic acid. Notably, the methanolic extracts showed a greater reducing power compared to acetone extracts from the same plant. These findings indicate that cassava leaves contain significant antioxidants, suggesting that further research could reveal their full potential as sources of antioxidant and antimutagenic agents[6].

*Allamanda cathartica*, also known as golden trumpet[7], common trumpetvine, or yellow allamanda, is a flowering plant native to Brazil and belongs to the Apocynaceae family. This plant is recognized for its medicinal uses, but all its parts contain allamandin, a toxic iridoid lactone[8]. Despite its milky sap having potential antibacterial and anticancer properties, it is poisonous if consumed in large quantities. A study revealed that the methanol extract of *Allamanda cathartica* contains several phytochemicals, including flavonoids, saponins, terpenoids, tannins, and alkaloids. The antioxidant activity of the extract was found to be dose-dependent, with

higher concentrations demonstrating greater activity. The extract exhibited moderate free radical scavenging ability in the DPPH assay[9]. Overall, the study concluded that *Allamanda cathartica* has an average total antioxidant capacity and a relatively weak ferric reducing potential compared to vitamin C[10].

Codiaeum variegatum, commonly known as garden croton or variegated croton, is a plant species in the Euphorbiaceae family. Research has identified several bioactive compounds in its extracts, including alkaloids, gums, tannins, and saponins. Phenolic compounds and flavonoids in Codiaeum variegatum have been linked to its antioxidant activity, particularly in scavenging singlet oxygen and free radicals. Plants containing gums, tannins, and saponins are known to be good sources of antioxidants. In one study, extracts of Codiaeum variegatum demonstrated notable scavenging activity against DPPH free radicals, a stable radical that can accept electrons from antioxidant compounds, thereby neutralizing its free radical properties[11].

## II. MATERIALS AND METHODS

### 2.1 Collection of materials

Different species of plant materials were collected from the local areas of Changanacherry and Alappuzha. Plants selected and parts used are the following. Leaves of *Manihot esculenta, Alamanda cathartica* and *Codiaeum variegatum* are collected.

### 2.2 Preparation of the Plant extracts

Plant parts are washed in tap water and then sterile distilled water. Then dried it in hot air oven at 40°C for few days. Dried leaves were powdered by grinding in a mixer grinder. Ig of powder was weighed and mixed with 50 ml of different solvents like- Distilled water, acetone and Petroleum ether. Then it was placed in an orbital shaker for overnight shaking. Filtered it and the extract was separated.

### 2.3 Screening of the Plant extracts

The extracts of plant parts were screen for comparing their overall antioxidant activity using the method of Oyaizu et al, 1986. Take solution of 500µg/ml conc. (0.5ml) in a test tube. Add 0.5ml of Phosphate buffer (0.2m, pH 6.6). Then add 2.5ml of Potassium ferricyanide [K+Fe (CN) & 1%]. To the test tube labeled blank add 1ml of Phosphate buffer and 2.5ml ofK3Fe (CN) 6. The mixture was incubated at 50°C for 20 minutes. After incubation, add 2.5 ml of 10% Trichloro acetic acid (10%). Centrifuge at 3000 rpm for 10 minutes. From it 5ml of supernatant was transferred to another test tube and was mixed with 5ml of distilled water. Add 1ml FeCl3 (0.1%). Absorbance was measured at 700nm. The increased absorbance of the reaction mixture indicated increased reducing power. Since the plant extract was showing a high activity and therefore the colour being non-measurable, it was further diluted from 500µg/ml to 31.25µg/ml. Then above procedure was repeated.

### Ascorbic acid Standard

Stock Concentration-10mg/5ml of distilled water. Using the standard of Ascorbic acid, various concentrations (2,1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml) was estimated with above procedure to compare the antioxidant activity of plant extracts.

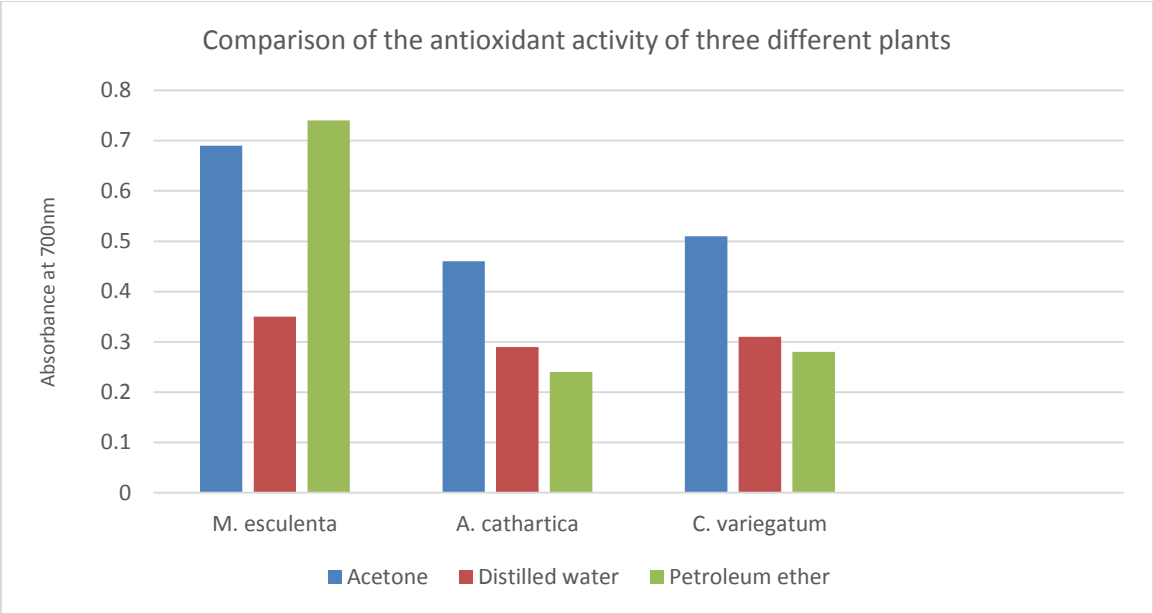
### 2.4 Estimation of total Phenolic contents

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue colored complex (Molybdenum blue). Weigh exactly 0.25g of the sample and grind it with mortar and pestle in 10-time volume of 80% ethanol. Centrifuge homogenates at 10,000 rpm for 20 minutes. Save the supernatant. Re-extract the residue with 5 times the volume of 80% ethanol centrifuge and pool the supernatants. Evaporate the supernatant to dryness. Dissolve the residue in a known volume of distilled water (10 ml). Pipette out different aliquots (0.05ml & 0.1ml) into test tubes. Make up the volume in each tube to 3ml with water. Add 0.5ml of Folin-Ciocalteau reagent. After 3 minutes, add 2 ml of 20% $Na_2$ CO; solution to each tube. Mix thoroughly, place the tubes in boiling water for exactly one minute, allow cool and measure the absorbance 650nm against a reagent blank. Prepare a standard curve using different concentrations of Catechol.

### 2.5 The antioxidant activity of three different plants

| No. | Plants | Absorbance at 700nm (concentration 0.05mg/ml) | | |
|---|---|---|---|---|
| | | Acetone | Distilled water | Petroleum ether |
| 1. | *Manihot esculenta,* | 0.69 | 0.35 | 0.74 |
| 2. | *Alamanda cathartica* | 0.49 | 0.29 | 0.24 |
| 3. | *Codiaeum variegatum* | 0.51 | 0.31 | 0.28 |

Table no. 2.1

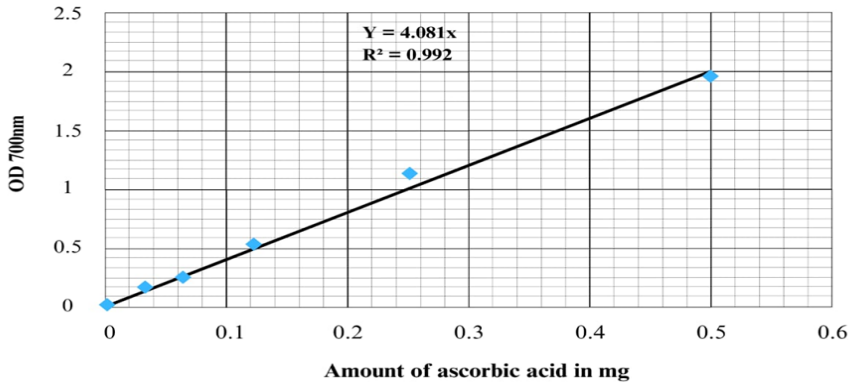



Graph 2.1

It can be understood from the above table that all the plants taken for study, *M. esculenta* has high antioxidant activity. All the plants have given positive results in all the two extracts. Plants show higher absorbance in Petroleum ether.

**2.6 Estimation of Antioxidant activity by using Ascorbic acid as standard**

Since the ascorbic acid at concentrations of 2mg/ml and 1mg/ml showing high activity and therefore the colour being non-measurable, values of them are not used in the standard curve.



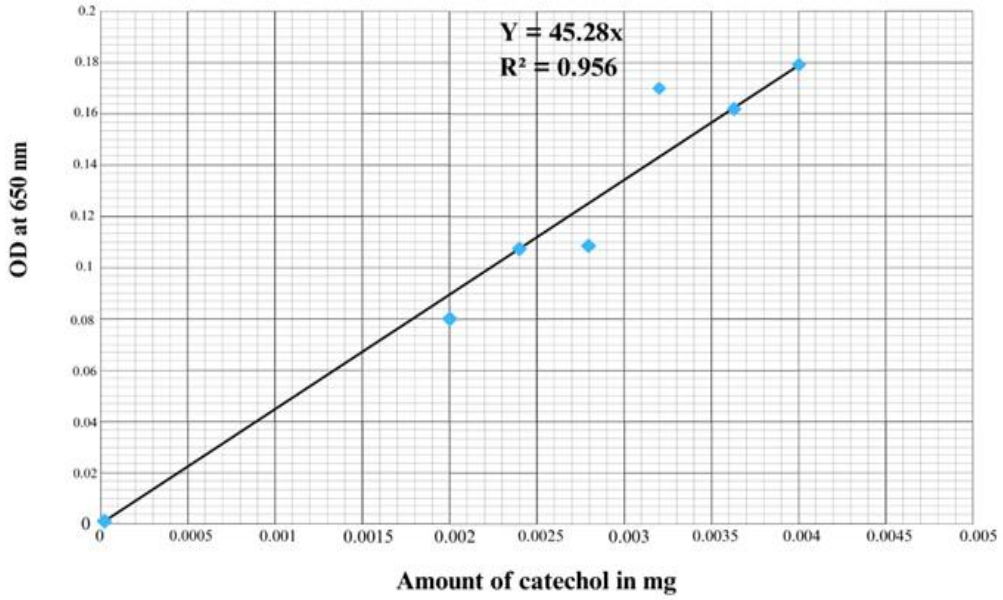

Graph 2.2 Standard curve using different concentrations of Ascorbic acid

| Sl. No. | Plants | OD at 700 nm in different solvents | | 0.5g leaf tissue is equivalent to following mg of Ascorbic acid |
|---|---|---|---|---|
| 1. | *Manihot esculenta* | Acetone | 0.69 | 1.6 |
| | | Distilled water | 0.35 | 0.8 |
| | | Petroleum ether | 0.74 | 1.8 |
| 2. | *Alamanda cathartica* | Acetone | 0.46 | 1.1 |
| | | Distilled water | 0.29 | 0.5 |
| | | Petroleum ether | 0.24 | 0.7 |
| 3. | *Codiaeum variegatum* | Acetone | 0.51 | 1.2 |
| | | Distilled water | 0.31 | 0.7 |
| | | Petroleum ether | 0.28 | 0.6 |

Table no. 2.2 Comparison of antioxidant activity of plant extracts with ascorbic acid standard.

**2.7 Estimation of Phenolic content of Selected plants**

Phenol content of the selected plant *Manihot esculenta*, was estimated using f-c method. The absorbance was read at 650 nm and it is expressed in terms of mg equivalent of pyrrocatechol. The result established is tabulated below.




Graph 2.3 Standard curve using different concentrations of Catechol

Calculation of Phenol content of leaf tissue from standard graph equation: X value of OD (650nm) 45.28mg of catechol Therefore the amount equivalence of catechol= (X/45.28) mg

| No | OD at 650 nm | Phenolic content in terms of equivalent of pyrrocatechol (mg per l gm of dry plant tissue) |
|---|---|---|
| $S_6$ | 0.65 | 5.74 |

Table no. 2.3

1 gm of *Manihot esculenta* leaf tissue is equivalent to phenolic content of 5.74mg of catechol.

## III. DISCUSSION

From the above results, in the selected three solvents plants show higher absorbance in Acetone and Petroleum ether. Manihot esculenta showing higher phenolic content when estimated at 650nm Other two plants also show high activity in acetone and petroleum ether. Since these plants are chosen randomly and they are common, we have found that they have antioxidant property. Thus they can be used for further studies and can be used in various fields. Plant derived secondary metabolites are receiving great attention in recent years due to their diverse biological activities.. In the present study, we have applied a wide range of established in vitro assays to evaluate the antioxidant and free radical scavenging activities of three plant extracts. In the estimation of total phenolic content, pyrrocatochol,is taken as a standard because, phenolic compounds hydrolyse to give catochol. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue coloured complex molybdenum blue.

## IV. CONCLUSION

Natural antioxidants have increasingly broad prospects of development because of its high demand in cosmetics, health products, food and pharmaceutical industries. The present study analyzed the antioxidant activity of 3 selected plants which are widely distributed in our locality. The highest antioxidant activity was shown in the plant extracts of acetone and petroleum ether. Moreover, we might speculate that the antioxidant activity of the plants could be related to the high concentration of phenolics. So it can be known that these plants may provide potential natural antioxidants for the medicine industry and other fields. In conclusion, these routinely used plants can be explored further as potential sources of natural antioxidants.

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