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ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL ACTIVITY OF *Adiantum lunulatum* burm.f.

P J Jenat[a] And S N Suresh[b]*

[a]PG and Research, Department of Biotechnology, Sree Narayana Guru College.
[b] PG and Research, Department of Biotechnology, Sree Narayana Guru College, K.G Chavadi, Coimbatore -641105.

***Corresponding Author Email: drsnsuresh78@gmail.com**

ABSTRACT

Medicinal value of Adiantum lunulatum due to the presence of the phytochemicals and this phytochemicals have major role in the antioxidant and antibacterial activity. DPPH assay and antibacterial activity analysis help to identify the medicinal potential of this fern. Agar well diffusion method and minimum inhibitory concentration analysis (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml and 1 mg/ml) carried out against bacterial strains such as Staphylococcus aureus, Klebsiella pneumonia, Proteus vulgaris and Escherichia coli. DPPH assay help to identify the free radical scavenging activity of plant extract. The result indicates that increase concentration of extract has direct influence corresponding to the percentage of free radical scavenging activity. Bactericidal activity analysis shows more bacterial growth inhibition at 0.8 mg/ml and 1 mg/ml against most of bacterial strains. Adiantum lunulatum show increased effectiveness against various diseases and need further research study.

KEY WORDS

Bactericidal; Fern; Phytochemicals; Radical; Strains

INTRODUCTION

Medicinal history of humans and animals depend on different variety of plants, which also include petridophytes. Petridiohytes are diverse member of plants and show distinct characteristics in development and growth. In many parts of world petridophytes used as source of food, medicine and for other essential purposes. *Adiantum lunulatum* is member of division pteridophyta and the genus *Adiantum* contains many species. *Adiantum* species of ferns show terrestrial / lithophytic in growth and are commonly found in moist shady places such as rock crevices, clay, near water streams and hillocks [1]

Medicinal plant contains many of the chemical constituents present in different plant parts [2]. This biochemical constituent's play major role in the plant as well as to other living things for their survival. Secondary metabolites produced by plant responsible for the prevention and curing of various diseases and show microbicidal activity, these secondary metabolites are produced as a part of primary metabolic pathway [3]. *Adiantacea* contain increased level of phytoconstituents and this presence of biochemical constituents purely seasonal [4]. Tribal peoples considered *Adiantum lunulatum* as curative to various diseases such as dysentery, leprosy, fever, centipede bite, blood related diseases and for other microbial diseases [5].

Adiantum lunulatum extract studied to determine the effect of phytochemical constituents against various microorganisms. In the field of drug discovery antimicrobial activity is very much necessary to study the activity of plant extract against various microbial strains and their growth. Different method commonly used for this purpose, such as a disc diffusion assay and agar well diffusion assay are important [6]. Oxidative damage to the cells is caused by hydroxyl, peroxyl, and

superoxide radicals produced as a part of various metabolisms. Various biochemical compounds present in plants show good antioxidant activity by scavenging reactive free radicals. Different antioxidant assays are widely used to analyse the antioxidant activity some important of them are DPPH and total antioxidant analysis.

MATERIALS AND METHODS

Preparation of plant material

Fresh *Adiantum lunulatum* collected from nearby places of Western Ghats during the month of October. The fern was identified in the department of biotechnology by the head of the department. To remove the soil particles and other contaminants, plant material properly washed in tap water. The leaves were kept at room temperature without exposure to sunlight.

Preparation of extracts

Dried plant material grinded into fine powder mixed in 10 g/100 ml in different solvents such as ethanol, methanol, chloroform and aqueous. This preparation was kept for 85 hours under room temperature with intermittent shaking. The filtrate prepared by using whatman No. 1 filter paper and evaporated to remove solvents.

Antibacterial activity of *Adiantum lunulatum* extracts

Bactericidal activity of *Adiantum* extract was analysed by agar well diffusion method using ethanol, methanol, and chloroform and aqueous extracts (10 g/100 ml). Muller Hinton agar media prepared, sterilized and poured into sterile petriplates. Five wells were punched in the solidified agar plates. These plates were swabbed with four bacterial species such as *Staphylococcus aureus, Klebsiella pneumonia, Proteus vulgaris and Escherichia coli.* Wells were added with extracts and chloramphenicol 1 mg/ml used as standard. Incubate overnight at 37^0c and then documented [7, 8]

Preparation of extracts

Dried plant material grinded into fine powder of different concentrations 0.02 g, 0.04 g, 0.06 g, 0.08 g, 0.1 g and mixed with 100 ml ethanol and kept it incubation 82 hours. After incubation filtrate was prepared by using whatman No 1 filter paper and solvent evaporation carried out to obtain the crude extracts.

Minimum inhibitory concentration of *Adiantum lunulatum* ethanolic extracts

Nutrient broth was prepared and sterilized by autoclaving; this sterilized broth was transferred into the sterile test tubes and cotton plugged. Test microorganisms were prepared and transferred into the nutrient broth. Ethanolic extract of different concentration added and incubate the tubes at 37^oc for 24 hours, then analysed the turbidity. Cultured tubes without turbidity after incubation taken as a positive result [9].

Preparation of *Adiantum lunulatum* extract for DPPH assay.

Methanolic extract of different concentration (0.2, 0.4, 0.6, 0.8 and 1 mg/ml) was prepared and its antioxidant activity analyzed by using DPPH and the phosphomolybednum method [10, 11].

2,2-diphenyl-1-picryl-hydrazyl assay

From the different concentration of methanolic plant extract prepared, 3 ml of plant extract was taken in test tubes. Then treated with 0.3 mM ethanolic solution of DPPH was prepared and 1 ml added to the extract in the test tube. The preparation was kept for 60 minutes at room temperature. Absorbance was measured by spectrophotometricaly at 517 nm. Blank preparation contains methanol and DPPH.

RESULTS AND DISCUSSIONS

Adiantum lunulatum extracts contains effective antibacterial activity against selected pathogenic bacterial strains and by forming clear zone of inhibition on bacterial culture.

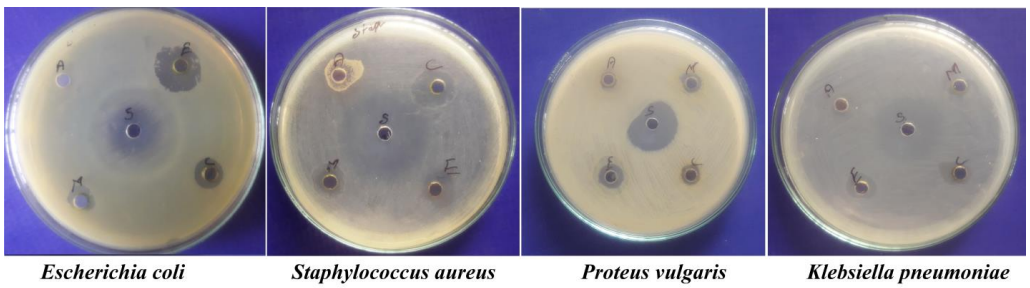


Fig 1: Antimicrobial activity analysis of different plant extracts

Table 1: Antimicrobial activity analysis of *Adiantum lunulatum*

Bacteria	Ethanolic extract	Methanolic extract	Chloroform extract	Aqueous extract	Chloramphenicol
Escherichia coli	8 ± 1	8.33 ± 1.527	10.33 ± 0.57	2.33 ± 4.04	25 ± 0
Staphylococcus aureus	12 ± 1	10.6 ± 0.58	10 ± 1	5 ± 4.35	18.33 ± 0.58
Proteus vulgaris	12 ± 1	9.66 ± 3.05	9 ± 2.6	6.66 ± 0.57	19.33 ± 0.57
Klebsiella pneumoniea	9 ± 1	11.33 ± 0.57	10.33 ± 2.51	2.33 ± 3.8	18.66 ± 1.95

Mean (mm) ± Standard Deviation (S D)

Table 2: Minimum inhibitory concentration of ethanolic extracts

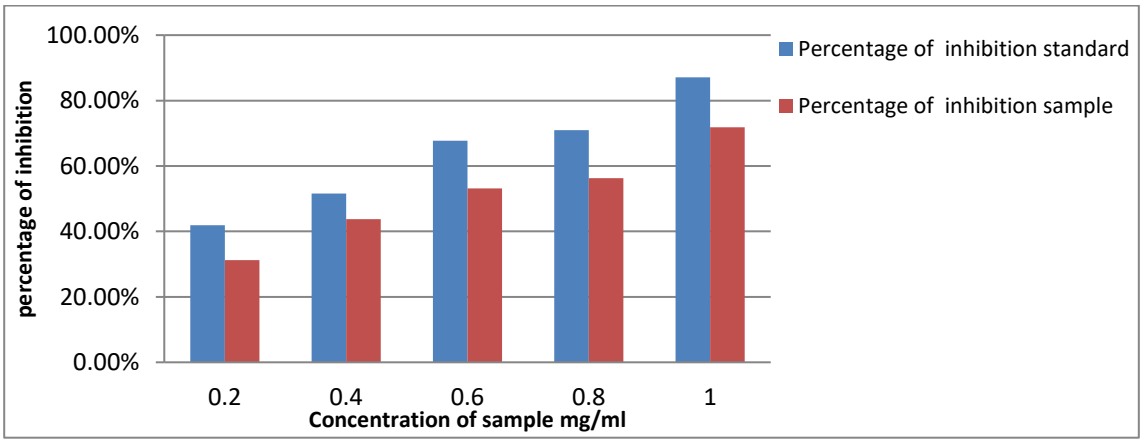
Bacteria	0.2 mg/ml	0.4 mg/ml	0.6 mg/ml	0.8 mg/ml	1 mg/ml
Staphylococcus aureus	+	+	+	+	-
Proteus vulgaris	+	+	-	-	-
Escherichia coli	+	+	+	-	-
Klebsiella pneumoniea	+	+	+	-	-

(+) presence of growth, (-) absence of growth.

DPPH Method

Table 3: DPPH analysis of *Adiantum lunulatum* extract

Sl no	Concentration of extract	Inhibition standard %	Inhibition sample %
1	0.2 mg/ml	41.93%	31.25%
2	0.4 mg/ml	51.61%	43.75%
3	0.6 mg/ml	67.74%	53.12%
4	0.8 mg/ml	70.96%	56.25%
5	1 mg/ml	87.09%	71.87%



Graph 1: *Adiantum lunulatum* antioxidant activity

Adiantum lunulatum extract contain many biochemical constituents; this constituent shows important medicinal properties such as antioxidant and antibacterial activity. Ethanolic extract contain effective antioxidant activity (31%-71%) with respect to increase in concentration. Studies on *Adiantum pedatum* show effective antioxidant activity and antimicrobial activity due to the presence of various biochemical compounds [12]. *Adiantum capillus-veneris* extract show significant antibacterial activity against many pathogenic microorganisms of *Proteus, Salmonella, Staphylococcus, Klebsiella, E. coli, Pseudomonas* and *vibrio* species [13]. Plant rich in various phytochemicals and this phytochemical have major role in health of living things. Some common phytochemicals such as alkaloids, flavanoids, glycosides, tannins, saponins, phenolics and terpenoids have effective medicinal roles [14]. Development of multidrug resistant bacterial species

led to the search for new source of drug to resist bacterial diseases. Plant phytochemicals shows effective antimicrobial mechanism in disease suppression [15].

CONCLUSION

Traditionally, peoples use this fern as a source of medicine to treat various diseases. In this study fern *Adiantum lunulatum* show better bactericidal and antioxidant activity. Different pathogenic bacterial strains *Staphylococcus aureus, Klebsiella pneumonia, Proteus vulgaris and Escherichia coli* show growth inhibition in presence of extract. Research studies on various other microorganisms and other medicinal studies necessary to develop new source of medicine.

Conflict of interest: nil

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***Corresponding Author:**
S N Suresh*
Email: drsnsuresh78@gmail.com