

# Comparision Of Antioxidant Activty Of Ethanolic Leaf And Fruit Extract Of Sapindus Mukorossi By Invitro Method

<sup>1</sup>K.Radhika, <sup>2</sup>A.Shashanka, <sup>3</sup>A.Pragathi  
<sup>1</sup>Assistant Professor, <sup>2-3</sup>Student  
 Gland Institute of Pharmaceutical Sciences

**Abstract - Antioxidant plays a major role in the prevention of carcinogenesis. In this study we evaluate the anti-oxidant activity by Hydrogen peroxide radical scavenging assay for the ethanolic leaf and fruit *Sapindus mukorossi*. The percentage yield of leaf and fruit extract was more for fruit than leaf respectively. These showed the positive test for Alkaloids, Tannins, and Phenolic compounds, Proteins, Flavonoids, Sterols and Saponins in phytochemical screening. For determination of antioxidant activity Ascorbic acid was used as standard by Hydrogen peroxide radical scavenging assay it was observed that fruit extract of *Sapindus mukorossi* showed more activity than leaf extract.**

**Keywords: Antioxidant, Carcinogenesis, Flavonoids**

## INTRODUCTION:

### HERBALMEDICINE:

Herbal medicine is the art or practice of using herbs and herbal preparations to maintain health and to prevent, alleviate or cure diseases. This is the plant or plant part or an extract or mixture of these used in herbal medicine. Herbal drugs are made from the roots, stems, leaves, bark, fruit, seeds, or flowers of various plants known or believed to have medicinal property. The word '**DRUG**' comes from French word '**DROGUE**' meaning '**DRIED HERB**'.

### Importance of Herbal medicine:

- Treat diseases and enhance general health and wellbeing
- Used for treatment of cancer
- Used for treating various infections like cold, dengue, malaria, helminthiasis, etc.
- Used to treat arthritis, heart diseases, atherosclerosis, etc.
- Used as cosmetics

**ANTIOXIDANTS:** Antioxidant is a substance that protect the body cells from damaging effects of oxidation. It can be accelerated by stress, cigarette smoking, and alcohol. When there are disruptions in the natural oxidation process, highly unstable and potentially damaging molecules called free radicals are created. Oxygen triggers the formation of these destructive little chemicals, and, if left uncontrolled, they can cause damage to cells in the body.

Examples: Vitamins E & C

Minerals	–	selenium, manganes	
Plant compounds	–	lycopene, beta carotene	Fruits
		pomegranate, apple, papaya	Vegetables – carrot, tomato, beetroot

### IMPORTANCE OF ANTI OXIDANTS:

- These are capable of preventing the oxidation of cells
- These prevent free radicals from oxidizing agent. Ex: vitamin E & C
- These also protect the body cells from damaging effects of oxidation Ex: vitamin E & C, beta carotene
- These are everything from disease fighters to memory protectors to the antidote to aging
- Donate electrons to free radicals, and make them more stable
- Detoxify the body from harmful molecules

### MATERIALS AND METHODS:

The plant material collected was leaves and fruits of *Sapindus mukorossi*. It was authenticated by Prof. P.Jayaram, Plant Anatomy Research Centre, PARC/2019/3949. The leaves were collected from Shangri-La farms, kothapet (V), Shivampet (M), Narsapur in the month of January 2019 and fruits was collected from local market of Narsapur.

All the collected plant materials were washed thoroughly with water to remove dust and were shade dried for about 1 week. Fruits were dried for about 2 weeks. These dried parts were made into fine powder in mixer grinder.

### **Method of Extraction:**

**Leaves** – 20gms of dried leaves powder was extracted by Soxhlation using 200ml of Ethanol at 50<sup>0</sup>C for 7hours. From the

obtained extract, the solvent was evaporated until the dried mass was obtained.

**Fruits** - By Soxhlation, 20gms of fine powdered fruit was extracted using 200ml of ethanol for 7hours at 50<sup>0</sup>C. The extract obtained was made into dried mass by evaporation.

### **PHYTOCHEMICAL SCREENING:**

#### **1. Detection of Alkaloids:**

##### **a) Mayer's Test:**

To 1ml of test solution Mayer's reagent (Potassium mercuric iodide solution) was added where white or creamy precipitate was observed.

##### **b) Hager's Test:**

1ml of test solution was taken into a test tube and two drops of Hager's reagent (saturated solution of picric acid) was added and yellow precipitate was observed.

##### **c) Wagner's Test:**

To 1ml of test solution, Wagner's reagent (iodine- potassium iodide solution) was added where reddish brown precipitate was observed.

#### **2. Detection of Carbohydrates:**

##### **a) Molisch's Test:**

1ml of the test solution was taken in a test tube and two drops of alcoholic solution of  $\alpha$ -naphthol (Molisch's reagent) was added and shaken vigorously. Then 1ml of conc. Sulphuric acid was added from the walls of the test tube where violet ring was observed at the junction.

#### **3. Detection of Proteins and Amino acids:**

##### **a) Millon's Test:**

To 2ml of sample, few drops of Millon's reagent was added. A white precipitate indicates presence of proteins.

##### **b) Biuret's Test:**

To the sample solution, 2ml drops of 4% NaOH was added and treated with two drops of 1% CuSO<sub>4</sub> solution and observed for the formation of pink colour.

##### **c) Ninhydrin's Test:**

To 1ml of sample, three drops of 5% Ninhydrin's reagent was added and heated in boiling water bath for 10minutes. Purple colour indicates presence of proteins.

#### **4. Detection of Sterols:**

##### **a) Libermann-Burchard's Test:**

To the test solution, 2ml of acetic anhydride, 2ml of chloroform were added and heated to boiling and cooled. Then 1ml of concentrated sulphuric acid was added from the walls of test tube which results in colour change indicating the presence of sterols.

#### **5. Detection of Flavonoids:**

##### **a) Alkaline Test:**

The sample solution was treated with 10% ammonium hydroxide solution. Yellow colour indicates presence of flavonoids.

#### **6. Detection of Phenolic compounds:**

##### **a) Ferric chloride Test:**

The sample solution was treated with few drops of neutral 5% Ferric chloride solution where a dark green colour observed indicating the presence of phenolic compounds.

##### **b) Lead acetate Test:**

To the sample solution 3ml of lead acetate was added which gives bulky white precipitate indicating the presence of phenolic compounds.

#### **7. Detection of Tannins:**

The sample solution was boiled with 2% Hydrochloride solution. Red precipitate indicates the presence of tannins.

#### **8. Detection of Saponins:**

##### **a) Foam Test:**

The sample was diluted with 20ml of distilled water and shaken for 15min in graduated cylinder and observed for formation on a layer of stable foam.

### **ANTI-OXIDANT ACTIVITY PROCEDURE:**

Anti-oxidant activity was performed by following *in-vitro* method:

- Hydrogen peroxide radical scavenging assay

#### **Hydrogen peroxide radical scavenging assay:**

Principle:

Hydrogen peroxide is a weak oxidizing agent and can inactivate few enzymes directly by oxidation of essential thiol (-SH) groups. Hydrogen peroxide crosses the cell membrane and reacts with Fe<sup>+2</sup> or Cu<sup>+2</sup> ions to form hydroxyl radicals which may be origin of many toxic effects.

**Reagents:**

- Phosphate buffer was prepared by dissolving 2.38gms of disodium hydrogen phosphate (ortho), 0.19gms of potassium dihydrogen phosphate and 8gms of sodium carbonate in 1000ml of distilled water. Adjust the pH, if necessary.

**Working procedure:**

Plant extract prepared in various concentrations was mixed with 0.6ml of 4mM H<sub>2</sub>O<sub>2</sub> solution prepared in phosphate buffer (0.1M pH 7.4) and incubated for 10min. the absorbance of the solution was taken at 230nm against blank solution containing H<sub>2</sub>O<sub>2</sub> without plant extract. All the analyses were performed in triplicate and results were averaged and ascorbic acid used as a positive control treated in the same way with H<sub>2</sub>O<sub>2</sub> solution. The percentage inhibition was measured by comparing the absorbance of control and test.

H<sub>2</sub>O<sub>2</sub> scavenging activity = [Abs control – Abs sample/ Abs control] \*100

Where Abs control – Absorbance of control Abs sample – Absorbance of sample.

**RESULTS AND DISCUSSIONS:****Physical status of extracts:****Table 1: Percentage yield of ethanolic leaf and fruit extract of *Sapindus mukorossi*.**

S.No.	Plant part	Method of Extraction	Colour and consistency	Weight (gms)	Extract (gms)	Percentage yield (%w/w)
1	Fruit	Soxhlation	Dark brown Semi solid	20	14	70
2	Leaf	Soxhlation	Dark green Semi solid	20	5.4	27

Based on the physical status (table 7.1) percentage yield of ethanolic fruit and leaf extracts was found to be 70% and 27% respectively. The colour of the fruit extract was dark brown in semisolid form while the colour of the leaf extract was dark green which is also observed in semi solid form.

**Preliminary phytochemical screening:**

Preliminary phytochemical screening was performed with standard analytical reagents and the results representing the presence of active constituents were listed below:

**Table 2: Presence (+) and absence (-) of active compounds in ethanolic leaf and fruit extracts of *Sapindus mukorossi* during phytochemical screening.**

Phytoconstituents	Fruit extract	Leaf extract
Alkaloids	-	-
Carbohydrates	+	+
Proteins	+	+
Sterols	+	+
Flavonoids	+	+
Phenolic compounds	+	+
Tannins	-	-

Saponins	+	+
----------	---	---

From the above results, fruit and leaf extracts contains Carbohydrates, Proteins, Sterols, Flavonoids, Phenolic compounds and Saponins.

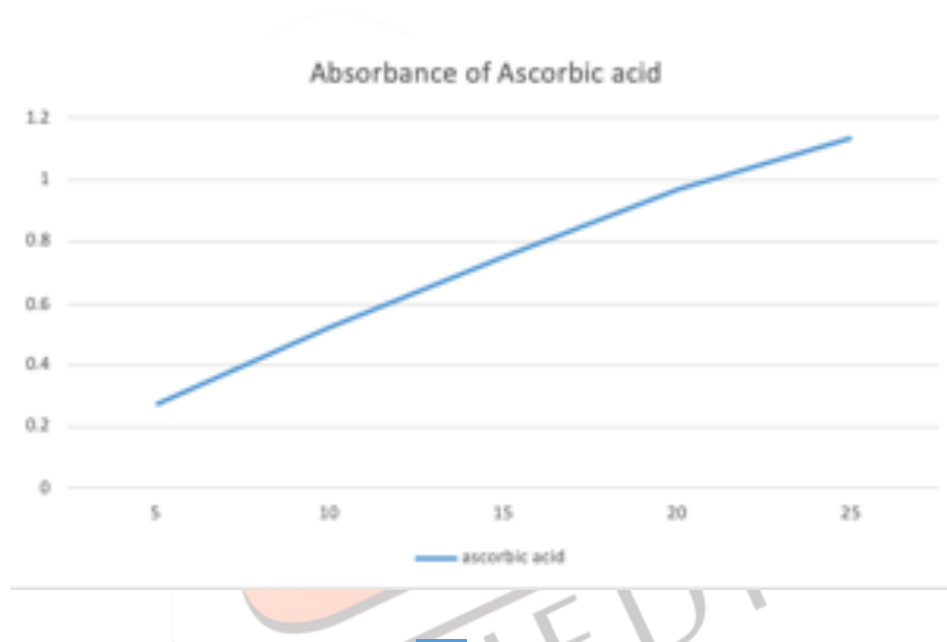
#### ANTIOXIDANT ACTIVITY:

##### Hydrogen peroxide radical scavenging assay:

Antioxidant activity was performed by Hydrogen peroxide radical scavenging assay in *In-vitro* method and results were observed as followed:

**Table 3: Absorbance of Ascorbic acid**

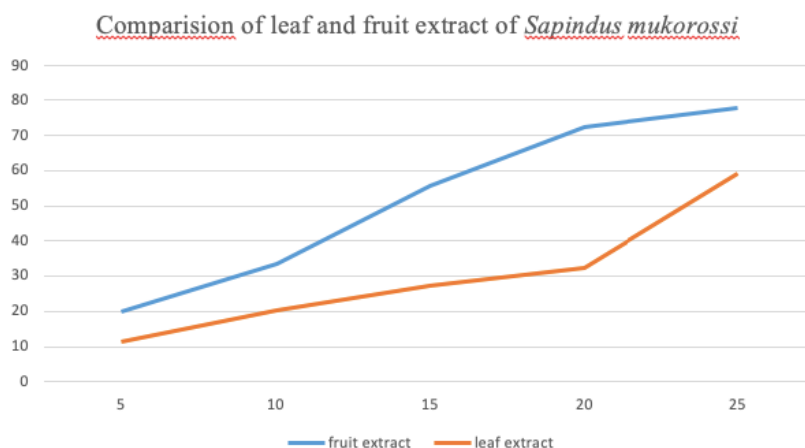
Concentration ( $\mu\text{g/ml}$ )	Absorbance
5	0.275
10	0.525
15	0.751
20	0.968
25	1.138



**Fig 1: Absorbance of Ascorbic acid in Hydrogen peroxide radical scavenging assay.**  
As the concentration of Ascorbic acid increased, absorbance also increased.

**Table 4: Effect of leaf and fruit extract of *Sapindus mukorossi* on Hydrogen radical scavenging assay**

Concentration ( $\mu\text{g/ml}$ )	Percentage inhibition	
	Fruit extract	Leaf extract
5	20	11.2
10	33.4	20.2
15	55.8	27.2
20	72.4	32.3
25	78.1	59.2

**Fig 2: Effect of leaf and fruit extract of *Sapindus mukorossi* on inhibition of Hydrogen peroxide radical scavenging assay****CONCLUSION**

The results of the present study showed antioxidant activity as that of standard Ascorbic acid by Hydrogen peroxide radical scavenging assay where fruit extract has more activity than leaf extract.

**REFERENCES**

- [1] Merriam webster.com; since 1828; herbal medicine
- [2] Bratman, Steven, and David J. Kroll. Clinical Evaluation of Medicinal Herbs and Other Therapeutic Natural Products. Roseville, CA: Prima Publishing, 1999.
- [3] Ernst, Edzard, ed. Herbal Medicine: A Concise Overview for Professionals. Oxford: Butterworth-Heinemann, 2000.
- [4] Fleming, Thomas, ed. Physicians' Desk Reference for Herbal Medicines. Montvale, NJ: Medical Economics Company, 1998.
- [5] Klein, Siegrid, and Chance Riggins. The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines. Boston: Integrative Medicine Communications, 1998.
- [6] Miller, Lucinda G., and Wallace J. Murray, eds. Herbal Medicinals: A Clinician's Guide. Binghamton, NY: Pharmaceutical Products Press, 1998.
- [7] Priyanka.B, Anitha.K, Shirisha.K, Jannipasha SK, Dipankar B, Rajesh, International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS) 3(2) 93-101: 2013: Hydrogen peroxide radical scavenging assay
- [8] Beth Fontenot; the Atlantis ; October 30 2011 Antioxidants and free radicals Singh et al. International Journal of Phytomedicine 8(1) 22-28; 2016; method of extraction
- [9] Meesala Srinivasrao, Mangamoori Lakshminarasu, A.Anjum, Mohammed Ibrahim: Annals of Phytomedicine 4(2): 93-97, 2015; method of extraction
- [10] Bharathi Arora, Preeti Bhadauria, Deepak Tripathi, Alok Sharma: Indo Global Journal of Pharmaceutical Sciences 2(3): 250-257, 2012; phytochemical screening procedure