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Phytochemical Studies with Comparative Anti-Oxidant Analysis and Method Development Using Analytical Techniques for Adiantum Lunulatum

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Abstract- Due to the increasing demand for medicinal plants in both developed and developing countries, they depend on traditional plants because of their non-toxic, and lack of side effects. As compared to the other plant genera ferns which are one of the primitive plant groups are less studied. "Adiantum Lunulatum" also known as hamsapadi an Indian medicinal plant with a small leafy green. It is used to treat throat infections, febrile conditions etc. First Phytochemical Analysis was done which shows the presence of most of the secondary Metabolites like phenol, saponins, tannins, flavonoids, steroids, and anthocyanin. This plant is also reported to have high concentrations of antioxidants, possibly contributing to disease prevention following human consumption. We are checking the concentration of antioxidants in different parts of the plant (Leaves & Midrib). Following analysis of Adiantum lunulatum leaves and midrib, we report the antioxidant content potential of these species using two comparable techniques assessing the consistency between the assays – the ferric reducing antioxidant power (FRAP) assay and the Phosphomolybdate (PMA) assay. We conclude that there is variation in Adiantum lunulatum antioxidant potential and that ferric reducing antioxidant power (FRAP) assay and Phosphomolybdate (PMA) assay are useful techniques for measuring antioxidants in this plant. The extracts showed a considerable antioxidant effect in Ethanol & Acetone and Methanol & Acetone from two different assays respectively. Thin-layer chromatography reveals the presence of different metabolites of all plant extracts.

Keywords- Hamsapadi,secondary metabolites, PMA,FRAP,TLC

I. INTRODUCTION

From thousands of years ago, the human races depend on plants for different purposes including food, shelter, fuel and medicine. In many parts of the world, tribal peoples considered plants as their only source of medicine. According to a world health organization report, 80% of developing countries' population still depends on traditional plant-based drugs. In the modern period, the pharmaceutical industry focused on drug discovery from plants. Medicinal plant demand increased in both developed and developing countries due to its non- toxic, lack of side effects, low prices and easy availability. However, it is very much necessary to investigate the medicinal potential of less studied plants.

Ferns are one of the primitive plant groups, that belong to Petridophyte, evolve millions of years ago and are scattered throughout the world. Humans use ferns for different purposes including medicinal and industrial aspects, but as compared to other plant genera they are less studied.

Hamsapadi is an Indian medicinal plant found throughout India at an altitude of 1200 m. It is an attractive small leafy fern and is also known as Adiantum lunuatum L. These plants usually occur in moist places on rocks and slopes of lower hills as well as on brown laterite soil or under the shade near swamps.

Hamsapadi Plant

The roots of Adiantaceae or Hamsapadi are considered carminative, tonic, diuretic and useful for treating bilious complaints. They are also prescribed for strangury and fever due to elephantiasis. A decoction of the roots is used to treat throat infections and febrile conditions in children and to treat discharge of blood in the urine (haematuria) among the Kondhs of southwestern Orissa. These people also use the root paste as an external application to promote the healing of bone fractures. In the Bhadrak District of Orissa, the crushing plant is applied to treat flatulence.

The botanical name of this Indian medicinal plant is Adiantum philippine Burm and popularly known as Hamsapadi in Sanskrit, Goyalelata and Kalijhant in Bengali, Walking Maidenhair Fern in English, Hansapadi, Hansraj, Mubarak and Mubarkhinipalo in Gujarati, hansapadi, kalijhant and paresiyavasan in Hindi, navalad in Kannada, Nilamparanta in Malayalam, Ghodkhuri, Hansraj, Kamsaraj and Ratkombada in Marathi, etc.

Antioxidants

Phosphomolybate Assay

This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically. It's a method used to analyse the antioxidant activity of plant extracts using a Phosphomolybdate reagent. Normal functioning of body systems and various other physiochemical conditions lead to the formation of free radicals. Because of the presence of unpaired electrons, they become very unstable and reactive. So, different plants produce antioxidants to varying degrees. In order to check, which plant is producing more antioxidants as compared to other plants, we use the Phosphomolybdate assay.

Principal: When a plant extract is added to the Phosphomolybdate reagent, antioxidants present in the plant extract, reduce the phosphomolybedenum. The reduced phosphomolybedenum gives the solution a different colour. The absorbance is taken and we plot a standard graph. So, this assay is very useful to predict the antioxidant activity of crude extracts on a total basis. The reducing capacity of extract was calculated by using the formula:

Final absorbance= Absorbance of sample - Absorbance of Blank - Absorbance of Extract.

(or)

%inhibition= (1- Absorbance of sample/absorbance of control) * 100

FRAP Assay

The ferric reducing antioxidant power (FRAP) assay is a typical ET-based method that measures the reduction of ferric ion (Fe3+)-ligand complex to the intensely blue- coloured ferrous (Fe2+) complex by

antioxidants in an acidic medium. The Sample to be used in this assay can be of plant origin, Serum, Plasma and other biological fluids. It is a type of Quantitative assay. Its sensitivity:

0.2 mM. The assay provides a quick, sensitive and easy way to measure the antioxidant capacity of a number of samples of biological origin.

Principal: The assay is used for the measurement of the antioxidant potential of various samples. Frap does this, by reducing the ferric ions to ferrous ions through antioxidants present in the samples. Once the ferric iron is reduced, a blue colour is developed which is read colourimetrically at 700 nm. Determination of the antioxidant potential of samples is done using a ferrous iron standard curve. The results are expressed as Fe2+ equivalents which are in uM or FRAP value.

The FRAP assay has been applied widely in nutritional science. Apart from measuring the "total antioxidant content" of various foods, the FRAP assay has been used also to explore the absorption of antioxidants from foods, such as soya milk, cocoa, and tea, and to investigate the effect of processing and cooking on the antioxidant content of foods. It is well recognized that transport to market, storage, and cooking practices affect the content of labile antioxidants in foods, and the World Health Organization (WHO) has taken this information into account in their recommendations for vitamin and mineral requirements in human nutrition.

II. MATERIALSAND METHODS

Collection and authentication of plant the plant Adiantum lunulatum was collected from the surrounding areas of Worli, Mumbai, Maharashtra, India, during the month of September - 2022.

Preparation of Plant Material

The collected plant was washed under tap water and dried in the shade without exposure of sunlight. Then dried plant material was ground into fine powder.

Extraction of Plant Material

Extract prepared by mixing separately 1g plant powder in 10ml each solvent (Ethanol, n-hexane, Chloroform, Ethyl acetate). This preparation was kept in a rotary shaker for 48 hrs. The filtrate is prepared by using Whatman No.1 filter paper.

Phytochemical Screening Phytochemical analysis of ethanol, n-hexane, chloroform, and ethyl acetate extract was carried out by standard methods.

Phenols

Nitric Acid Test

To the 2ml of each extract treated with 2ml of diluted nitric acid solution. The appearance of the reddish to yellowish colour indicates phenolics.

Flavanoids

Sodium hydroxide test

1 ml of each extract was treated with 1 ml of 10% sodium hydroxide. The formation of yellow colour indicates flavonoids.

Alkaloids

Mayer's test

To the 2ml of each extract treated with Mayer's reagent. The development of yellowish colour indicates alkaloids.

Tannins

Braymer's Test

The 2ml of each extract was treated with 2ml of H2O and 2-3 drops of 5% ferric chloride solution, which resulted in the formation brownish green precipitate that gradually changes to bluish-black detecting the presence of tannins.

Saponins

Foam Test

The 5ml of each extract was treated with an equal volume of distilled water and placed in a boiling water bath. The appearance of the froth indicates saponins.

Steroids

Salkowski Test

To the 2ml of each extract treated with 2ml of chloroform, then add a few drops of con H2So4

appearance of a reddish brown colour ring at the junction indicates steroid presence.

Carbohydrates

Benedict Test

The 2ml of each extract was treated with Benedict's reagent and heated in boiling water bath for 5 minutes and cooled. The development of an orange-red precipitate indicates the presence of carbohydrates.

Anthocyanins

Each 2 mL of the plant extract is treated with 2 mL of 2 N HCl. The appearance of a pink-red colour that turns purplish blue after the addition of ammonia indicates the presence of anthocyanins.

Antioxidant Activity

Preparation of the Extracts

Plant material was collected from the field, washed under tap water, and dried in the shade without exposure to sunlight. Then dried plant material was ground into fine powder.

- The first set of the extract is prepared by mixing separately 1g of plant leaves in 10 ml of each solvent (Ethanol, Methanol, Acetone, Ethyl acetate).
- Similarly, the Second set of the extract is prepared by mixing separately 1g plant midrib powder in 10ml each solvent (Ethanol, Methanol, Acetone, Ethyl acetate).
- This preparation was kept in a rotary shaker for 48 hrs. The filtrate is prepared by using Whatman No.1 filter paper and evaporated to remove solvents.

Phosphomolybdate Assay (Total Antioxidant Capacity)

The total antioxidant capacity of the fractions was determined by the phosphomolybdate method using ascorbic acid as a standard. An aliquot of 0.1ml of sample solution was mixed with 1 ml of PMA reagent solution.

- 0.6 M sulphuric acid - is made with 0.6M sulphuric acid in 50ml of distilled water.
- 28 mM sodium phosphate - is made with 28 mM sodium phosphate in 50ml of distilled water.
- 4 mM ammonium molybdate - is made with 4 mM of ammonium molybdate in 50ml of distilled water

The above solution is made separately with a volume of 50 ml in a scotch bottle. The tubes were capped and incubated in a water bath for millimoles or 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. A typical blank contained 1 ml of the reagent solution and the appropriate solvent volume and was incubated under the same conditions. Ascorbic acid was used as standard.

FRAP Assay (Ferric Reducing Antioxidant Powder)

FRAP assay is done by making

- phosphate buffer saline or PBS: concentration of 0.2 M & pH 6.6
- 1% potassium ferricyanide: 1g of potassium ferricyanide is dissolved in distilled water and volume is made to 100 ml with distilled water.
- 10% trichloroacetic acid, TCA: 10 g of TCA is dissolved in distilled water and the volume is adjusted to 100 ml with distilled water.
- 0.1 per cent ferric chloride: 1. g of ferric chloride is dissolved in distilled water and makes up the volume of 100 ml distilled water keep all the chemicals in air-tight containers and store them in dark conditions to prevent oxidation and other unnecessary activities. After following the protocol, this would give us a bluish-colour formation. Then take the measurement and at 700nm, a sample with more concentration will show higher absorbance; the opposite is true. positive control: use a certain antioxidant molecule like ascorbic acid, citric acid or butylated hydroxytoluene.

Adjust the volume to one 1ml & same protocol is followed.

Negative Control: Instead of adding any sample, in this tube, we only put 1ml distilled water.

HPTIC

For HPTLC we have used 3 Mobile phases with different combinations, 7

Standards and 3 plant extracts which are mentioned as follows,

- MP 1 : Chloroform: Methanol [24:6]
- MP 2 : [15:9:6.18]
- MP 3 : [15:15:3]

Standards:- Flavanoids:
Quercetin
Alkaloids: Quenin sulphate Tannins: Gallic acid
Steroids: Prednisolone tablet Sapponins: digoxin
Carbohydrates: dextrose Tannins: L-ascorbic acid
Plant Sample:- Methanol Acetone Ethyl acetate.

III. RESULTS AND DISCUSSION

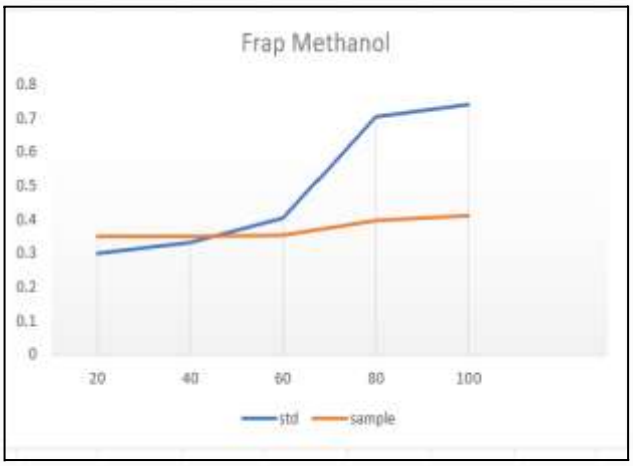
1. Phytochemicals Analysis

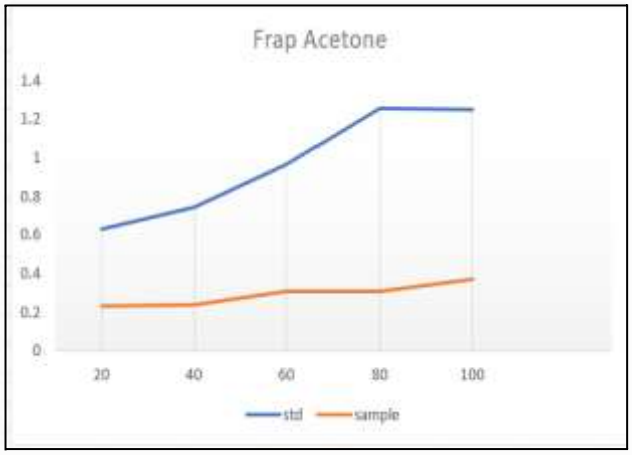
The preliminary phytochemical evaluation of Adiantum lunulatum is shown in Table l.

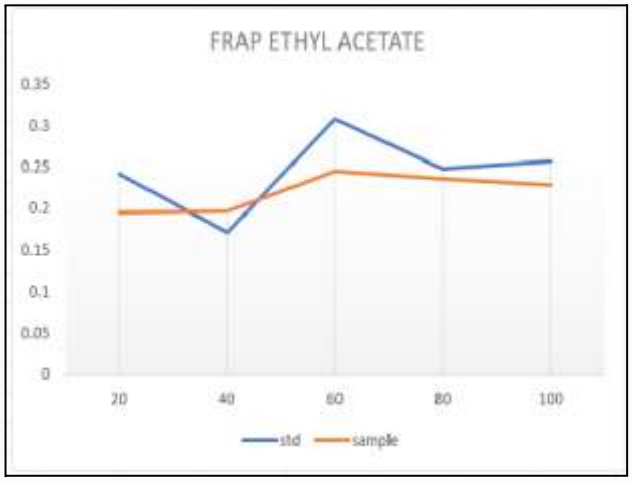
A. lunulatum extracts contain the presence of important phytochemicals such as saponins, tannins, carbohydrates, steroids, and anthocyanin.

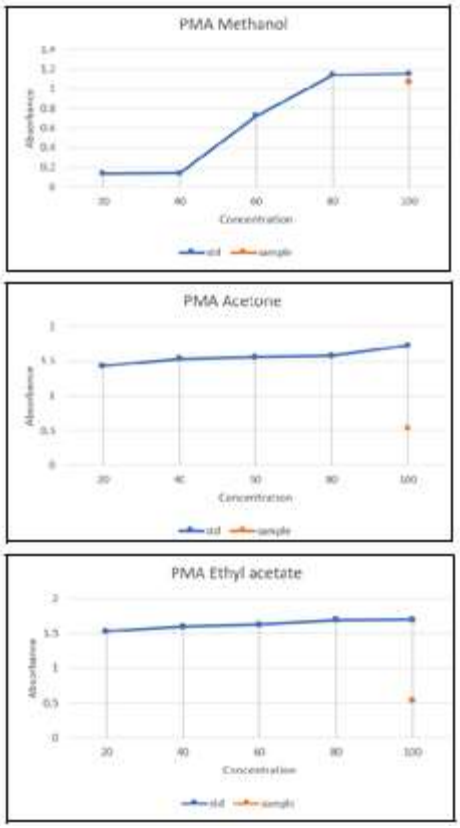
Table 1. Results of Phytochemical analysis (-) Absent (+) Present

Standard	Methanol	Ethyl Acetate	Acetone
Tannins	+	+	-
Carbohydrate	+	-	-
Flavonoids	-	-	-
Phenols	-	-	-
Steroids	+	-	-
Anthocyanin	+	-	-
Saponins	+	-	-
Alkaloids	-	-	-
Glycoside	-	-	-
Reducing Sugar	-	-	-
Terpenoids	-	-	-
Protein	-	-	-









Antioxidant Activity

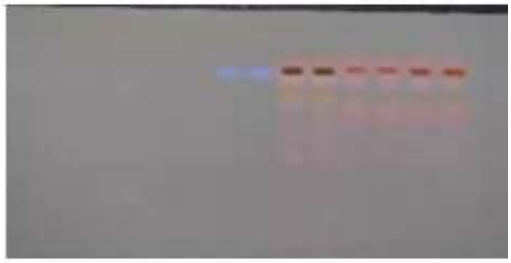
Adiantum lunulatum extract contains many biochemical constituents; this constituent shows important medicinal properties such as antioxidant and antibacterial activity. Various combinations of solvent types and extraction techniques were investigated to determine optimum conditions for plant tissue extraction. To check the antioxidant property/potential of "Adiantum Lunulatum" we have done comparative studies between PMA and FRAP Assay Method.

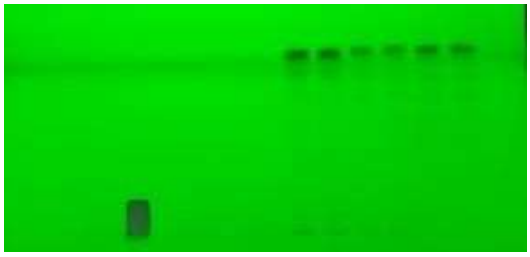
The solvents used in these methods were Methanol, Acetone, and Ethyl acetate.

In the PMA assay, the highest level of antioxidant activity were shown in Methanol compare to Acetone & Ethyl acetate. The graphs shows that Methanol extracts of the plant sample gives better extraction than that of other solvents.

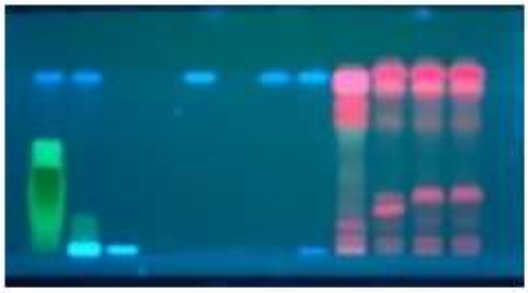
Similarly in FRAP Assay also, Methanol & Ethyl acetate shows the highest level of antioxidant activity as compared to that of Acetone. The graphs shows that the Methanol and Ethyl acetate extracts of the plant samples gives better extraction than that of the other solvents.

HPTLC

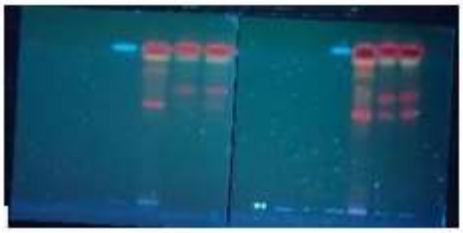


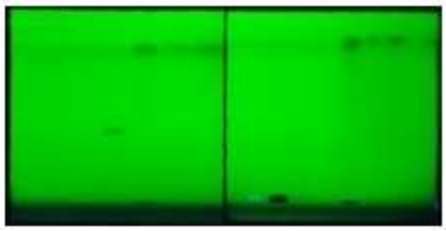


MP 2



MP 3





IV. CONCLUSION

The results of current research revealed that "ADIANTUM LUNULATUM" has potential antioxidant activity due to the presence of certain phytoconstituents such as Tannins, Flavonoids, Carbohydrates, Steroids, Anthocyanins and Saponins, etc. Hence conclude that "ADIANTUM LUNULATUM" is an effective and potential source of novel drug to improve the treatment of diseases associated with antioxidative agents. The future direction of this study is to perform 'in-vivo' studies and find its anti-inflammatory activity for product formulation.

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