International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 6 Issue III, March 2018- Available at www.ijraset.com

Phytochemical Screening and Antibacterial Evaluation of the Leaf and Root Extracts of Cassia Alata

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Abstract: ***Cassia alata plant has a significant reservoir of medicinal compounds and secondary metabolites as an important source for the drug discovery. But medicinal compounds details and their action mechanism remain less explored. Isolation of different endophytic bacterial species were carried out and among that six positive colonies and one negative colony were identified. The purpose of this work is to characterize and identify the polyphenolic compounds from its leaves and stem by crude sample, distilled water, and hexane extraction. UV spectrophotometry analysis of C. alata extract was done. Furthermore the phytochemical compound were analysed using FTIR and identified different functional groups in three extract samples. The antibacterial activity of C. alata samples were tested against E.coli, Bacillus, Pseudomonas and S. Aureus bacterial cultures.***
Keywords: Medicinal Compound, Endophytic species, UV Spectrophotometry, FTIR, Antibacterial activity.

I. INTRODUCTION

Cassia alata contain rich source of natural products displaying a broad spectrum of biological activities and also associate with endophytic microbes which continues to increase significance in drug discovery. Many medicinal plants are reported to be harbouring endophytic microorganisms. It was found that the medicinal properties of some medicinal plants are attributed to the endophytes present in the tissues. Thus property may be the result of the chemical compounds produced by these endophytes. The most important bioactive compounds from medicinal plant includes Terpenes, Alkaloids, Phenolic compound steroidal compounds and Flavonoids (Kusari and Spiteller., 2012). Many endophytes have the potential to synthesize various bioactive metabolites that may directly or indirectly be used as therapeutic agents against numerous disease (Strobel et al., 2002; Kharwar et al., 2011; Kusari and spiteller et al., 2009). Occasionally endophytes that produce host plant secondary metabolites with therapeutic value or potential have been discovered some examples include paclitaxel (also known as taxol) podophyllo-toxin (Eyberger et al., 2006) deoxypodophyllotoxin (Kusari et al., 2009) camtothecin and structural analogs Kusari et al., 2009). Hypericine and emodin and azadirachtin (Kusari et al., 2009). The “balanced antagonism “ hypothesis (Schulz and Boyle., 2005) was initially proposed to address how an endophyte’s avoids activating the host defences ensures self-resistance before being incapacitated by the toxic metabolites of the host and manages to grow within its host without causing visible manifestation of infection or disease (Schulz and Boyle et al., 2005). In fact, endophytes act as chemical synthesizers inside the host plant, producing a plethora of bioactive secondary metabolites (Strobel et al., 2003, Owen and Hundley et al., 2004). Cassia alata is often called ring worm bush because of it very effective fungicidal and bactericidal property, C. alata is a shrub that belongs to the family Fabaceae. C. alata has been reported to have very high medicinal values like, (Igwe and Onwu et al., 2015). Leaf extract is also credited for the treatment of constipation, inguinal hernia, intestinal parasitosis, syphilis and diabetes. Leaf extract is a good antioxidant. Antimicrobial activities of compound biosynthesized by the plant endophytes have been reported only by a few group (Li et al., 2005; Harper et al., 2003) Endophytes can also be beneficial to their host by producing a range of natural products that could be harnessed for potential use in medicine, agriculture or industry (Schulz and Boyle et al., 2005; Schulz et al., 1993). Medicinal plants are growing gaining global attention owing to the fact that the herbal drug are cost effective easily available and with negligible side effects. (Maroof Ahmed et al., 2012),

II. MATERIALS AND METHODS

A. Glass ware and chemicals

All chemicals and solvents used were of analytical AR grade and methods were aseptically performed.

B. Collection And Preparation Of Leaf Samples From Cassia Alata

Healthy and mture plant C*assia* alata was carefully chosen for sampling. Samples were treated with sodium hypochlorite (NaOCl - 12%) was used as sterilization agent. Sterilization agent and time period required for surface sterilization were optimized by trial and error method.

C. Isolation of Endophytes And Confirmation Test

Alata stem sample were cut into long 0.5-1cm pieces, whereas leafs were cut into 4-5mm pieces. The sample tissues were surface sterilized by immersion in 75% alcohol for 5 mins and Then 80% sodium hypochlorite (NaOCl) for 1 min.

The surface sterilized plant tissues are placed in Nutrient Agar (NA) plates and Potato Dextrose Agar (PDA) plates and plates were incubated for bacterial growth at 37 ˚C for 24hrs. PDA plates were incubated for fungal growth at room temperature for 3-5 days. The bacterial plates were taken for further analysis and confirmation test. The gram staining and motility test were performed. Biochemical test (IMVIC) were done for the bacterial cultures.

D. Extraction of sample and antimicrobial Activity

Crude extract, distilled water extract and Hexane solvent extracts - The fresh leaves are collected and chopped into pieces (50g) and shade dried at 32-35^{O}C at constant weight for 5 days.

They were coarsely powdered using a mortar and pestle and finely powdered using electric blender. Powder sample (25g) was mixed with 100ml deionised water in a conical flask and shaken at 120 rpm for 30 mins and extracted by warming at 60^{O}C in a shaker water bath at 50 rpm for 24 hrs.

The sample was extracted with 100 ml of hexane solvent in a shaker counter bath at 50 rpm for 2hrs and filtered again. The samples was allowed to cool and filtered rapidly through four layer of gauze.

Aseptically emulsify a colony from the plate in the sterile saline solution were used.

Treat the swab with test organism and inoculate in the Muller Hinton agar (MHA) plate or nutrient agar (NA) plate for a lawn of growth. Antibiotic disc were placed on cultured plates using sterilized forceps.

The plates were inoculated and incubated for 24 hrs at 37ºC. After the incubation, the diameter of the zone of inhibition for each of the antibiotic used where measured. Antibiotic results were compared with the standard table to determine the sensitivity zone (Zone of Clearence).

E. Characterization of Extract samples - UV Spectrophotometry and FTIR Analysis

Crude extracted samples were analysed for the peak of absorbance at 420nm in UV – spectrophotometry. The Fourier Transform Infrared Spectroscopy (FTIR) analysis were carried out in the plant samples for the aqueous and the solvent extract and it was performed to identify the structural composition of the given sample to know the different functional groups present in the plant extracts.

III. RESULTS AND DISCUSSION

A. Gram Staining Method

Isolated endophytic bacteria colonies exhibited different the morphological characterization - diverse colony, different shapes, colour, margins surface including round shape to irregular shape colonies. Out of 6 isolated colonies, 3 Rod and 3 cocci are purple and Pink (5:1) respectively are given in the figures (1.1 – 1.6). Regarding cell shape and gram staining, 3 were Gram-positive cocci, 3 were Gram-negative rod, and 6 were indicated on table no 1. In total 3 isolates showed possibly belonging to the genus Bacillus (Nawed et al. 2015, Elvia Perez et al. 2017).

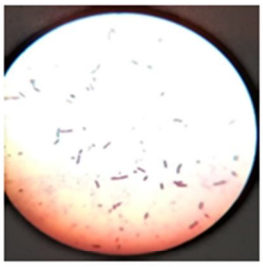


Fig 1.1 C-01 gram-ve (Chain)

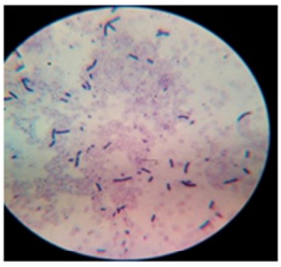


Fig 1.2 C-02 gram+ve (Cluster)

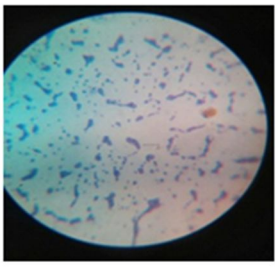


Fig 1.3 C-03 gram +ve (Chain)

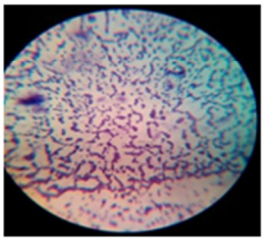


Fig 1.4 C-04 Gram +ve (Cluster)

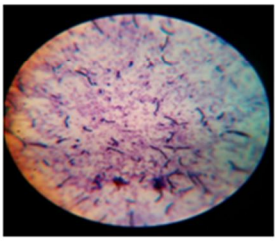


Fig 1.5 C-05 - Gram +ve (Chain)

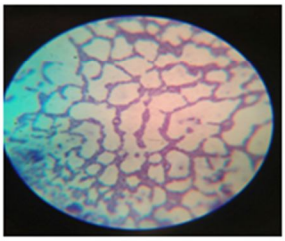


Fig 1.6 C-06 - Gram +ve (Both)

S.No	Bacterial Strain	Shape of Organisms	Colour	Characteristics
1	C-01	Rod	Pink	Single, Chain
2	C-02	Cocci	Purple	Single, Cluster
3	C-03	Rod	Purple	Single, Chain
4	C-04	Cocci	Purple	Single, Cluster
5	C-05	Rod	Purple	Single, Chain
6	C-06	Cocci	Purple	Single, Chain, Cluster

Table no 1: The bacterial strains identification from Gram staining method

A. Preliminary Characterization Of Endophytic Bacteria

Biochemical test were done for endophytic bacterial strains obtained from both root samples and leaf samples tests. The results indicates methyl red test - 3 +ve for root and 1 +ve, 2 –ve for leaf strains. Voges – Proskaue test results were shown as negative for root strain and 2+ve, 1-ve for leaf samples. Indole test were indicated negative for both leaf and root colonies. Citrate results shown as 1+ve, 2 -ve for root colonies and 2+ve, 1-ve for leaf colonies (Wu et al. 2016).

Table no - 2.1: Biochemical Results Of C.Alata Colonies Isolated From Roots.

S.n	Colonies	Mr	Vp	Indole	Citrate	Oxidase	Catalase	SIM	H_2s production
1.	C_1	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve
2.	C_2	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve
3.	C_3	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve

Table no – 2.2: Biochemical Results Of C. Alata Colonies Isolated From Leaf.

S.n	Colonies	MR	Vp	Indole	Citrate	Oxidase	Catalase	SIM	H_2s production
1.	C_4	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
2.	C_5	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve
3.	C_6	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve

The result indicates that they can produce catalase and oxidase enzyme (1-Root, 2-Leaf) and (3- Root, 2 - Leaf) respectively. The Sulfide Indole Motility result indicates (2- Root, 1-Leaf). Sulfur compound positivity were indicated as (1- Root, 1- Leaf). The culture characteristics of isolated endophytic bacteria are given in Table 2.1 and 2.2.

B. Antimicrobial activity of C.alata Leaf extracts

C.alata extract were tested for antibacterial activity (Zone of inhibition) against different bacterial strains (E.coli, Pseudomonas aeroginosa, Bacillus, Staphulococcus aureus). Zone of inhibition for hexane solvent has higher value against 19mm and 23mm for 25 µl and 50 µl respectively. The results for distilled water extracted samples was higher 18mm and 20mm for 25 µl and 50 µl respectively. Zone of inhibition values were given in table no 3.

Table no-2.3: Zone inhibition produced by solvent extract of C.alata against selected pathogenic organisms with reference antibiotic disc.

S.No	Sample	Organisms	Diameter of the Zone (mm)	
			25µl	50µl
1	Hexane	E.coli	13mm	15mm
2		Pseudomonas.aeroginosa	13 mm	18 mm
3		Bacillus	12 mm	19 mm
4		Staphylococcus aureus	19 mm	23 mm
1	Distilled water	E.coli	18 mm	20 mm
2		Pseudomonas.aeroginosa	12 mm	15 mm
3		Bacillus	16 mm	18 mm
4		Staphylococcus aureus	14 mm	17 mm

On the basis of antibacterial activity of *C.* alata solvent extract, it was evident that they show significant antibacterial activity against all the selected pathogenic bacteria. By increasing concentration both the solvents can create more zone of inhibition and antibacterial activity results were shown in the figure 3.1-3.4 and 3.5-3.8 for hexane and distilled water extract samples respectively.

Figure 2.1

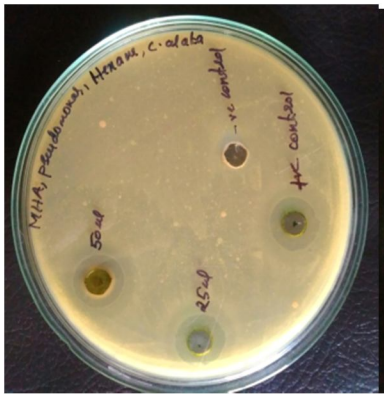


Figure 2.2

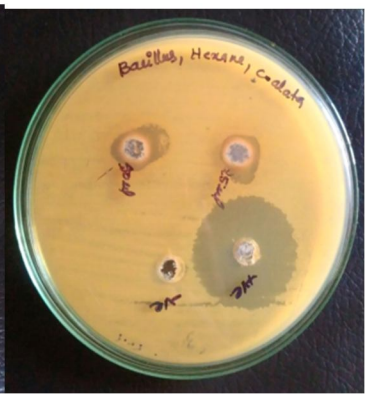


Figure 2.3

Figure 2.4

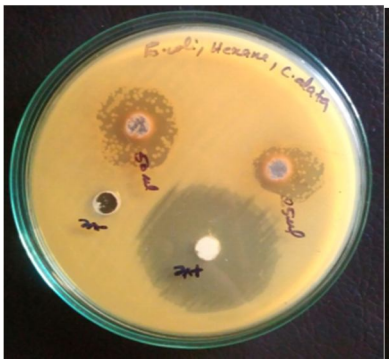


Figure 2.5

Figure 2.6

Figure 2.7

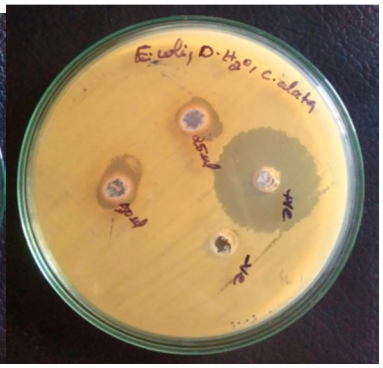
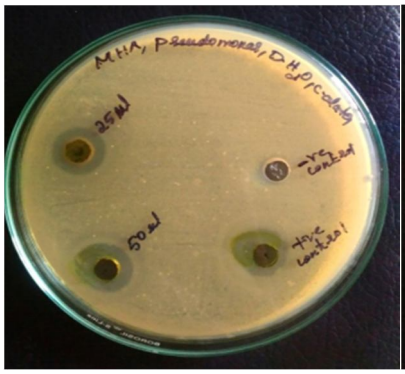
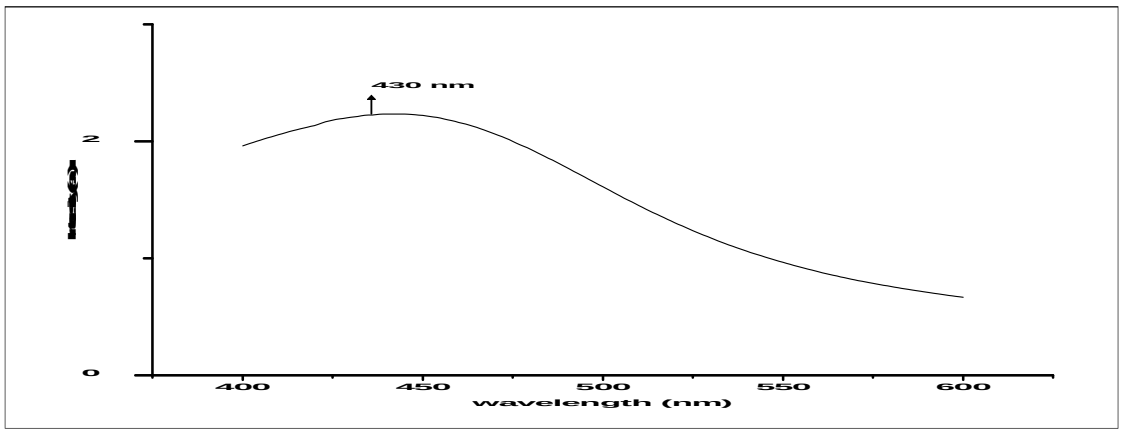


Figure 2.8





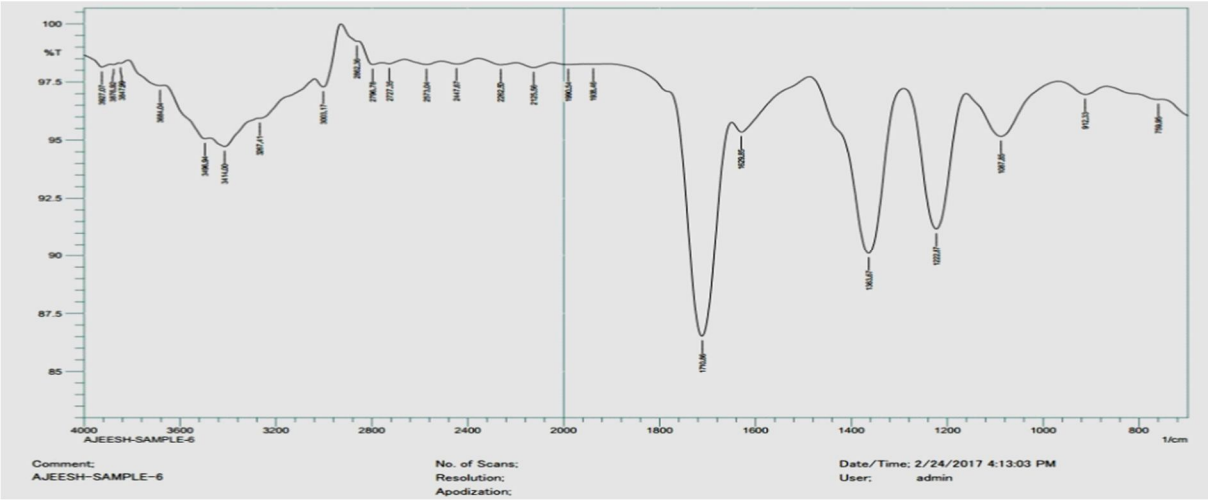


Figure 4.3 – Aqueous extract sample of Cassia alata

From the FTIR results of C. alata –Aqueous extract (figure-4.3) - the function groups as the following.N-H primary and secondary Amines and Amides ,Stretch, O-H (H- bonded);O-H(H-bonded); N-H primary and secondary Amines and Amides ,Stretch, O-H (H-bonded);O-H (H- bonded); C-H Alkanes (Stretch); C-H Aldehyde; O-H Carboxylic acid; O-H Carboxylic acid; X=C=Y Allenes, Ketenes,Isocyanates, Isothiocyanates; X=C=Y Allenes, Ketenes,Isocyanates, Isothiocyanates, Carboxylic acid; C=C Alkane, N-H (Bend); C-X Fluoride, C-O Alcohol, Esthers, Ethers, Carboxylic acid, Anhydrides; C-H(Out of plane bend) (Tan RX et al. 2001, Yuanting et al 2017).

IV. CONCLUSION

Cassia alata plant has various medicinal compounds ranging from mild to severe infectious and non-infectious diseases. The research work was focused on to identify the endophytic bacterial and also comparing the antimicrobial activities of the aqueous and hexane extracts of Cassia alata on bacteria strains for antibacterial activity and their phenolic compounds in Cassia alata samples. The relationship between the host and the endophytes may range from symbiotic to near pathogenic. Medicinal plants are gaining global attention owing to the fact that the herbal drugs cause effective, easily available and with negligible side effects. The production of bioactive compounds by endophytes, especially those exclusive to their host plant it is not important for an ecological perspectives but also from a bio-chemical stand point. Plants have long provided mankind with a source of medicinal agent with natural product once serving as source of all drugs. The antimicrobial activity of the aqueous and hexane extracts of Cassia alata leaves has been evaluated. The extracts exhibited more antibacterial properties. Aqueous leaf extract had better antibacterial activity against Escherichia Coli and Bacillus. Sp. while hexane leaf extract was better on Pseudomonas aeruginosa. The most important of these bioactive compounds are alkaloids, tannins, saponins, anthraquinones, anthocyanosides, phenolic flavonoids, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides and phlobatannins. Plants can be used to discover and screen these bioactive natural organic compounds which may be beneficial for the development of new pharmaceuticals that address today's therapeutic needs.

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