

# PHARMACOGNOSTIC FEATURES, PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF THE LEAF *ALOCASIA MACRORRHIZA*.

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**ABSTRACT:** *Alocasia macrorrhiza* also known as giant taro is a large herbaceous plant belonging to the family Araceae. In this study, the leaves were evaluated for pharmacognostic study and physicochemical parameters. The phytochemical screening was determined to know the phytoconstituents present in the plant. The methanolic extract of the leaves was investigated for quantification of total phenolic and total flavonoid content. The total phenol and flavonoid content was found to be 360mg GAE/g and 80mg QE/g. An *in-vitro* antioxidant activity has been determined by using DPPH radical scavenging activity, reducing power and nitric oxide assay. The plant extract showed a potential result in inhibiting the free radical scavenging activity. This may be due to the significant amount of phenol and flavonoid content in the plant which may act as a vital source for the treatment of various human ailments.

**Keywords:** Pharmacognostic study, Phytochemical screening, DPPH, Reducing power, Nitric oxide, *Alocasia macrorrhiza*.

## I. INTRODUCTION

The benefits of traditional medicines antedate the discovery of modern drugs all over the world since the ancient time (Akinyemi et al.,2005). The different parts of the plant such as leaves, roots, bark, stem, seeds, flowers, fruits, wood etc. contain numerous bioactive compounds. These bioactive compounds called secondary metabolites are responsible for regulation of various health problems (Sanjeev et al.,2017). Flavonoids and other phenolic compounds are commonly known secondary metabolite that act as a natural antioxidant activity reported to have numerous pharmacological activities. The plants containing these compounds are associated to reduce the risk of cancer, cardiovascular disease, diabetes, immune system modulator, inflammation and age related disease (Tungmunnithum et al.,2018).

*Alocasia macrorrhiza* belonging to Araceae family is an herbaceous plant also known as giant taro. It has been reported that people are using traditionally to treat abdominal pain, jaundice, spleen inflammation, ring worm, snakes, insect bites, toothache and also use as a source for digestion, diuretic, laxative, astringent, malaria, typhoid, tuberculosis (Sandoval et al., 2013).

Oxidative stress is a condition that occurs due to the over production of Reactive Oxygen Species (ROS) such as hydroxyl radical, superoxide anion radical, hydrogen peroxide, non-free radical species and singlet oxygen responsible for various degenerative disease in humans. When human antioxidant defense mechanism is insufficient to protect against the oxidative damage, the dietary intake of antioxidant compounds is required to neutralize the excess reactive oxygen species. Antioxidant compounds serve by terminating the free radical species or by oxidative degradation of lipids resulting in cell damage (Kurutas, 2016). Medicinal plants, fruits, grains and vegetables have been reported to have high content of antioxidants (Zhang et al., 2015).

This work was undertaken to study the pharmacognostic parameters, phytochemical screening, determination of total flavonoid and total phenolic content and to investigate the *in-vitro* antioxidant activity by using DPPH (2, 2-DIPHENYL-1-PICRYLHYDRAZYL) reducing scavenging activity, Reducing power and Nitric oxide assay.

## II. MATERIALS AND METHODS:

### 2.1 Plant material:

The fresh leaves of *Alocasia macrorrhiza* was collected from the adjacent place around Zemabawk Venglai, Aizawl, Mizoram. The collected leaves were washed and dried under the shade at room temperature for 3 weeks. The dried leaves were further grounded into coarse powder and kept in container until use.

### 2.2 Preparation of plant extract:

The powdered crude material was extracted successively with the solvents in the increasing order of polarity i.e. Petroleum ether, Chloroform and methanol respectively by hot continuous percolation using Soxhlet extractor. Then, methanol extract was evaporated to dryness in the water bath. The crude extract was kept in refrigerator until further use.

### 2.3 Pharmacognostic study:

In organoleptic evaluation, various sensory of the plant materials such as color, odor and taste of the leaves is recorded. The following macroscopic characters for the fresh leaves were noted: size, shape, color, apex, margin and base (Kokate et al., 47<sup>th</sup> edition).

### 2.4. Physicochemical parameters:

The dried leaves were determined for various physicochemical parameters like moisture content, total ash, acid insoluble ash and water soluble ash, extractive value- alcohol extractive value and water extractive value with reference to standard methods (Kokate et al., 47<sup>th</sup> edition).

### 2.5. Phytochemical screening:

The phytochemical screening of the crude extract has been carried out as per the standard method to determine the presence of various chemical constituents such as alkaloids, glycosides, tannins, flavonoids, steroids, saponins, proteins, amino acids, volatile oil, reducing sugars, carbohydrates, fats and fixed oils (Kokate et al., 47<sup>th</sup> edition).

### 2.6 Determination of total phenolic content:

The total phenolic content was determined based on Follin-Ciocalteu's reagent assay by using spectrophotometer (Malsawmtluangi *et al.*, 2014). To an aliquot 1ml (50µg/ml) of extract 5ml of Follin-Ciocalteu's reagent was added and shaken. After 3 minutes 4ml of 0.7M sodium carbonate solution was added to the mixture and the mixture was allowed to stand at room temperature for 1 hour. Similarly, for calibration curves 1 ml aliquot of different concentration of the standard Gallic acid solution (20, 40, 60, 80, 100µg/ml) was prepared. After 1 hour, the absorbance was measured against the blank at 765nm using UV-Vis spectrophotometer (ThermoFisher Scientific 201). The total phenol content was expressed as GAE (mg/g) of the extract.

### 2.7 Determination of total flavonoid content:

The total flavonoid content was measured by the aluminum chloride colorimetric assay (Chang *et al.*, 2002). An aliquot of 1ml (50µg/ml) of extract and standard solution of Quercetin of different concentration (20, 40, 60, 80, 100 µg/ml) was mixed with 2ml of methanol. After 5 minutes 3ml of 5% sodium nitrite and 0.3ml of 10% aluminum chloride was added and kept for 6 minutes. Then 2ml of sodium hydroxide was added and volume was made up to 10ml with methanol. After 1 hour, the absorbance was measured against the blank at 510nm using UV-Vis spectrophotometer. The total flavonoid content expressed as milligram quercetin equivalent (QE)/gram of extract.

### 2.8 Evaluation of *in vitro* antioxidant activity:

#### 2.8.1 Determination of 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity:

The free radical scavenging activity of the plant extract was measured by employing the stable radical DPPH by using ascorbic acid as a positive control (Chauhan *et al.*, 2019). 3ml aliquots each of different concentrations (20, 40, 60, 80, 100µg/ml) of both the extract and standard were taken in a test tube and added

0.5ml of 0.1mM DPPH to each test tube. The mixture was shaken and then incubated at 37°C for 30 minutes. After which the absorbance was measured using the UV-Vis spectrophotometer at 517nm against the DPPH control containing 3ml of methanol in place of extract. The percentage of scavenging activity was calculated using the expression:

$$\% \text{ scavenging activity} = [A_c - A_s / A_c] \times 100$$

Where, A = Absorbance of control, A = Absorbance of sample

### 2.8.2 Determination of reducing power:

The ferric reducing capacity of methanolic extract of the leaves and ascorbic acid as a positive control was determined by using potassium ferricyanide - ferric chloride method (Malsawmtluangi et al., 2014). 1ml each of various concentration (20, 40, 60, 80, 100 µg/ml) of both extract and standard were mixed with 2.5ml of phosphate buffer (6.6pH) and 2.5ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 30 minutes after which the reaction was stopped by addition of 2.5ml of 10% trichloroacetic acid. 2.5ml of supernatant was diluted with 2.5ml of methanol after the mixture was centrifuged at 3000rpm for 10 minutes. Then 0.5ml of freshly prepared 0.1% ferric chloride was added and measured absorbance at wavelength of 700nm.

### 2.8.3 Determination of nitric oxide:

The nitric oxide scavenging activity of the extract was estimated by using Griess reagent. Griess reagent was prepared by mixing 1% sulphanilamide, 0.1% naphthylene diamine dihydrochloride and 2% phosphoric acid immediately before use. 0.5ml of 10mM sodium nitroprusside in phosphate buffered saline was mixed with 1ml each of various concentrations of the extract and standard and incubated at 25°C for 3 hours. The mixture was then mixed with equal volume of freshly prepared Griess reagent (Panda *et al.*, 2009). Control was prepared without the extract but with equal volume of buffer similar with the test sample and absorbance was measured at 546nm using UV-Vis spectrophotometer.

## III. RESULTS AND DISCUSSION:

### 3.1 Pharmacognostic study:

The pharmacognostic study of the leaves were evaluated by organoleptic and macroscopic character. Organoleptic character was done by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure quality of a particular drug. The leaves were found to be dark green in color with pleasant odor and bitter taste. The macroscopic study was determined by describing the morphological parts of leaves seen by which by naked eye. The size of the leaves was 90-180cm x 60-120cm with broadly ovate shape, slightly undulate margin, pointed apex and arrow shaped base.

### 3.2 Physicochemical parameters:

The parameters studied are moisture content, total ash, acid-insoluble ash, water soluble ash, alcohol and water-soluble extractive values. The quality and purity of crude drug was determined by ash value content that indicates the presence of various impurities like carbonate, oxalate and silicate. The amount of inorganic compound and earthly material like silica present in drugs was estimated by water soluble and acid insoluble ash. The moisture content of drugs should be minimum to prevent the growth of microorganisms on storage. The amount of active constituents was estimated by extracting the plant material with a particular solvent. Table no. 1 shows the physicochemical parameters.

Table No. 1: - Physicochemical parameters of *Alocasia macrorrhiza*.

Sl. No.	PARAMETERS	AMOUNT
1.	Total Ash value	10.8%
2.	Acid insoluble ash	15.3%
3.	Water soluble ash	56%

4.	Moisture content	20.86%
5.	Water soluble extractive	9.76%
6.	Alcohol soluble extractive	4.66%

### 3.3 Phytochemical screening:

The methanolic extracts of *Alocasia macrorrhiza* leaves were investigated for different qualitative phytochemical analysis and the result obtained are shown in Table 2. The methanolic extract showed the presence of alkaloids, glycosides, flavonoids, steroids, phenols.

Table No. 2: - Phytochemical screening of methanolic extract of *Alocasia macrorrhiza*

Sl. No.	PHYTOCONSTITUENTS	RESULTS
1.	Alkaloids	+
2.	Amino acids	-
3.	Glycosides	+
4.	Flavonoids	+
5.	Phenol	+
6.	Saponin	-
7.	Steroids	+
8.	Tannins	-

### 3.4 Determination of total phenolic content:

The total phenolic content of the methanolic extract of the leaves was determined by using Folin-Ciocalteu's reagent. The total phenolic component exhibit antioxidant activity through adsorption and neutralization of the free radicals (Bhalodia et al., 2013). The total phenolic content was found to be 360mg GAE/g expressed as mg of gallic acid equivalent /gm.

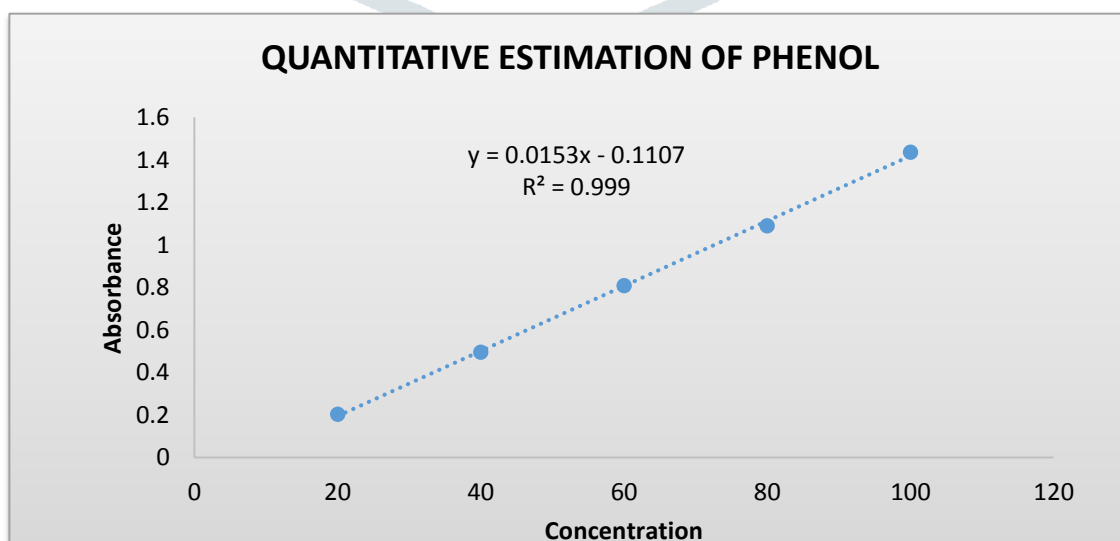


Fig.1: -Standard calibration curve for total phenolic content

### 3.5. Determination of total flavonoid content:

The flavonoid content of the extract was determined by using Aluminum chloride colorimetric assay. The flavonoid content showed antioxidant activity through scavenging reactive oxygen species or chelating iron (Deng et al., 1996). The total flavonoid content was found to 80mg/g of dried extract equivalent to Quercetin.

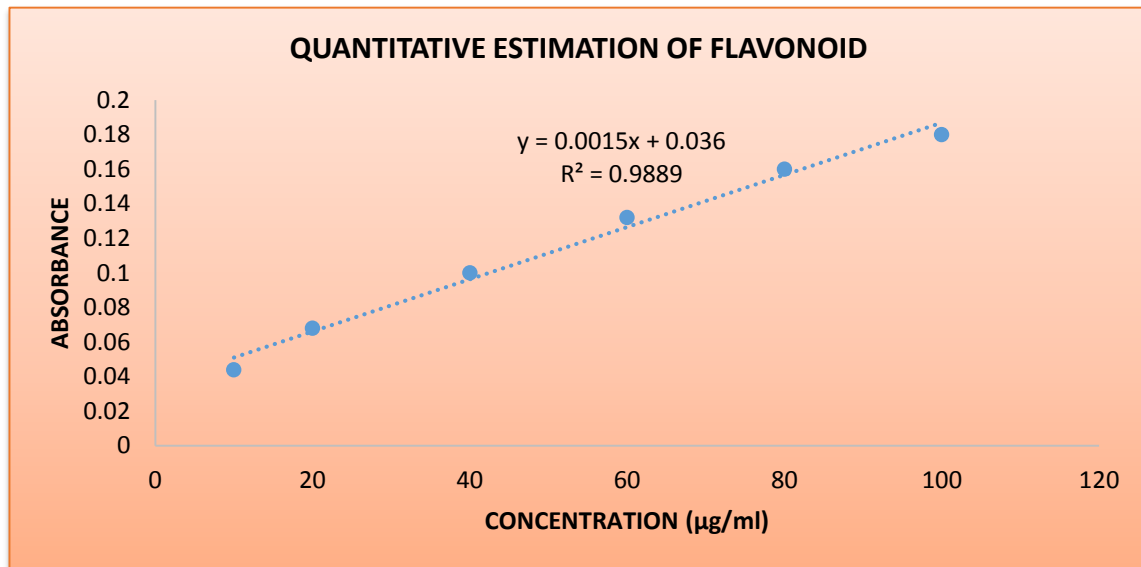


Fig. 2: - Standard calibration curve for total flavonoid content

### 3.6. Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity:

The stable free radical DPPH has been used to test the free radical scavenging ability of the methanolic extract of the plant by using Ascorbic acid as a standard. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517nm induced by the antioxidant (Gangwar et al., 2014). The 50% inhibition concentration was calculated by plotting the % inhibition against concentration. The figure shows steady increase in the antiradical activity on increasing concentration. The IC<sub>50</sub> value of the methanolic extract showed significantly higher scavenging activity as compared to the standard which exhibited 29.89µg/ml in extract and 83.51µg/ml in case of standard.

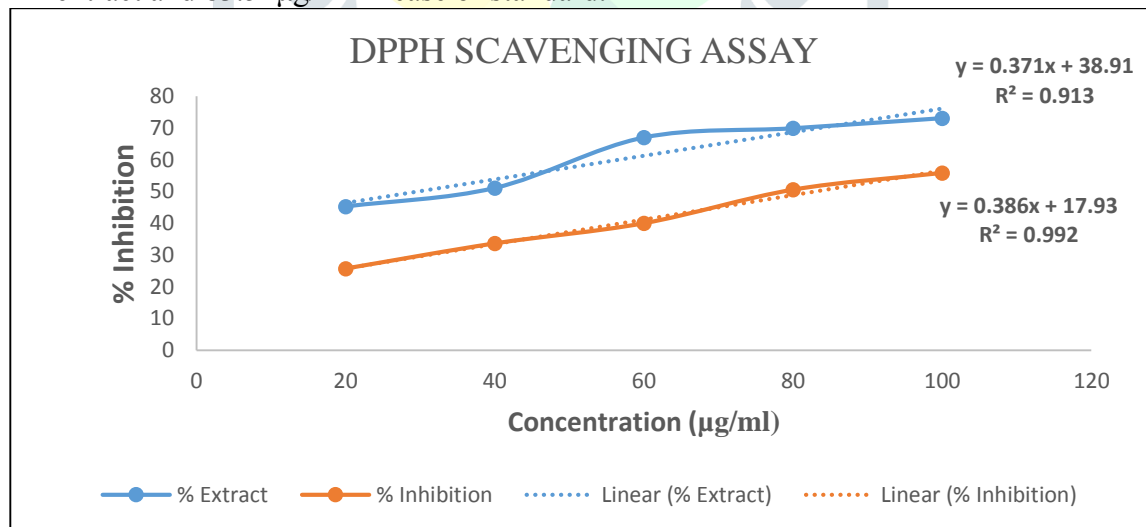


Fig. 3: - 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) scavenging for *Alocasia Macrorrhiza* and Ascorbic acid

### 3.7. Determination of reducing power:

The antioxidant activity of the methanolic extract of *Alocasia macrorrhiza* leaves were determined by reducing power assay. In this assay, the reducing potential of the extract react with potassium ferricyanide to form potassium ferrocyanide which then reacts with ferric cyanide complex to form ferrous cyanide complex (Bhalodia et al., 2013). The reducing power increase significantly with increasing concentration and the extract shows reductive capabilities suggesting that some compound in the extracts may be able to donate hydrogen atom to break the free radical chain reaction. However, the reducing power of the extract was lower when compared to the standard ascorbic acid shown in fig.4.



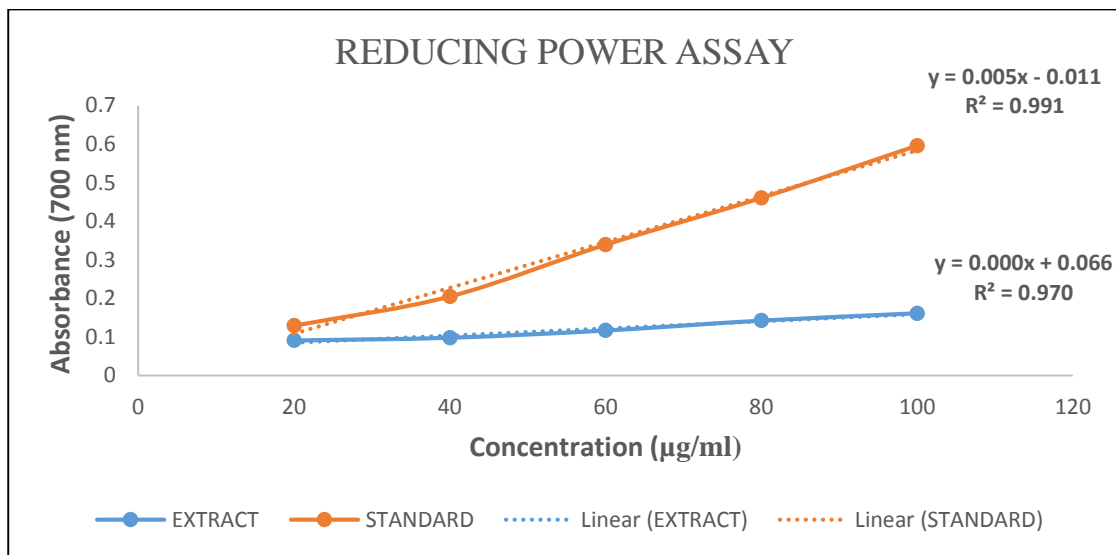


Fig. 4: - 2, 2- Diphenyl-1-Picrylhydrazyl (DPPH) scavenging for *Alocasia Macrorrhiza* and Ascorbic acid

### 3.8. Determination of nitric oxide:

Nitric oxide assay was carried out on methanolic leaves extract of *Alocasia macrorrhiza* using Ascorbic acid as a standard. Nitric oxide scavenging capacity was determined by decrease in the absorbance at 546nm, induced by antioxidants. The antiradical activity increased with an increase in the concentration of the extract (Parul et al., 2013). The maximum free radical scavenging activity and potency were interpolated in fig. 5. The IC<sub>50</sub> values of the extract was found to be 42.93µg/ml as compared to the standard which was 31.71µg/ml. The results indicate that the *Alocasia macrorrhiza* exhibited antiradical activity. However, the standard ascorbic acid showed significant higher scavenging activity than the extract.

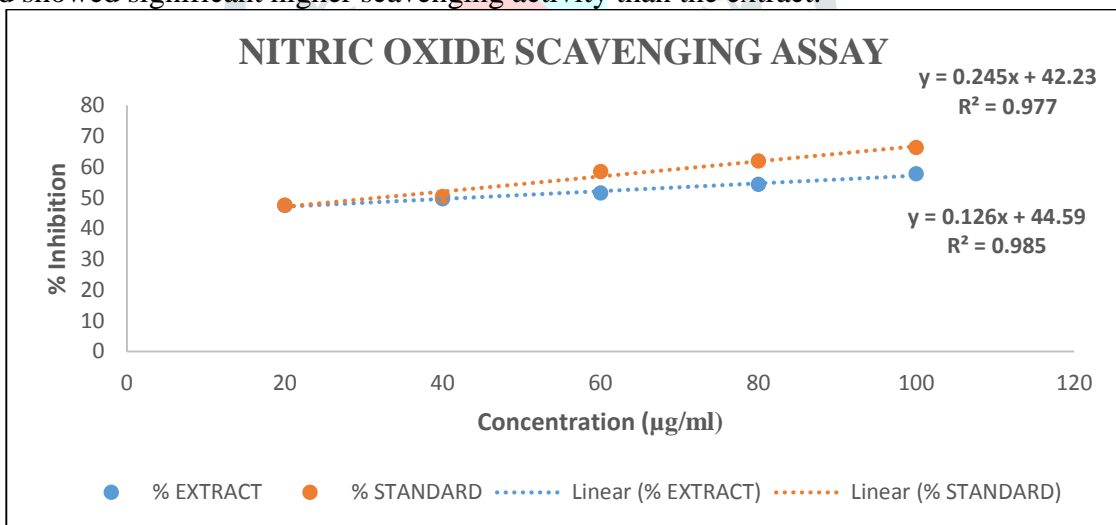


Fig. 5: - 2, 2- Diphenyl-1-Picrylhydrazyl (DPPH) scavenging for *Alocasia Macrorrhiza* and Ascorbic acid

## IV. CONCLUSION:

The extraction of the leaves of the plant was obtained by sequential extraction using petroleum ether, chloroform and methanol respectively. The study concludes that the information obtained from pharmacognostic study and physicochemical parameters can be used for the identification of plant, purity and quality of the plant material. The phytochemical screening also shows the presence of alkaloids, flavonoids, glycosides, phenols, and steroids which may lead to the discovery of new drug development. The determination of TPC and TFC shows considerable amount of phenolic and flavonoid content which are responsible for exhibiting various pharmacological activities. The in-vitro antioxidant assay of the methanolic extract of *Alocasia macrorrhiza* revealed significant scavenging activity which might suggest its importance as a potentially useful source of natural antioxidant.

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