WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 3, 640-648. Research Article ISSN 2277– 7105

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF FERN ADIANTUM LUNULATUM BURM .F

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Article Received on
06 December 2017,

Revised on 27 Dec. 2017,
Accepted on 17 Jan. 2018

DOI: 10.20959/wjpr20183-10810

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ABSTRACT

Phytochemical studies on the fern Adiantum lunulatum are very much necessary to identify the presence of phytoconstituents. Different phytochemical tests and antimicrobial assay help to evaluate, the medicinal potential of the phytochemicals present in this fern. Phytochemical screening for major phytochemicals such as Phenols, terpenoids, saponins, tannins, glycosides proteins and carbohydrates were carried out by using ethanol, methanol, chloroform and aqueous plant extracts. Antimicrobial study was performed by using agar well diffusion method, with four bacterial species (Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli). Presence of phytochemicals such as phenols, tannins, terpenoids, saponins, glycosides, proteins was confirmed in Adiantum lunulatum extracts. Fern extracts also show good bactericidal activity, against selected bacterial species by forming clear zone of inhibition. So this plant has promising future in the medicinal field and drug development.

KEYWORDS: Phytochemical, Antimicrobial, Bactericidal, confirmed.

INTRODUCTION

From thousands of years ago, human races depend on plants for different purposes including food, shelter, fuel and medicine. In many part of the world, tribal peoples considered plants as their only source of medicine. According to world health organization report, 80% of developing countries population still depends on traditional plant based drugs. In modern

period, pharmaceutical industry focused on drug discovery from plants. Medicinal plant demand increased in both developed and developing country due to non toxic, lack of side effect, low price and easily availability.[1] However, it is very much necessary to investigate medicinal-potential of less studied plants.

Ferns are one of the primitive plant groups, belong to Petridophyte, evolve millions of years ago and 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. Adiantum lunulatum is kind of fern belongs to family of Adiantacia.[2] Different members of this family is widely distributed in different regions based on the susceptibility to climatic conditions such as temperature, wind, moisture content of the soil and presence of sunlight. In India this plant present predominantly in southern plains.[3] In different parts of India Adiantum lunulatum has been used medicinally by different communities, for the purpose of curing dysentery, ulcer, burning sensation, chest pain and snake bite.[4,5] Detailed study of this fern is very much necessary for the determination of medicinal value. Phytochemicals analysis of the fern extracts help to confirm the presence of phytochemicals and focused it influence on the disease suppression. So the present investigation deals with detection of phytochemicals and antibacterial activity.

MATERIALS AND METHODS

Preparation of the extracts

Plant material was collected from the field, washed under tap water, dried in the shade without the exposure of sunlight. Then dried plant material was grinded in to fine powder. Extract prepared by mixing separately 20g plant powder in 200ml each solvent (ethanol, methanol, chloroform, distilled water). This preparation was kept in incubator shaker for 57 hrs. The filtrate prepared by using whattman No.1 filter paper and evaporated to remove solvents.

Phytochemical screening

Phytochemical analysis of ethanol, methanol, chloroform, aqueous extracts were carried out by standard methods.[6,7,8]

Phenols

Nitric acid test

To the 2ml of each extract treated with 2ml of diluted nitric acid solution. Appearance of the reddish to yellowish color indicates phenolics.

Flavanoids

Sodium hydroxide test

To the 1 ml of each extract treated with 1 ml of 10% sodium hydroxide. Formation of yellow color indicates flavanoids.

Alkaloids

Mayer's test

To the 2ml of each extract treated with Mayer's reagent. Development of yellowish color indicate alkaloids.

Tannins

Braymer's test

To the 2ml of each extract treated with 2ml of H_2O and 2-3 drops of 5% ferric chloride solution, which result in to the formation brownish green precipitate gradually changes to bluish black detect the presence of tannins.

Terpenoids

To the 2ml of extract treated with 2ml of chloroform, placed in water bath to evaporate and add 2ml of con H_2So_4 then heated for 2 minute. Which result in to the formation of reddish brown color.

Saponins

Foam test

To the 5ml of each extract treated with equal volume of distilled water and placed in boiling water bath. Appearance of the froth indicates saponins.

Steroids

Salkowski test

To the 2ml of each extract treated with 2ml of chloroform, then add few drops of con H_2So_4 appearance of reddish brown color ring at the junction indicate steroid presence.

Carbohydrates

Benedict test

To the 2ml of each extract treated with Benedict's reagent (sodium carbonate+sodium citrate+coppersulphate) and heated in boiling water bath for 5 minute and cooled. Development of orange red precipitate indicates presence of carbohydrates.

Glycosides

Borntrager's test

To the 2ml of each extract treated with 10% ferric chloride and placed in the boiling water bath for 5 minutes. This preparation was allowed to cooled and treated with benzene. Addition of ammonia solution result in the formation of rose pink color in ammonical layer indicates glycosides.

Coumarins

To the 2ml of each extract treated with 3ml of 10% NaOH and placed in boiling water bath. Formation of shining yellow color when viewed under UV light indicates coumarins.

Proteins

Biuret test

To the 2ml of each extract treated with 2ml of 10% sodium hydroxide and added with 0.7% copper sulphate solution, then heated for 5 minutes result in to the formation purplish violet color denote proteins.

Emodins

To the 2ml of each extract treated with 2ml of ammonium hydroxide and added with 3ml of benzene. Development of red color shows presence of emodins.

Antibacterial activity

Agar well diffusion method is used to determine the antibacterial activity. Four bacterial strains (Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli) were used to test antibacterial activity of different plant extracts (methanol, ethanol, chloroform, aqueous). Muller Hinton agar media poured into sterile petriplates and solidified. Five wells of 0.5mm were punched on the medium for aqueous, ethanol, methanol and chloroform extracts. Media containing plates were swabbed with different bacterial strains and wells prepared in the media filled with different extracts. Chloramphenicole used as

standard (1mg/ml). For each bacteria method performed in triplicate. Finally, these plates were kept under the incubation overnight at 37^0c. Antibacterial activity measured by identifying clear zone of inhibition.[9,10]

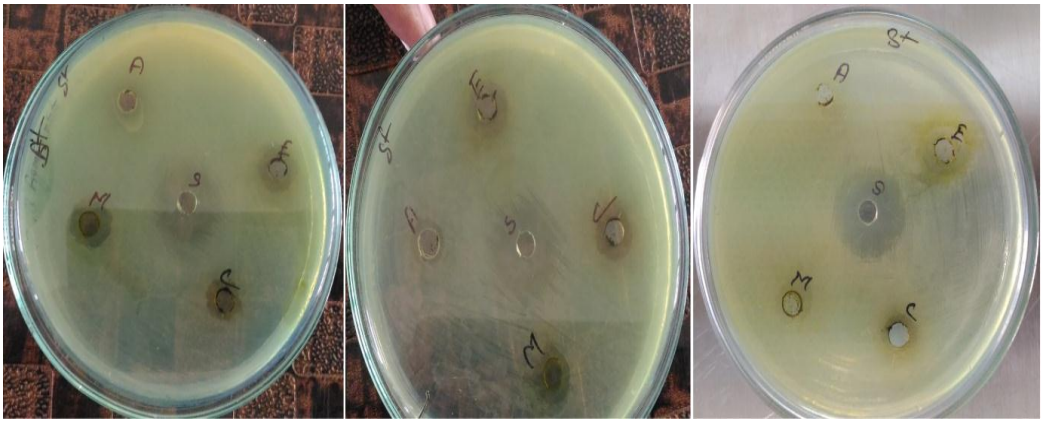

Fig 1: Staphylococcus aureus.

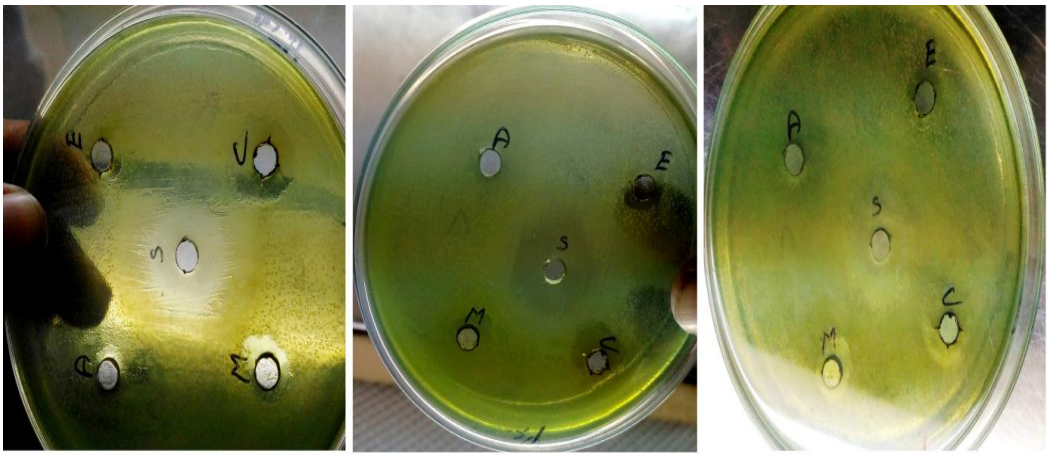

Fig 2: Pseudomonas aeruginosa.

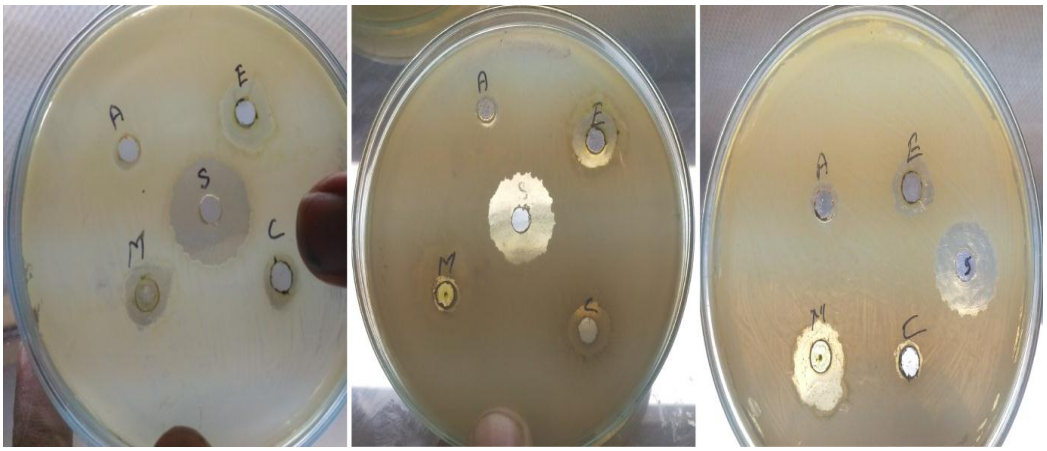

Fig 3: Proteus vulgaris.

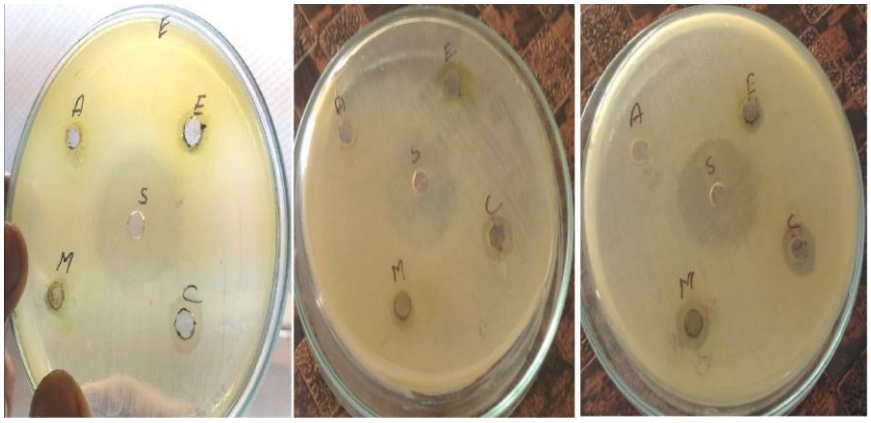

Fig 4: Escherichia coli.

RESULTS AND DISCUSSION

Table 1: Result of phytochemical evaluvation of fern Adiantum lunulatum.

Phytochemicals	Aqueous	Ethanol	Methanol	Chloroform
Phenols	+	+	+	+
Flavanoids	-	-	-	-
Alkaloids	-	-	-	-
Tannins	+	+	+	-
Terpenoids	-	+	+	+
Saponins	-	-	-	+
Steroids	-	-	-	-
Carbohydrates	-	-	-	-
Glycosides	+	+	+	+
Coumarins	-	-	-	-
Proteins	+	-	-	-
Emodins	-	-	-	-

(+) ⟶ *Presence* (-) ⟶*Absence*

Table 2: Result of antimicrobial activity of fern Adiantum lunulatum.

Bacteria	Ethanolic extract (mm)	Methanolic extract (mm)	Chloroform extract (mm)	Aqueous extract (mm)	Chloramphenicol (mm)
Staphylococcus aureus	13 ± 11	11 ± 10	11 ± 9	8 ± 0	19 ± 18
Pseudomonas aeruginosa	13 ± 8	12 ± 7	14 ± 12	7 ± 0	20 ± 18
Proteus vulgaris	13 ± 11	13 ± 7	11 ± 6	7 ± 6	20 ± 19
Escherichia coli	9 ± 7	10 ± 7	11 ± 10	7 ± 0	25

Adiantum lunulatum plant extracts contain the presence of important phytochemicals such as phenols, tannins, terpenoids, saponins, glycosides and proteins explained in Table. 1. Both phenols and glycosides were present in all solvent extracts, but tannins presence undetected in chloroform extract. Terpenoids present in all extracts except aqueous extract. Saponins and

proteins presence detected only in single extract, former present in chloroform extract and later present in aqueous extract.

These secondary plant metabolites have medicinal importance and Table. 2 indicate antibacterial activities of plant extracts against different pathogenic bacterial strains (Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli). In present study ethanolic plant extract show high clear zone of inhibition (13 ± 11) against Staphylococcus aureus as compared to other plant extracts "Fig.1". In case of Pseudomonas aeroginosa chloroform extract had high clear zone of inhibition (14 ± 12) "Fig. 2". Both the ethanolic and methanolic extract active against Proteus vulgaris form (13 ± 11, 13 ± 7) zone of inhibition respectively "Fig. 3". Chloroform extract was more active against Escherichia coli as compared to other plant extracts "Fig. 4". So the result indicates presence of phytochemicals in the Adiantum lunulatum extract has major role against harmful pathogens. Earlier studies pointed out the importance of this phytochemicals such as Terpenoids have anti-inflammatory activity.[11] Tannins present in the plant extract have anticancer, antioxidant, antifungal activity and antimicrobial activity.[12,13,15] Saponins have anti-carcinogenic, immunostimulatory activity and anti-mutagenic activity.[14,15] Glycosides have cardio protective action against cardiac arrest and play major role in immune stimulant activity.[16,17] Phenolics have effective action in human health to protect against pathogenic microbes, anti-allergic, anti-infamatory and antioxidant actions.[18] Therefore, the presence of these secondary metabolites in Adiantum lunulatum increases the medicinal value of this fern.

CONCLUSION

Studies on Adiantum lunulatum reveals this plant carry many phytochemicals which present in plant extracts. This phytochemicals act against the Gram positive as well as Gram negative pathogenic bacteria that cause infectious diseases to humans and animals. This fern is more dependable for the wide variety of uses in the field of medicine. So further studies based on the chemical constituents and clinical trials will be very much necessary for development of new beneficial drugs.

ACKNOWLEDGEMENT

I am greatly thankful to department of biotechnology SNGC, Coimbatore for providing facilities to carry out this research study.

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