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Phytochemical Screening and In Vitro Antioxidant Activity of *Amorphophallus paeoniifolius*



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ABSTRACT

Antioxidant is a molecule that inhibits the oxidation of the other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. Present investigation is of the antioxidant activity of methanolic extract of *Amorphophallus paeoniifolius* tuber extract. The extract was screened for possible antioxidant activity by DPPH method. The results showed that *Amorphophallus paeoniifolius* tuber extract possesses antioxidant properties when compared with standard antioxidant such as Ascorbic Acid.



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INTRODUCTION

Antioxidants

Antioxidants are substances which help to neutralize free radicals in your body by stabilizing their chemical structure. Antioxidants provide extra electrons to the free radicals and prevent them from causing to the body's cell and tissue. They are believed to reduce the physical effects of aging, and possibly prevent disease. The human body produces some antioxidants naturally, while food and health supplements provide others. Well known antioxidants include enzymes such as catalase, superoxide dismutase and other substance such as vitamin C, vitamin E and beta carotene and to trace elements including selenium and zinc.

Classification of antioxidants

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation. These compounds may be synthesized in the body or obtained from the diet. The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed. Some antioxidants are only found in a few organisms and these compounds can be important in pathogens and can be virulence factors. The relative importance and interactions between these different antioxidants are very complex question, with the various metabolites and enzymes systems having synergistic and interdependent effects on one another. The action of one antioxidant may therefore depend on the proper function of other members of the antioxidant system. The amount protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts. Some compounds contribute to antioxidant defense by chelating transition metals and preventing them from catalyzing the production of free radicals in the cell. Particularly important is the ability to sequester iron, which is the function of iron-binding proteins such as transferrin and ferritin. Selenium and zinc are commonly referred to as antioxidant nutrients, but these chemical elements have no antioxidant action themselves.

Enzymes	Antioxidants	Role	Remarks
	Superoxide Dismutase(SOD) Mitochondrial Cytoplasmic Extracellular	Dismutase O_2^{7-} to H_2O_2	Contains manganese (Mn, SOD) Contains copper & zinc (Cu, Zn, SOD) Contains copper(Cu, SOD)
	Catalase	Dismutase H_2O_2 to H_2O	Tetrameric hemoprotein present in peroxisomes.
	Glutathione peroxides (GSH.Px)	Removes H_2O_2 and lipid peroxidases	Selenoproteins (contains Se^{+2}) Primarily in the cytosol also mitochondria Uses GSH
Vitamins	Alpha tocopherol	Breaks-lipid peroxidation, lipid peroxide and O_2^{7-} and OH scavenger.	Fat soluble vitamin
	Beta carotene	Scavenges OH, O_2^{7-} and per-oxy radicals. Prevents oxidation of vitamin A Binds to transition metals	Fat soluble vitamin
	Ascorbic acid	Directly scavenges O_2^{7-} , OH, and H_2O_2 neutralizes oxidants from stimulated neutrophils contributed to regeneration of vitamin E.	Water soluble vitamin

THE ROLE OF FREE RADICALS IN DISEASES Reactive oxygen species (ROS)

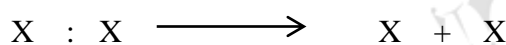
➤ Reactive oxygen species (ROS) are highly reactive ions and free radicals (chemicals containing atoms with an unpaired electron in its outer orbit) involving oxygen molecules. Free radicals are present that do not contain oxygen, but ROS refers to free radicals containing oxygen molecules.

Source of ROS's

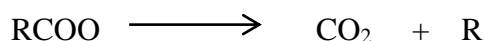
- By product, if cellular respiration presence of redox cycling compounds.
- Synthesized by enzyme systems – phagocytic cells, neutrophils and macrophage (NADPH oxidase myeloperoxidase).
- Exposure to ionizing radiation.
- Smoking, herbicides, pesticides, fried foods etc.

Production

- Chain reaction, free radical steals from nearby compound forming a new free radical. Free radicals may steal electrons from cellular structures or molecules.
- By normal cellular respiration-electron transport system often oxygen is the terminal electron acceptor in the cell mitochondria.
- Hemolysis of covalent bond in presence of thermal or radiant energy.



- Loss of neutral molecule from free radical.



- Oxidation, reduction reactions.



- Biogenesis of free radicals.

Free radicals are natural by products of many biochemical processes within and among cells and essential part of aerobic life and metabolic processes. They continuously produced by the body's normal use of oxygen such as respiration and some cell mediated immune functions. They are also found or generated through stress, exercise, consuming alcohol, exposed to ultraviolet light and fatty diet.

Functions and purpose

- Necessary for production of some hormones (thyroxin).

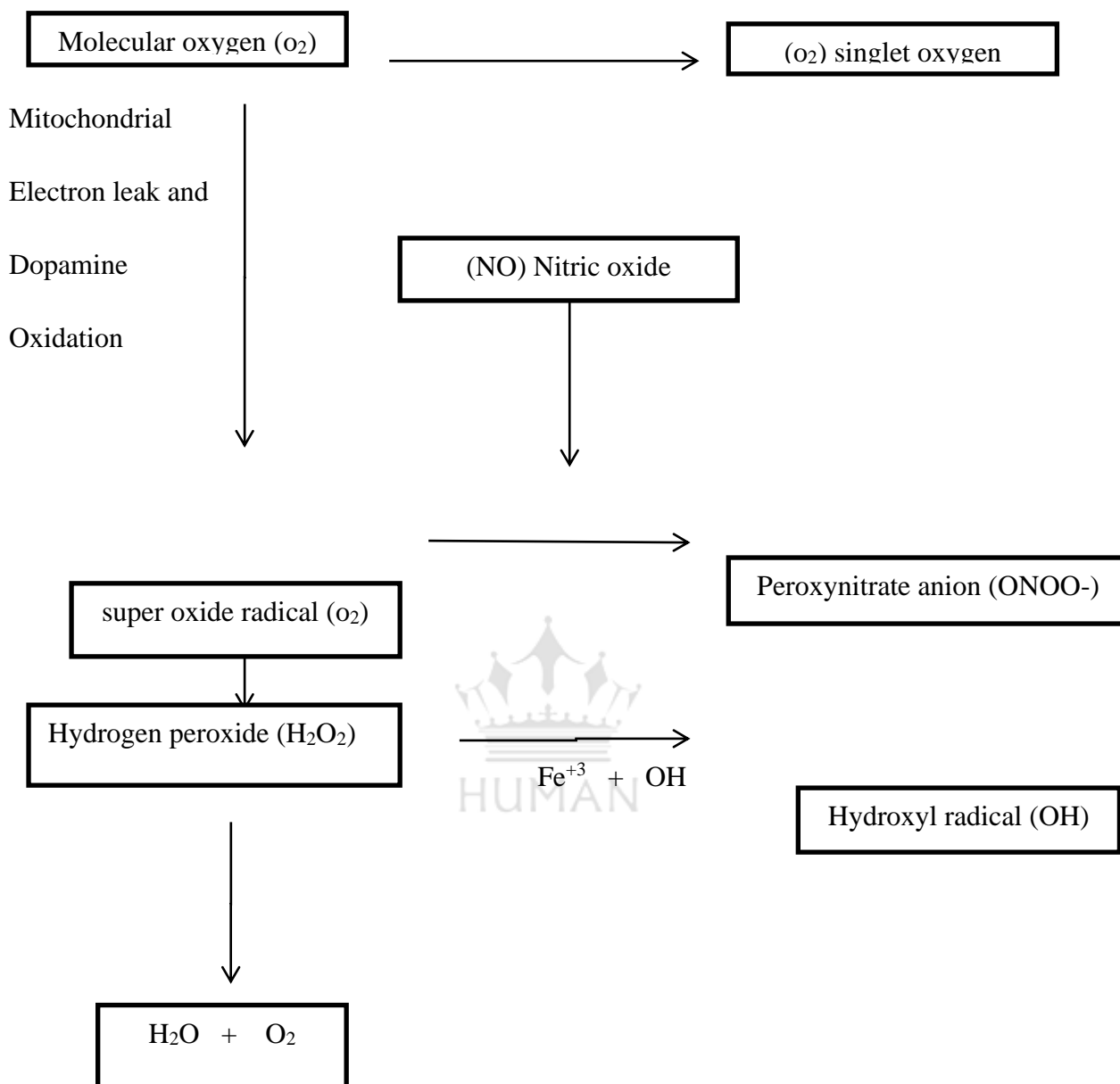
- Generated to kill some type of bacteria and engulfed pathogens.
- Normal cell functions and cell signaling.

The formation of free radicals or imbalance in antioxidant defense within our body may leads to disease include-

- Inflammatory joint disease
- Asthma
- Diabetes mellitus
- Cancer
- Atherosclerosis
- Dementia
- Degenerative eye diseases
- Gastric ulcer
- Parkinson's disease
- Brain trauma, shock, brain damage



Schematic diagram illustrating the formation of oxygen free radicals:



Sources

Antioxidants are found in vegetables, fruits, grain, cereals, eggs, meat, legumes, and nuts. Some such as lycopene and ascorbic acid can be destroyed by long-term storage or prolonged cooking. Other antioxidant compounds are more stable, such as the polyphenolic antioxidants in foods such as whole-wheat cereals and tea. Processed food contains fewer antioxidants than fresh and uncooked foods, as preparation exposes food to oxygen. Foods containing high levels of these antioxidants vitamin C (tocopherols, tocotrienols), vegetable oils polyphenolic antioxidants (resveratrol, tea, coffee, soya, fruit, olive oil, chocolate, cinnamon, flavonoids)

oregano carotenoids (lycopene, carotene, lutein) fruit, vegetables and eggs. Other antioxidants are not vitamins and are instead made in the body. For example, ubiquinol (coenzyme Q) is poorly absorbed from the gut and is made in humans through the mevalonate pathway.

Uses

- Antioxidants are frequently added to industrial products. A common use is as stabilizers in fuels and lubricants to prevent oxidant and in gasolines to prevent the polymerization that leads to the formation of engine fouling residues. They are widely used to prevent the oxidative degradation of polymers such as rubbers, plastics and adhesives that causes a loss of strength and flexibility in these materials.
- Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. However, as oxygen is also important for plant respiration, storing plant materials in anaerobic conditions produces unpleasant flavors and unappealing colors. Consequently, packaging of fresh fruits and vegetables contain 8% oxygen in atmosphere. Antioxidants are an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food. These preservatives include natural antioxidants such as ascorbic acid (AA, E300) and tocopherols (E306) as well as synthetic antioxidants such as propylgallate (PG E310), tertiary butyl hydroquinone (TBHQ), butylated hydroxyl anisole (BHA, E320) and butylated hydroxyl toluene (BHT, E321).
- The most common molecules attacked by oxidation are unsaturated fats; oxidation causes them to turn rancid. Since oxidized lipids are often discolored and usually have unpleasant taste such as metallic or sulfurous flavors, it is important to avoid oxidation in fat-rich foods. Thus, these foods are rarely preserved by drying; instead they are preserved by smoking, salting or fermenting. Even fewer fatty foods such as fruits are sprayed with sulfurous antioxidants prior to air drying. Oxidation is often catalyzed by metals, which is why fats such as butter should never be wrapped in aluminum foil or kept in metal containers. Some fatty foods such as olive oil are partially protected from oxidation by their natural content of antioxidants, but remain sensitive to photo-oxidation. Antioxidant preservatives are also added to fat-based cosmetics such as lipstick and moisturizers to prevent rancidity.

- Antioxidants – rich foods may help prevent various cancers, heart disease, and disease of aging. Vitamin C and E, carotenoids (including beta-carotene), and the mineral selenium are all powerful antioxidants found in food. Vitamin C in the diet (90%) comes from fruits and vegetables. However, since vitamin C is water soluble, cooking can destroy than vitamin C in the food. Vitamin E, also known as tocopherol, is fat. Because vitamin E is found in oils, people who follow a low-fat diet may not get enough. Beta- carotene is a member of the carotenoid family. Found in many plants, carotenoids provide the vibrant red, yellow, green, and orange colors of fruits and vegetables, with carrots being a major contributor of beta-carotene.

Selenium helps in maintain healthy hair and nails. In garlic, seeds, Brazil nuts, meat, immunity works with vitamin E. Eggs, poultry, seafood it reduces the risk of cancer. Beta-carotene keeps skin healthy, helps prevent night red, yellow-orange and leafy carotene blindness and infections, promotes growth and green vegetables and fruits in bone development. Vitamin C destroys free radicals inside and outside cells. Pepper, tomatoes, citrus fruits helps in the formation of connective tissue, and juices, berries, broccoli in healing the wounds, and iron absorption and also spinach, cabbage, potatoes, help to prevent bruising, keep gums healthy and mango, papaya may reduce risk of cataracts, heart diseases and cancer.

PLANT PROFILE

Amorphophallus paeoniifolius (Dennst.) Nicolson

(Syn. *Amorphophallus campanulatus* Blume ex Decne.) of family Areaceae

Figures 1 and 2 are a tuberous plant commonly used in Ayurvedic medicines as well as tribal medicines in India.



Figure No. 1: *Amorphophallus paeoniifolius* tuber



Figure No. 2: Plant of *Amorphophallus paeoniifolius*

Scientific classification

Kingdom: Plantae

Phylum: Magnoliophyta

Order: Alismatales

Family: Araceae

Genus: *Amorphophallus*

Species: *A. Paeoniifolius*

Binomial name *Amorphophallus paeoniifolius* (Dennst.) Nicolson

Synonyms

A. campanulata or Elephant foot yam or Whitespot giant arum or Stink lily,

General description

English Name: Elephant foot yam

Bengali Name: Ol

Sanskrit Name: Suranah

Telugu Name: PulaGandha

Hindi Name: Suran, Jamikand

Parts Used: Corms

Traditional Uses

The corms are acrid, astringent, thermogenic, irritant, anodyne, anti-inflammatory, anti-haemorrhoidal, haemostatic, expectorant, carminative, digestive, appetizer, stomachic, anthelmintic, liver tonic, aphrodisiac, emmenagogue, rejuvenating and tonic. They are useful in vitiated condition of *Vata* and *Kapha*, arthralgia, elephantiasis, tumors, inflammations, haemorrhoids, haemorrhages, vomiting, cough, bronchitis, asthma, anorexia, dyspepsia, flatulence, colic, constipation, helminthiasis hepatopathy, splenopathy, amenorrhoea, dysmenorrhoea, seminal weakness, fatigue, anaemia and general debility.

MORPHOLOGY OF PLANT

A stout herbaceous plant with underground hemispherical depressed dark brown corm; leaves compound, large, solitary, petiole stout, mottled, 60-90 cm long, leaflets 5-12.5 cm long of variable width, obovate or oblong, acute, strongly and many nerved; male and female inflorescences contiguous, neuters absent, appendage of spadix sub-globose or amorphous, equal or longer than the fertile region, spathe campanulate, pointed, strongly, closely veined, greenish-pink externally, base within purple, margins recurved, undulate and crisped, male inflorescence subturbinate, female 7.5 cm or more long; fruits obovoid 2-3 seeded red berries.

Distribution

The plant is cultivated largely throughout India and also found wild from Punjab to West Bengal, Assam, Konkan, dekkon, Rampa Hills. It is also cultivated in Srilanka.

AYURVEDIC PROPERTIES

- Rasa- Katu, Kashaya
- Guna- Ruksha, Tikshna, Guru, Vishada, Laghu
- Vipaka- Katu
- Veerya- Ushna
- Prabhava- Arshaghana
- Dose- Powder 3-6 g.

FORMULATION AND PREPARATIONS

- Avahela and Paka- Sri Bahusala Guda
- Churna - Samudradya Churna
- Vatika - Suranavatica
- It is media in the preparation of *Tamra Bhasma*
- *Loha - Suranava Loha, Surana Modaka*

SIDDHA PROPERTIES

- Siddha name- *Karunai Kilangu*
- *Suvai* (Taste)- *Kaarppu* (Pungent)
- *Gunam* (Pharmacological actions)- *Thuvarppi* (Astringent), *Ul Azhal Atrri*(Demulcent)
- Siddha pharmaceutical Preparations- *Karunai Kilangu Lehyam*
- Uses- Used in treatment of anorectal abscess, hemorrhoids.

PHYTOCHEMICALS SCREENING

Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are:-

- ❖ Flavonoids
- ❖ Carbohydrates
- ❖ Amino Acids
- ❖ Proteins
- ❖ Alkaloids
- ❖ Carotenoids
- ❖ Antioxidants
- ❖ Tannin and Phenolic compounds.

Various methods are used to detect Antioxidant activity some of them are:-

- ❖ Oxygen radical absorbance capacity (ORAC)
- ❖ Total peroxy radical trapping antioxidant parameter (TRAP)
- ❖ B-Carotene bleaching assay (B-CBA)
- ❖ Crocin bleaching assay (CBA)
- ❖ Total phenolic content (TPC)
- ❖ Ferric ion reducing antioxidant power assay (FRAP)
- ❖ 2,2-diphenyl-1-picrylhydrazyl (DPPH)
- ❖ Trolox equivalent antioxidant capacity (TEAC)
- ❖ Cereals, cupric reducing antioxidant capacity (CUPRAC)
- ❖ Lipid peroxidation inhibition assay (LPIA)

- ❖ Hydroxyl radical averting capacity (HORAC assay)
- ❖ Nitric oxide free radical scavenging activity (NOFRSA)
- ❖ Potassium ferricyanide reducing power (PFRP)
- ❖ Thiobarbituric acid reactive substances (TBARS)
- ❖ N,N-dimethyl-p-phenylenediamine (DMPD).

MATERIALS AND METHODOLOGY

MATERIALS

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH)

Ascorbic Acid

Methanol

Tuber powder (*Amorphophallus Paeoniifolius*),

Ninhydrin solution

Molisch reagent

Concentrated Sulphuric acid

2% NaOH Solution

Diluted acid,

Chloroform

Acetic acid

Mayers reagent, Benedicts reagent, Fehlings reagent

Millions reagent, Wagners reagent, Hagers reagent

Dragendroff's reagent, Ferric chloride



Apparatus

Soxhlet Apparatus

UV visible spectroscopy

Methodology

Collection of the sample:- The tubers of *A. paeoniifolius* were collected from the local vegetable market of Karimnagar district, Telangana India. Initially, the tubers were washed with tap water to remove the surface contamination and then sliced into small pieces. The pieces were shade dried and were powdered using an electrical grinder.

Soxhlet extraction:- 10 g of the powder was weighed using an electrical balance and made into 8 packets using filter paper. Petroleum ether, chloroform, methanol, and water were used as solvents for Soxhlet extraction in the increasing order of polarity. The distillation process was carried out at a low temperature of 40°C. After evaporation of solvents, corresponding residues were obtained and stored in the refrigerator for further use.

PHYTOCHEMICALS SCREENING

Test for Carbohydrates

Fehling's Test; Boil a mixture of Fehling's solutions A and B equal volumes and add it to crude plant extract. If a red color precipitate appears it includes the presence of reducing sugars.

Benedict's Test; Boil 2ml of Benedict's reagent with crude extract, a reddish brown colour indicates the presence of the carbohydrates.

Molisch's Test; Shake 2ml of Molisch's solution with crude plant extract then add 2ml of concentrated sulphuric acid and pour it carefully along the side of the test tube. A violet ring appeared at the interphase of the test tube indicates the presence of carbohydrate.

Test for Alkaloids

Mayer's Test; Mayer's reagent (2-3drops) was added to 1ml of various extract. Cream color precipitate is observed in alkaloids.

Dragendoff's Test; In a test tube containing 1ml of extract, few drops of Dragendoff's reagent (potassium bismuth iodide solution) was added, alkaloids show reddish brown precipitate with the same.

Wagner's Test; Wagner's reagent was added to the various test solutions containing 1ml of extracts. Alkaloids show a reddish-brown precipitate with Wagner's reagent.

Hager's Test; Presence of alkaloids is confirmed due to formation of yellow color precipitate with Hager's reagent.

Test for Proteins and Amino Acids

Millon's Test; Mix 2ml of Millon's reagent with the entire plant crude extract, if white precipitate appeared, which upon gentle heating turned into red color indicates the presence of protein in the plant.

Ninhydrin Test; Boil 2ml of 0.2% of Ninhydrin solution with the entire plant crude extract, if Violet color appears it indicates the presence of proteins and amino acids.

Test for Phenols and Tannins

Gelatin Test; 1% gelatin solution with 10% sodium chloride was added to the test solution containing 2ml of extract white precipitate gives the presence of tannins.

Ferric Chloride Test; Presence of blue green color with ferric chloride shows positive test for tannins.

Test for Flavonoids

Shinoda Test; Mix the pieces of magnesium ribbon and concentrated hydrochloric acid with crude plant extract after few minutes pink colored scarlet appears which indicates the presence of flavonoids.

Alkaline Reagent Test; Mix 2ml of 2% of NaOH solution with plant crude extract, intensive yellow color is formed, which turns into colorless when added to 2 drops of diluted acid, this result indicates the presence of flavonoids.

Test for Saponins

Froth Test; Add 5ml of distilled water to crude plant extract in a test tube and shake it vigorously. The foam formation indicates the presence of saponins.

Test for Glycosides

Legal's Test; Treat the extract with pyridine and add alkaline sodium nitroprusside solution blood red color appears.

Bromine Water Test; Test solution when treated with bromine water gives yellow precipitate.

Test for Steroids and Terpenoids

Liebermann-Burchard's Test; 2mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green color indicates the presence of steroids.

Salkowski's Test; 2mg of dry extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of test tube. Formation of red color indicated the presence of steroids.

IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF AMORPHOPHALLUS PAOENIIFOLIUS

Preparation of Standard Solution

Ascorbic acid was used as standard for antioxidant activity. The weight equivalent to concentration 20, 40, 60, 80, and 100 μ g/ml was weighed and dissolved in methanol.

Preparation of Test Solution

Stock solutions of samples were prepared by dissolving 10mg of test sample in 10ml of methanol to give concentration of 1mg/ml. From the above stock solution, the concentration of 20,40,60,80 and 100 μ g/ml were prepared equivalent quantity in methanol.

Preparation of DPPH Solution

4.3mg of DPPH was dissolved in 3.3ml methanol. It was protected from light by covering the test tubes with aluminum foil.

Protocol for Estimation of DPPH Scavenging Activity

DPPH solution was added to 3ml methanol and absorbance was taken immediately at 516nm for control reading. Different concentration levels of test samples 20, 40, 60, 80 100 were screened and made 100 of each dose level by dilution with methanol up to 3ml, 1ml of DPPH solution was added to each test tube.

Absorbance was taken at 516nm in UV- visible spectrophotometer after 15min using methanol as blank.

The % Reduction is Calculated as Follows

The free radical scavenging activity (*FRSA*) was calculated by the following equation;

$$\% \text{ antiradical activity} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

RESULTS AND DISCUSSION

TABLE NO. 1: PHYTOCHEMICAL SCREENING

Phytoconstituents	Test	Results
Carbohydrates	Molischs Test	+ve
	Benedicts Test	+ve
	Fehlings Test	+ve
Alkaloids	Mayers Test	+ve
	Dragendroffs Test	+ve
	Wagners Test	+ve
	Mayers Test	+ve
Tanins and Phenols	Gelatin Test	-ve
	Ferric Chloride Test	-ve
Flavonoids	Shinoda Test	+ve
	Alkaline Reagent Test	+ve

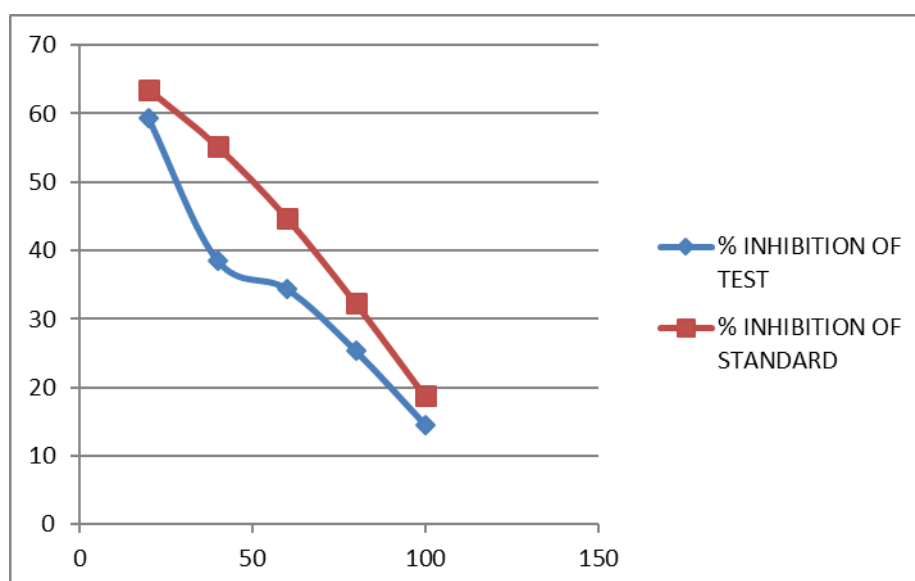
TABLE NO. 2: *IN VITRO* EVALUATION OF ANTIOXIDANT ACTIVITY TEST

Concentration	Absorbance of Test	% Inhibition of Test
20	0.392	59.37
40	0.594	38.54
60	0.632	34.37
80	0.728	25.12
100	0.823	14.58

TABLE NO. 3: *IN VITRO* EVALUATION OF ANTIOXIDANT ACTIVITY STANDARD

Concentration	Absorbance of Standard	% inhibition of Standard
20	0.356	63.54
40	0.437	55.20
60	0.537	44.79
80	0.652	32.29
100	0.782	18.75

COMPARISION OF *IN VITRO* ANTIOXIDANT ACTIVITY OF EXTRACT AND STANDARD



- The various phytochemical screening test for Carbohydrates, Alkaloids, Proteins and Amino Acids, Tannins and Phenols, Flavonoids, Glycosides, Steroids and Terpenoids, Saponins was performed that showed positive results for Carbohydrates, Alkaloids, Flavonoids, Steroids and Terpenoids, and Negative results for Proteins and Amino Acids, Taninns And Phenols, Glycosides, Saponins.
- *In vitro* evaluation of antioxidant activity by DPPH method, the extract was showed absorbance from 0.392 to 0.823 and % inhibition from 14.58 to 59.37 and standard showed absorbance from 0.356 to 0.782 and % inhibition from 18.75 to 63.5.

CONCLUSION

- In the present investigation *Amorphophallus paeoniifolius* extract was prepared by using methanol as solvent by soxhlet apparatus.
- *Amorphophallus paeoniifolius in vitro* antioxidant activity was evaluated by using DPPH method.
- By comparing with standard antioxidant such as ascorbic acid, *Amorphophallus paeoniifolius* was showed better results. So finally, we conclude that *Amorphophallus paeoniifolius* having antioxidant activity.

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