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## Antioxidant potential of *Meyna laxiflora* Robyns collected from the Western Ghat of Maharashtra

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**Abstract**

The *Meyna laxiflora* Robyns distributed in wild all over the Western Ghat and the Fruits are as raw by the tribes as it rich source of Nutrition. The plants have potential benefits from immune to many diseases. This richest source of secondary metabolites with highest antioxidant properties have collected from the parts of Western Ghat of Akole Tehsil of Ahilyanagar District (MH) and evaluated the Phytochemical and Antioxidant properties in the Methanol extract.

**Keywords:** *Meyna laxiflora*, antioxidant activity, methanol, Fruit, Maharashtra

**Introduction**

*Meyna*, a genus distributed in all over the tropical and subtropical region. *Meyna* Earlier it was *Vangueria spinosa*. Recently the plants has been separated and classified into eleven different species of *Meyna* with the help of molecular phylogenetic. The species are *Meyna grisea* Robyns, *Meyna hainanensis* Merr., *Meyna laxiflora* Robyns, *Meyna parviflora* Robyns, *Meyna peltata* Robyns, *Meyna pierrei* Robyns, *Meyna pubescens* (Kurz) Robyns, *Meyna spinosa* Roxb. ex Link, *Meyna tetraphylla* (Schweinf. Ex Hiern) Robyns, *Meyna tetraphylla* subsp. *Comorensis* (Robyns) Verdc, *Meyna velutina* Robyns.

The *Meyna laxiflora* Robyns and *Meyna spinosa* Roxb.ex Link are the only \ twospecies distributed in India which is considered as inseparable having difference only in flowers. The *Meyna spinosa* Roxb.ex Link have flowers crowded into fascicles with shorter pedicels and petioles as compared to *Meyna laxiflora* Robyns. However the *Meyna laxiflora* (Rubiaceae) is a wild edible fruit found in many parts of the Western Ghats and the local tribe consume these wild edible fruits of the plant available in the season (Patil *et al*, 2012) <sup>[18]</sup>.

Western Ghats zone is varied in climatic and edaphic factors which affects fruit plants distribution at the fluctuated altitude elevation. The tribal peoples acquired the knowledge of wild edible plant species based on trials in the fields and knowledge of wild edible fruit plants can be used to solve food insecurity and malnutrition problems. (Kumbhojkar *et. al*, 1988) The dependence on these fruits has steadily declining as more exotic fruits have been introduced. But many people in tribal areas still use them as a supplement of their basic need of food. Some of them are preserved for use in dry period or sold in local market.

Apart from their traditional use of food, potentially they have many advantages they are immune to many diseases and often used in different formulation of „Ayurveda“ in Indian Folk medicine and traditional medicines. They are edible and having fortified nutritional food value, which provides the minerals like sodium, potassium, magnesium, iron, calcium, phosphorus as well as vitamins, proteins and carbohydrates and microelements. These are richest source of secondary metabolites with highest antioxidant properties. Wild edible fruits today are needs to be recommended for cultivation due to this, they can serve as food material for ever increasing population (Valvi *et. al*, 2011) <sup>[14]</sup>.

These fruits are reached source of antioxidant because of they contain compounds which Inhibit oxidation a chemical reaction that can produce free radicals. (Dhenge *et. al*, 2024) <sup>[1]</sup> They are natural molecules that help neutralize harmful Free radicals in our bodies. Antioxidants are compound that inhibits oxidation a chemical reaction that can produce free radicals. As the body requires the compound to inhibit the Free radical many industrial

products, such as polymers, fuels and lubricants added Antioxidant very frequently to extend their usable time.

Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fiber to protect against diseases. They are non-nutritive compounds. These phytochemicals are the secondary metabolites present in smaller quantities in higher plants and they include the alkaloids, Steroids, flavonoids, Tannin, Carbohydrate, proteins, glycosides and Gums, tannins and many others. Phytochemicals are naturally occurring biochemical in plant that give plants their colour, flavour, smell and texture. They are bioactive non-nutritive plant compounds in fruits, vegetables, grains and other plant foods that have health related effects. Free radicals are compound with one or more unpaired electrons in its outer most orbital. They are extremely reactive and are known to be the underlying cause of several degenerative diseases (Bag et. al, 2016)<sup>[9]</sup>.

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant in the body. Antioxidants are agents which scavenge free radicals. Many synthetic antioxidants are available but caused toxicity to human. Many studies have found that plants are potential source of immense chemicals for the treatment of various ailments and known to be rich source of natural antioxidant.

The present investigation is carried out to study the Antioxidant potential and constituents of the Fruits of plants *Meyna laxiflora* Robyns by phytochemical analysis process. The Fruits were collected by random method from the different 05 locations of the Akole Tehsil of Ahilyanagar District. This area is compress as a part of Western ghat and fall inside the Kalsubai-Harishchandra gad Sanctuary area.

## Materials and Methods

### Study area

Ahilyanagar district is located between 18°02" and 19°09" north latitude and 73°09" and 75°05" east longitude. Geographically Ahilyanagar is the largest district in the state of Maharashtra. It is dividing into 14 talukas in Ahilyanagar district. The district is bounded on the north by Nasik district, on the north-east by Aurangabad district, on the east by Beed and Osmanabad districts, on the south by Solapur district and on the west by Pune district, on the north-west it is surrounded by Thane district. Akole taluka is on western hilly region western part of the Ahilyanagar district. Akole taluka is located in 19° 15" 14" N to 19° 44" 59" N latitude and 73° 37" 00" E to 74° 07" 24" E longitudes. The study conducted in well surrounded with the mountains of Sahyadri under ranges of Kalsubai - Harishchandragad Sanctuary 1. Bhandardara 2. Ghatghar 3. Pachanai, 4. Vihir, and 5. Kumshet Villages of Akole taluka of Ahmednagar district, Maharashtra. The collection of Fresh Fruits of plant material from the selected 5 villages was done in the Month of June and July, 2024 (Source: Google Census 2011)

### Plant Material Collection

The collected specimen of plant and fruits was identified using Flora of Maharashtra (Singh, N.P., Lakshminarasimhan, P., Karthikeyan, S. and Prasanna, P.V. 2001.)<sup>[20]</sup> Flora of Bombay Presidency (T. Cook, 1903) and further authenticated by using the available literature on Google Scholar and Available online resources.

**Description of plant:** Small or medium size tree, Bark light black, smooth. Leaves opposite or whorled, 3.5-15 X 1.2-10 cm, elliptic oblong, shining, glabrous. Flower greenish yellow, in axillary cymes on leafless wood. Fruits nearly globose, fleshy, smooth, purplish when rip.

### Chemicals

All the required chemicals used in this study were procured from Department of Botany and Chemistry Laboratory of Adv. M. N. Deshmukh College, Rajur, Tal Akole, Dist. Ahilyanagar.

### Analysis of samples

All samples were cleaned thoroughly to remove any attached soil and other impurities and were blotted dry. For analyzing the nutrient parameters various standard methods as mentioned below were followed.

### Estimation of Ash

Sample, each of 5 gm, was weighed in a silica crucible and heated in muffle furnace for about 5-6 h at 500 °C. Further, it was cooled in desiccators and weighted. Again it was heated in the furnace for half an hour. Cooled and weighed. Constantly, this was repeated till the weight became (Ash became white or grayish white) Weight of Ash gave the ash content (Vanessa et al., 2016)

### Estimation of Moisture:

Sample each of 2 gm was taken in flat bottom dish and kept overnight in an air oven at 100-110 °C and weighed. The weight loss was regarded as a measure of moisture content (Vanessa et al., 2016; Ping et al., 2016)<sup>[11]</sup>

### Preparation of plant extract:

Fresh Fruits were washed repeatedly to remove adhered dust particles, blotted, shade dried, powdered using an electric blender and stored in air tight container. About 300g of the Fruit powder was subjected to methanol extraction under reflux for 1 hour. The procedure was repeated thrice until complete extraction was achieved.

### Estimation of total phenolic content:

The total phenolic content of the methanolic Fruit extract was determined by Folin-Ciocalteu reagent (Singelton and Rossi, 1965). Briefly, 40 µl of the methanol solution of the extract (1 mg/ml), 200 µl of Folin-Ciocalteu reagent was mixed with 2.4 ml of distilled water and the contents were mixed thoroughly. After 1 min, 600 µl of sodium carbonate (20% Na<sub>2</sub>CO<sub>3</sub>) was added and the volume was made up to 4.0 ml with distilled water. After 2 h of incubation at room temperature, the reduction of the Folin-Ciocalteu reagent by phenolic compounds under alkaline conditions resulted in the development of a Brown colour, the absorbance was measured at 765 nm and compared to a gallic acid calibration curve. Results were expressed as mg of gallic acid equivalents (GAE) /g of extract.

### Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to Benzie et al., (1996) with slight modifications suggested by Firuzi and Lancana 2005). FRAP reagent was prepared and kept at 37 °C for 5-10 minutes prior to use. In a 96 well plate, 175µL of FRAP reagent was added and the plate was pre read at

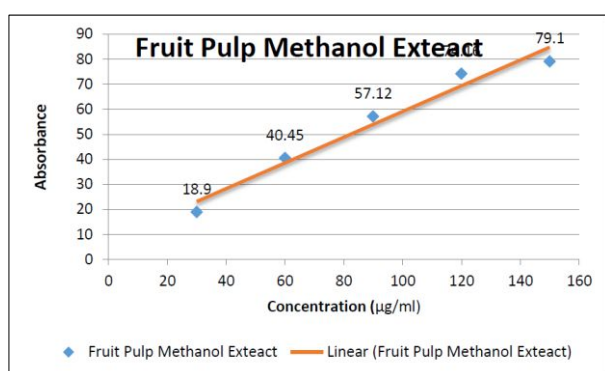
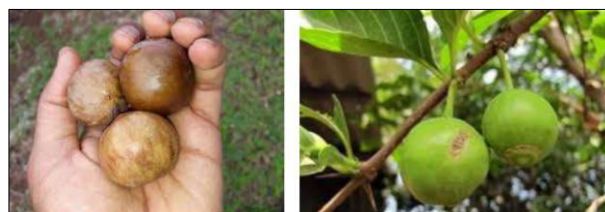
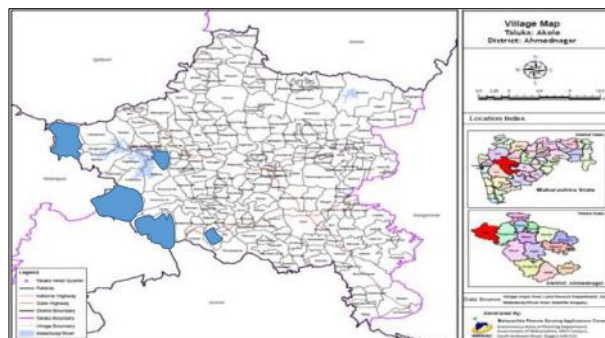
593 nm. To each well, 25  $\mu$ L of sample solution was added and contents were mixed well using a pipette. Absorbance was measured at 593 nm at 4, 15, 30 and 60 minutes, using methanol as blank. Change in absorbance at different time intervals was then translated into a FRAP value using a standard trolox curve.

### Total antioxidant activity

The total antioxidant activity of methanolic Fruit powder extract was determined by using phosphomolybdenum (Prieto *et al.*, 1999). An aliquot of 0.4 mL of methanolic Fruit (1 mg/mL) was mixed with 4 mL of the phosphomolybdenum reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in water bath at 95° C for 90 minutes, cooled and the absorbance was read at 695 nm. The antioxidant activity of the sample was determined using a standard curve of ascorbic acid. The reducing capacity of the extract was expressed as mg of ascorbic acid equivalents (AAE)/g of extract.

### Nitric oxide scavenging assay

About 1 mg of the methanolic Fruit powder extract was dissolved in 20 $\mu$ L of methanol and the volume was made up to 1 mL with distilled water. Further dilutions were done to final concentrations of 25, 50, 100, 250, and 500 $\mu$ g/mL using vehicle buffer. Nitric oxide was generated from sodium nitroprusside and was measured by the Griess reagent. The reaction mixture contained 50  $\mu$ L of 10 mM sodium nitroprusside, 50  $\mu$ L of distilled water and test solution of various concentrations. The reaction mixture was preincubated at room temperature for 15 minutes in the presence of light, followed by the addition of 125  $\mu$ L Griess reagent and incubated for 10 minutes at room temperature. The absorbance was measured at 546nm.



### Results and Discussion

The mineral and nutrient present  $\mu$ g/ml in the Fruit powder of *Meyna laxiflora* is as flows:

| Plant Name             | N     | P      | K      | S     | Ca    | Mg     | Zn   | Na   | Fe   | Mn   | Cu   | B    |
|------------------------|-------|--------|--------|-------|-------|--------|------|------|------|------|------|------|
| <i>Meyna laxiflora</i> | 0.058 | 135.09 | 498.16 | 34.16 | 80.12 | 214.24 | 1.43 | 6.10 | 12.9 | 1.13 | 1.08 | 0.38 |

#### 1. Estimation of Ash

The 5 gm of Sample of *Meyna laxiflora* weight in silica crucible and heated in muffle furnace for about 5 hr. then after cool the sample material weight has been taken. Repeated the till weight of ash equal to the weight of sample content taken then, The total Ash content of dried fruit of *Meyna laxiflora* is estimated as (0.49 $\pm$ 0.02)%.

#### 2. Estimation of Moisture

The 2 gm fruit powder were kept in oven overnight at 105°C then the weight loss counted according to the method of Vanessa. The moisture of dried Fruit powder is estimated to be (3.07 $\pm$ 0.094)%.

#### 3. Estimation of total phenolic content

The mean phenolic content of the methanolic Fruit extract was 140.53 $\pm$ 0.40 mg/g of extract. This indicates that *Meyna laxiflora* significantly rich in phenolic contents. Presence of flavonoid as a major phytoconstituent has been reported in *Meyna laxiflora*. It is well established that flavonoids containing hydroxyl functional groups contribute to the antioxidant potential of the plant extracts. Result indicates that methanol extract of fruit pulp of *M. laxiflora* showed higher total phenolic content than other extract while for

seed, leaf, and other parts of plant extract. Total phenolic content than methanol extract is 140.53 $\pm$ 0.40 mg/g.

#### 4. Ferric reducing antioxidant power (FRAP) assay

The reagents were mixed and absorbance was immediately read at 734 nm using blank run without test sample

**Table 1:** Phytochemicals present fruits of *M. laxiflora*

| Phytochemicals           | Test performed        | Fruit pulp |
|--------------------------|-----------------------|------------|
| Alkaloids                | Hager's test          | Absent     |
| Carbohydrates            | Benedict's test       | Present    |
|                          | Fehling's test        | Present    |
| Amino acid               | Ninhydrin test        | Absent     |
| Proteins                 | Xanthoproteic test    | Present    |
|                          | Beuret test           | Absent     |
| Flavonoids               | Alkaline reagent test | Present    |
| Phenolic compounds       | Lead acetate test     | Present    |
| Tannins                  | Lead acetate test     | Present    |
| Steroids & terpenoids    | Salkowski's test      | Present    |
| Saponins                 | Froth test            | Present    |
| Cardiac glycosides       | Keller Killiani test  | Present    |
| Oil & fats               | -                     | Present    |
| Phlobatanin              | -                     | Absent     |
| Diterpene Copper acetate | -                     | Present    |

**Table 2:** Total antioxidant activity in µg/ml of extract (in AAE)

| Sr. No. | Concentration (µg/ml) | Fruit Pulp Methanol Extract |
|---------|-----------------------|-----------------------------|
| 1       | 30                    | 18.9                        |
| 2       | 60                    | 40.45                       |
| 3       | 90                    | 57.12                       |
| 4       | 120                   | 74.16                       |
| 5       | 150                   | 79.10                       |

## Conclusion

The present study highlights that the wild edible ripe and unripe fruit of *Meyna laxiflora* collected from the Akole Tehsil of Ahilyanagar District, Maharashtra, were rich source of various diet supplementary factors like protein, reducing sugars, phenol, ascorbic acid, antioxidants and minerals analysis. These supplementary factors and minerals are required for normal body metabolism which helps in normal growth and development of human being. The extensive consumption of unripe and ripe fruit of *Meyna laxiflora* which in turn can lead to reduced health costs, prevention and cure of a number of prevalent diseases, and an improved diet. Simultaneously, the population can be made aware of the consumption values of these plants as a staple food owing to their good nutritional qualities and adequate protection can be obtained against disease arising from malnutrition. The present investigation point out the importance of fruits and fruit pulp of *Meyna laxiflora* in the diet as rich source of Antioxidant potential. The fruits are available for very short time period during the season of Monsoon. The fruit are required to come under Horticulture practices so that this fruit can available in all season and longer duration.

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