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Antibacterial screening and phytochemical powder isolated from dorsal side of leaves of Cocos nucifera (Arecaceae)

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Abstract

The antibacterial activity of powder extract, isolated from the dorsal side of the leaves of Cocos nucifera, family Arecaceae, had studied against Escherichia coli using antibiotic ciprofloxacin 10 mm disc (concentration 1µg) as standard, by disc diffusion method on Muller Hinton agar medium. The extraction of powder was carried out by soxhlet extraction apparatus using water, 70% ethanol, chloroform and diethyl ether as solvent. Among the four extracts ethanol and water extracts were found to have antibacterial activity. The 70% ethanol and aqueous extract of powder shows zone of inhibition 5.0 mm and 3.5 mm respectively compared to ciprofloxacin 10mm and control. The phytochemical screening of crude extracts shows the presence of tannins, lignin, flavonoid, as secondary metabolites, which may contribute to the antibacterial activity.

Keywords-Phytochemical Screening, Antibacterial, Soxhlet Extraction, Ciprofloxacin, Secondary Metabolites.

INTRODUCTION

Traditional method of treatment with plants shows better therapeutic outcome with minimum or no toxicity. Phytochemical screening of plant extracts reveals the secondary metabolites like alkaloids, tannin, lignin, flavonoid, reducing sugar, saponins, steroid, which may have therapeutic property when ingested or applied locally.[1]

Literature study reveals that the coconut tree or its parts widely used in different diseases, commercially or industrially for preparation of household products and cosmetics. Coconut shell, leaves, husk, sheeth, oil, leaves, wood flower possess wide physiological activity in human body. Methanolic extract of coconut shell possess anticancer activity [2]. Ethanolic extract of leaves shows anti Alzheimer's activity [3]. Various antibiotics had developed from solvent extract of Cocos nucifera [4].

The antibacterial screening of powder isolated from the dorsal side of the leaves of Cocos nucifera was done using different extracts (aqueous, 70% ethanol, chloroform and diethyl ether) by disc diffusion method. The phytochemical screening was carried out to determine the secondary metabolites. [5]

MATERIALS AND METHODS

CHEMICAL, REAGENTS AND EQUIPMENT

The analytical grade chemicals and reagents (hydrochloric acid, phloro glucinol, sodium chloride, gelatine, chloroform, acetic anhydride, ethanol and sulphuric acid) obtained from sigma Aldrich, were used for phytochemical screening and antibacterial study. Laminar air flow (CLEAN AIR instrument), micro tip (tarsons), Petri plates (SD Fine chem.Ltd), glass wares (Borosil), sterile swab (Hi media), micropipette (VARI pipettes-Hi tech lab), Muller Hinton agar (Hi media), 10mm sterile disc (Hi media)

COLLECTION AND EXTRACTION

The powder was collected from the dorsal part of leaves of Cocos nucifera, and spread on a butter paper, dried in shade to remove the traces of water. The dried powder is stored in desiccator and was used for soxhlet extraction.

The powder (Figure.1) divided in to four each containing 50gm, packed in soxhlet apparatus and are extracted with solvents like water, 70% ethanol, chloroform and diethyl ether for 4-6hrs. The extracts were collected and stored in refrigerator at 4^0C and diluted for further studies. [6]

Figure.1 Showing The Powder On The Dorsal Side Of Leaves Along With Leaf Sheeth Of Cocos nucifera, Family-Aracaceae

Figure.2 Showing the Powder Obtained From The Dorsal Side Of Leaves Of Cocos nucifera

Phytochemical analysis

Test for lignin: A solution of hydrochloric acid and Phloroglucinol is used for the detection of lignin. A brilliant red colour develops, owing to the presence of lignin.

Test for tannin: To a solution of tannin, aqueous solution of gelatin and sodium chloride are added. A white buff coloured precipitate is formed, indicate presence of tannins.

Test for flavonoid: To dry powder or extract, add 5 ml of 95% ethanol, few drops of conc. HCl and 0.5 g magnesium turnings formation of Pink colour indicate presence of flavonoid [7,8,9]

Antibacterial screening of powder extract

Preparation of nutrient agar medium

Muller Hinton agar 38gm was weighed and added to 1000ml distilled water, sterilized in an autoclave at 121^0C for 30minutes and transferred in to sterile Petri dish. Broth culture of Escherichia coli was prepared and transferred on petri dish and incubated for 15minutes at room temperature [10]

Determination of antimicrobial activity

The prepared extracts (20 µl) were added to the sterile disc using a micro pipette in aseptic condition, allowed to dry the solvent, and procedure is repeated until the sterile disc saturated with the respective extracts.[11,12,13] The solvents evaporated and the disc were placed in inoculated media using sterile forceps. Ciprofloxacin disc of conc. 1µg kept as standard and 20µl of aqueous, ethanolic, chloroform and diethyl ether extracts as sample water, ethanol, chloroform and diethyl ether was used as control. The plates were kept in refrigerator for 30 minutes to allow the diffusion of sample to the surrounding agar medium. The plates were incubated at 37 ± 2^0c for 24 hours and observed for antibacterial activity. The ratio of diameter of zone of inhibition of sample and control were measured and compared with that of standard the values[14,15]

RESULT AND DISCUSSION

Phytochemical screening:

The phytochemical screening of powder extract shows the presence of tannins, lignin and flavonoids as the secondary metabolites in the crude extract (Table-1). These secondary metabolites might contribute to the antibacterial activity of the crude extract.

Anti-Bacterial activity

By combining the zone of inhibition sample and standard and blank, it was found that water extract and Ethanolic extract of powder isolated from dorsal side of leaf of Cocus nucifera is having anti-bacterial activity. There zone of inhibition seen in water extract and ethanolic extract (3.5 mm and 5 mm) compared to zone of inhibition of antibiotic ciprofloxacin (10mm). (Figure-3 and Table-2)

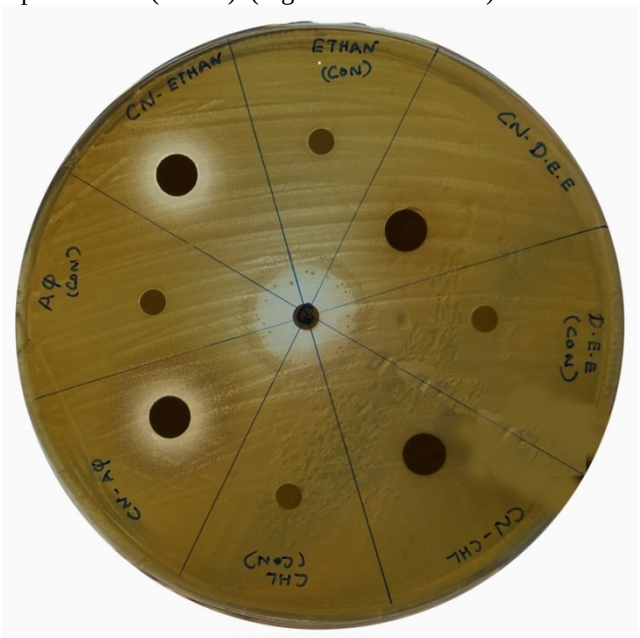

Figure (3) Antibacterial Screening Of Powder Extract Against Gram Negative Organism *Escherichia Coli*

CN AQ-Cocos powder aqueous extract
AQ-CON --Aqueous control (distilled water)
CN-ETHAN-Cocos powder ethanol extract **ETHAN CON** – 70%Ethanol control
CN D.E.E-Cocos powder diethyl ether extract
D.E.E CON-Diethyl ether control
CN CHL Cocos powder chloroform extract
CHL CON-Chloroform control

Table 1- Phytochemical Screening Result

Secondary metabolite	Observation	Result (alcohol and water extract)
Test for reducing sugar	No characteristic change	(-)
Test for alkaloids	No characteristic change	(-)
Test for tannins	Formation of white buff colour	(+)
Test for lignin	Formation of red colour	(+)
Test for flavonoids	Formation of pink colour	(+)
Test for steroids	No characteristic change	(-)
Test for saponins	No characteristic change	(-)

(-) Absence secondary metabolites, (+) presence of secondary metabolites

Table -2. Details Of Zone Of Inhibition Of Powder Exract Against Gram Negative Organism (E.coli)

Particulars	**Zone of inhibition in Millimeter, against E coli**
Water extract	3.5mm
Ethanol extract	5.3 mm
Chloroform extract	1mm
Diethyl ether extract	1mm
Ciprofloxacin(Antibiotic standard)	**10mm**
Control	
Control water	Nil
Ethanol control	0.3mm
Chloroform control	1mm
Diethyl ether control	1mm

CONCLUSION AND FUTURE SCOPE

The current work, extract of powder isolated from the dorsal side of Cocos leaves were found to possess secondary metabolites like tannin, lignin and flavonoid. These secondary metabolite might contribute to the antibacterial efficacy of the crude powder extract. The powder is used traditionally as antiseptic and as coagulant when applied locally to wounds, which foresight the screening of powder towards its coagulant and antiseptic property.

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