Asian Journal of Multidisciplinary Studies

ISSN: 2321-8819 (Online)
2348-7186 (Print)
Impact Factor: 1.498
Vol.4, Issue 8, July 2016

Protein Profile Studies – Effect of Flavonoids and Glycosides from *Caesalpinia Coriaria* (JACQ) Willd as Bactericidal Compound.

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ABSTRACT

Scope: The scope of the present study was to identify the bioactive compounds and to investigate the mode of action as antibacterial compound (Protein Profile Studies) from Caesalpinia coriaria (Jacq) Willd.

Materials and Methods: Isolation of flavonoids and glycosides from the ethanolic extract of Caesalpinia coriaria was carried and phytochemicals were analyzed by TLC. Flavonoids and glycosides compounds were treated with E.coli, Staphylococcus aureus and Klebsiella pneumonia resulted in the disturbance in protein synthesis which may act as bactericidal effect of the medicinal plant. .

Conclusion: Based on the current findings, the action model of these two bioactive compounds results in the disturbance of protein synthesis. Hence the bioactive compounds enter the inner membrane and inactivate protein profile thus inhibiting the growth of cells. Thus the result suggests that Caesalpinia coriaria was a potential candidate plant for the management of organism for antibacterial activity.

Key Words: Antibacterial activity, *Caesalpinia coriaria, E.coli, Staphylococcus aureus, Klebsiella pneumonia,* flavonoids, glycosides, TLC-Thin Layer Chromatography, SDS- PAGE.

1.1 INTRODUCTION

In India, around 17,000 species of higher plants, 7500 are known for their medicinal uses. This proportion of medicinal plants is the highest at any country of the world for the existing flora of that respective country. Ayurveda, the oldest medicinal system in Indian sub-continent has alone reported approximately 2000, medicinal plant species, followed by Siddha and Unani. The Charak Samhita which is an age-old written document on herbal therapy has reported on the production of 340 herbal drugs and their indigenous uses (Prajapati *et al*., 2003). In modern pharmacopeia, approximately 25 % of drugs are derived from plants and many others are synthetic analogues built on prototype compounds isolated from plant species (Rao *et al*., 2004). Apart from the human, animal husbandry also uses many plant species as its primary source of healthcare in India (Amal *et al*., 2009). India in its long history has accumulated a rich body of empirical knowledge in the use of medicinal plants for the treatment of various diseases. Chemical studies of Indian medicinal plants provide a valuable material base for the discovery and development of new drugs of natural origin (Qin and Xu, 1998). In contrast to the synthetic drugs, antimicrobial of plant origin are not associated with many side effects and have enormous therapeutic ability to heal many diseases (Iwu et al., 1999). Numerous research works have been identified compounds within herbal plants that are effective antibiotics (Basile et al., 2000). The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids etc. that are present in these plants (Sher, 2009). Some of these phytochemicals are physiologically active and are being exploited for human and animal use as they are being succeeded for vast therapeutic properties and as source of new drugs

1.2 MATERIALS AND METHODS

1.2.1. Standard strains- *Escherichia coli, Methicillin resistant Staphylococcus aureus, Klebsiella pneumoniae* were from the Royal Research Laboratory, and they were maintained for further study.

1.2.2 Chemicals and reagents

All the chemicals and reagents for the project used were purchased from Hi-Media, Merck, Qualigens and Loba chemie

1.2.3.Plant sample collection.

The Sample leaves of *Caesalpinia coriaria* were collected form Captain Srinivasa Murti Drug and Ayurveda Research Institute, Chennai and were identified from Dr. Jayaraman. The samples were allowed to dry under the shade completely. The leaves were ground into powder which was used for further extraction.

1.2.4.Extract preparation

About 1 kg of dry sample powder was weighed and macerated with 1000 ml of ethanol solvent and kept overnight in shaker. The extract was collected after filtration using Whatmann No. 1 filter paper and was stored. 1000 ml of solvent was added to the residual mixture and incubated in shaker for 24

hours and the extracts were collected again using Whatmann No.1 filter paper. This procedure was repeated again and the extracts were evaporated below 40 ^{0} C, which was used for further pytochemicals extract preparations.

1.2.5.Extraction of flavonoids (Amal et al., 2009).

The ethanol extract powder was defatted with petroleum ether (40-60^{0}C). The extract was then percolated with methanol until exhaustion at 40^{0}C by rotary evaporator. The condensed was partition using ethylacetate. This ethylacetate extraction contained crude flavonoids (Amal et al., 2009).

1.2.6.Extraction of glycosides (Aya *et al.*, 2011).

The ethanol extract powder were extracted three times with methanol at 25 ^{0}C for 24 hours and then concentrated in vacuum. The extract was washed with n-hexane and then the methanol layer was further concentrated to a gummy mass. The later was suspended with water and extracted with equal volume of ethyl acetate to give glycosides extract of the plant (Aya *et al.*, 2011).

1.2.7. Partial characterization of phytoconstituents from the leaves of *C.coriaria* (Gaw et al., 2002; Srivastava et al., 1996)

The partial characterization of the different phytoconstituents of *C.coriaria* was done on precoated silica gel TLC plates (Merck, USA). The efficient solvent system used for the flavonoids and glycosides was as follows:

1. Flavonoid Extract: Ethyl acetate: formic acid: acetic acid and water in 10:1.1:1.1:2.7 ratio.

2.Glycoside extract: Chloroform, ethylacetate, methanol and water in 1.5:4:2.2:1 ratio

The developed chromatogram plates were viewed under UV 240nm and 360 nm and day light (Gaw et al., 2002; Srivastava et al., 1996)

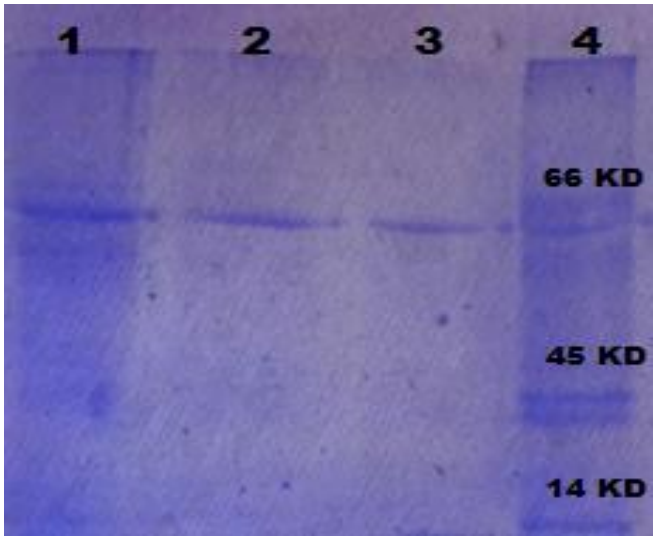
1.2.8.Protein profile studies – SDS PAGE (Laemmie, 1970).

SDS-Poly acrylamide gel electrophoresis was performed on slab gel with separating and stacking gels (12 and 4 %) by the method of (Laemmie, 1970). The test sample was mixed with an equal volume of sample buffer, boiled in a water bath for 3 min, cooled and added to the wells then the power supple was connected with cathode in the upper tank and anode in the lower tank. Electrophoresis was carried out at room temperature with constant voltage. The current supple was maintained until the tracer dye reached 0.5 cm above the lower end. At the end of electrophoresis gel was removed and stained with Coomassie brilliant blue. The molecular mass of the test samples were determined on SDS-PAGE. Purified protein samples were run on SDS-PAGE with concurrent run of standard protein markers consist of phosphorylase B, Bovine serine Albumin, Ovalbumin, Carbonic anhydrase and lactoglobulin. After separation the gels were stained with Coomassie brilliant blue.

1.3. RESULTS AND DISCUSSION

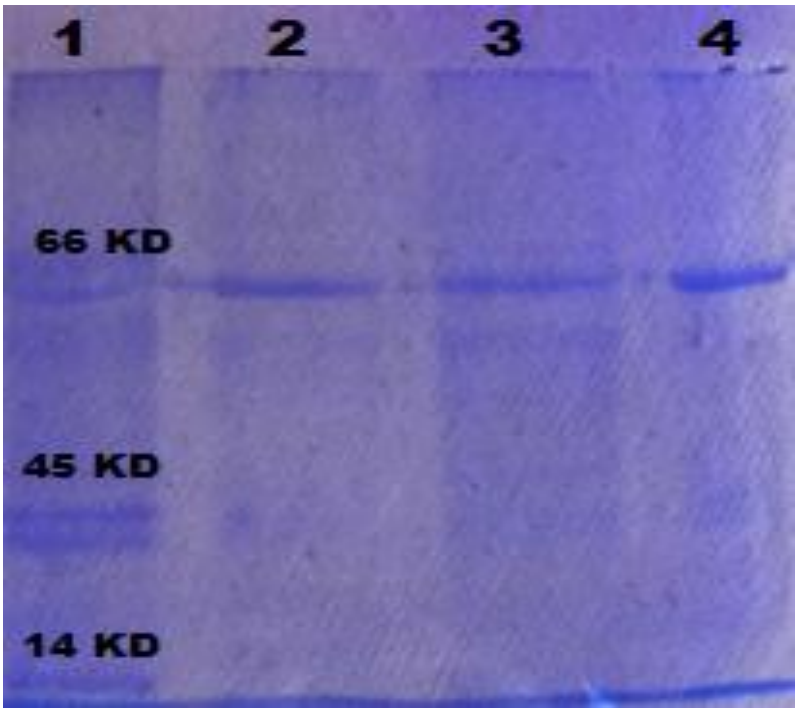
Detection of protein inhibition by SDS-PAGE was an important step for protein analysis. From our studies, it was evident that there was inhibition in the protein profile of bacteria treated with glycosides and flavonoids of *Caesalpinia coriaria*. As illustrated (Fig 1, 2, 3, 4, 5, 6) variations in the intensities of protein bands were detected. It was observed that all the control samples were characterized by more numbers of bands as that of markers. The treated samples showed decreased number of bands and hence it might be predicted that the protein types were failed to synthesize by the antibacterial by the way of inhibition.

Fig 1: SDS PAGE – TOTAL PROTEINS ISOLATED FORM PATHOGENIC BACTERIA *(E.coli)*TREATED WITH GLYCOSIDES COMPOUNDS OF *C.CORIARIA*



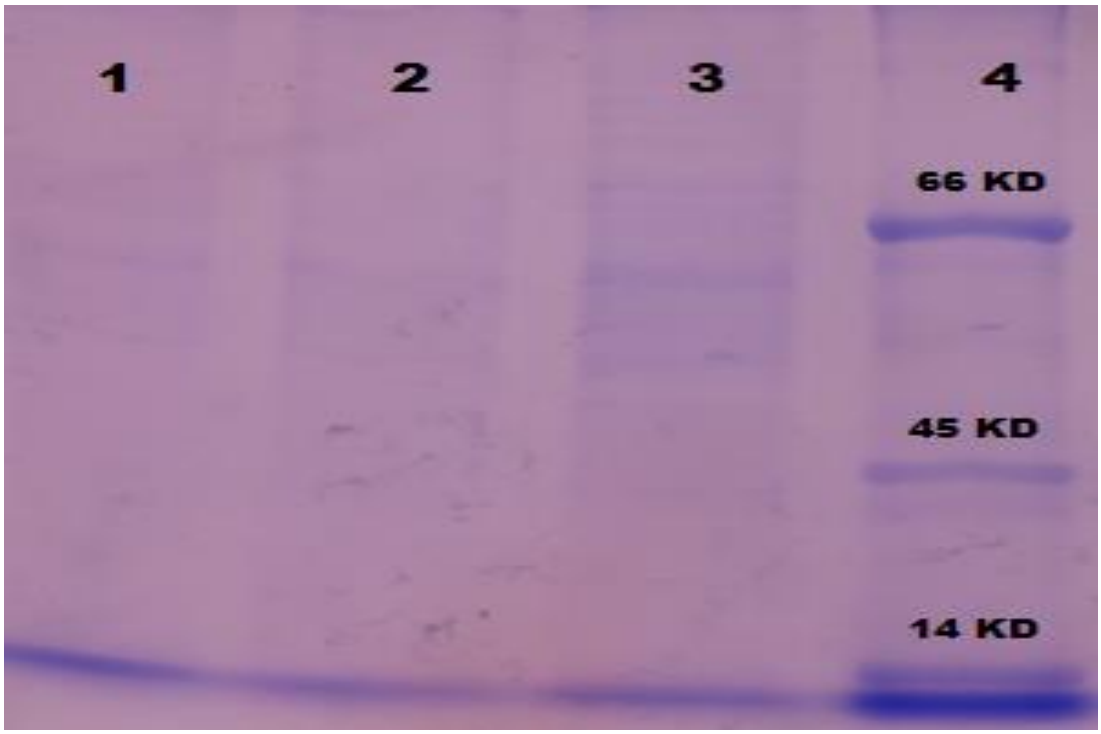
***E.coli* Lane 1- Control, Lane 2- 10 µg/ml glycoside extract, Lane 3- 20µg/ml glycoside extract, Lane 4- Standard Protein Marker.**

Fig 2: SDS PAGE – TOTAL PROTEINS ISOLATED FORM PATHOGENIC BACTERIA *(E.Coli)* TREATED WITH FLAVONLIDS COMPOUNDS OF *C.CORIARIA*



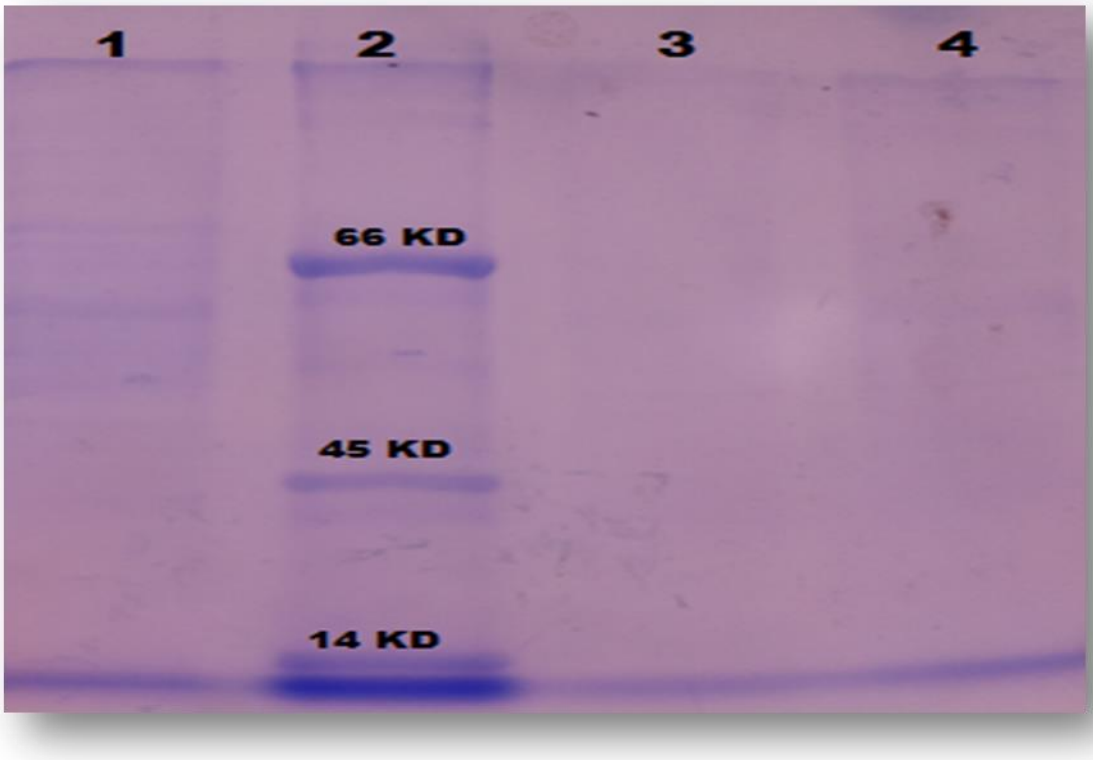
***E.coli* Lane 1- Standard Protein Marker, Lane 2- 10 µg/ml flavonoid extract, Lane 3- Control Lane 4- 20µg/ml flavonoid extract**

Fig 3: SDS PAGE – TOTAL PROTEINS ISOLATED FORM PATHOGENIC BACTERA TREATED WITH GLYCOSIDE COMPOUNDS OF *C.CORIARIA*



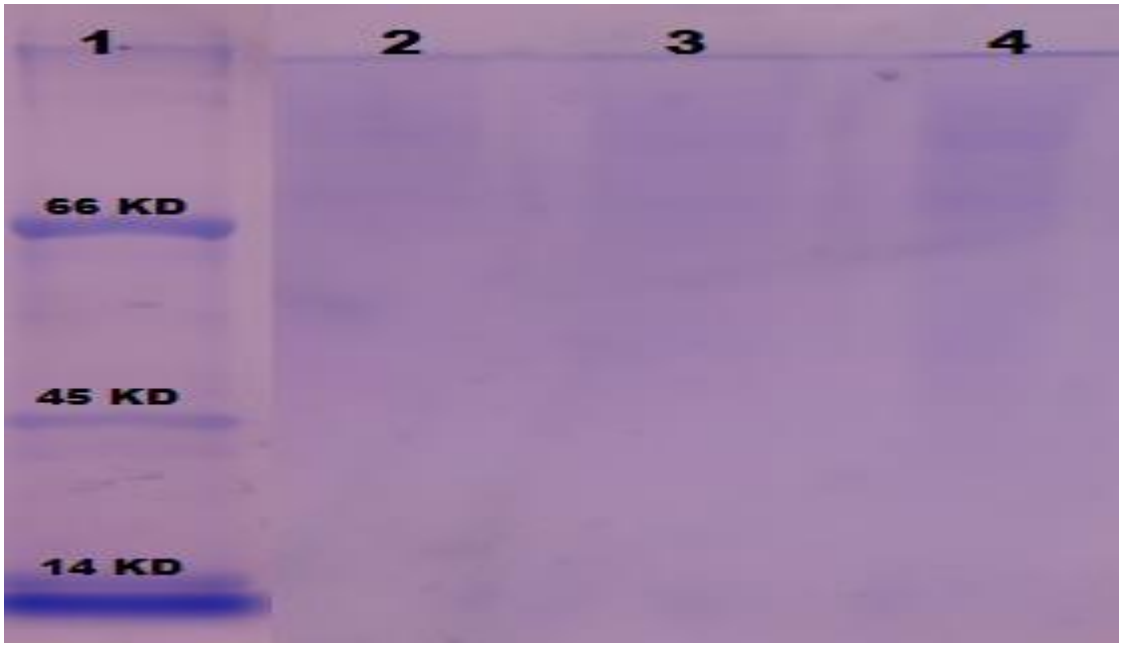
***Staphylococcus aureus* Lane 1- 10 µg/ml glycoside extract, Lane 2-20µg/ml glycoside extract, Lane 3- Control, Lane 4- Standard Protein Marker.**

Fig 4: SDS PAGE – TOTAL PROTEINS ISOLATED FORM PATHOGENIC BACTERA TREATED WITH FLAVONLID COMPOUND OF *C.CORIARIA*



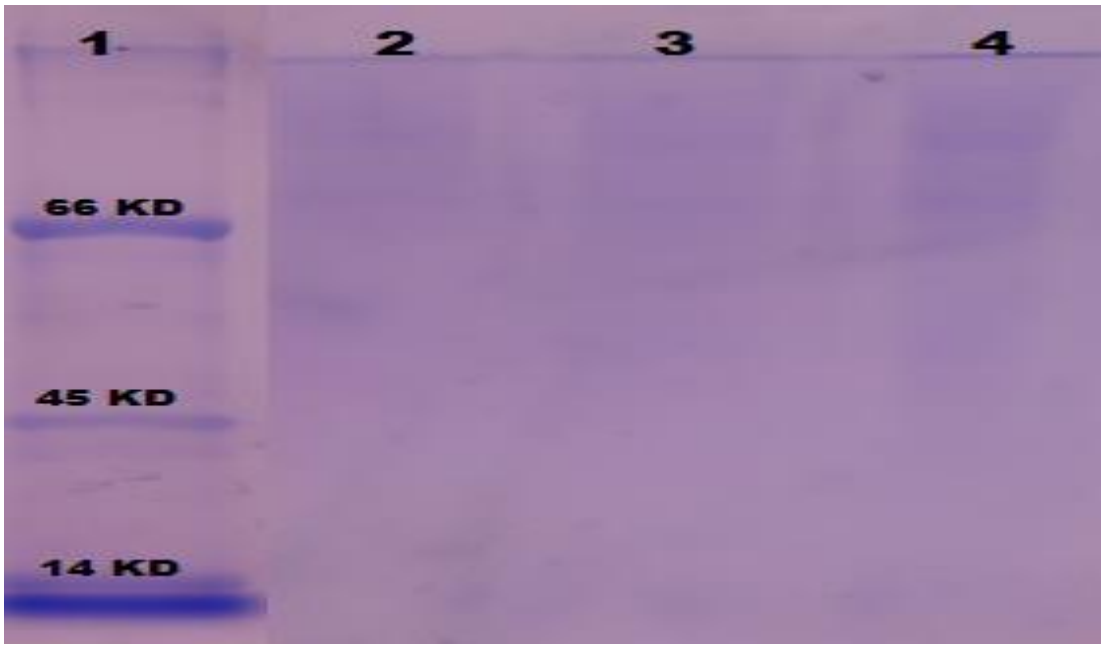
Staphylococcus aureus **Lane 1- Control, Lane 2- Standard Protein Marker, Lane 3- 10µg/ml flavonoid extract Lane 4- 20µg/ml flavonoid extract.**

Fig 5: SDS PAGE – TOTAL PROTEINS ISOLATED FORM PATHOGENIC BACTERA TREATED WITH GLYCOSIDE COMPOUNDS OF *C.CORIARIA*



Klebsiella pnumoniae**Lane 1- Standard Protein Marker, Lane 2-10 µg/ml glycoside extract, Lane 3-20µg/ml glycoside extract, Lane 4-Control**.

Fig 6: SDS PAGE – TOTAL PROTEINS ISOLATED FORM PATHOGENIC BACTERA TREATED WITH FLAVONLIDS COMPOUNDS OF *C.CORIARIA*



***Klebsiella pnumoniae*Lane 1- Standard Protein Marker, Lane 2- 10 µg/ml flavonoid extract, Lane 3- 20µg/ml flavonoid extract, Lane 4- Control.**

1.4. DISCUSSION

Protein profile obtained from SDS-PAGE analysis showed variation in the number of bands in protein of antibacterial treated bacterial cell, when compared with control and standard marker and it was supported by Piller et al, (2008) in their study on antibiotic- resistant bacteria inhibited by extracts from Brazilian marine sponger similar reports were made by Gilmour et al,(2000) and Shariff et al,(2001) in their work on honeybee as strong antibiotic against bacteria isolated from patients of burn-wound respectively.

1.5 CONCLUSION

The bioactive compound enters the membrane of the cells and inactivates the protein synthesis or activates the protein degradation. In conclusion it was evident that the glycosides and flavonoids interfere with the protein system at several different levels, thereby preventing the native protein structure from functioning as a proper template. Effect of flavonoids and glycosides from *C.coriaria* were found to be effective against *E.coli*, *S.aureus* and *K.pnuemoniae* at the concentration of 10-20 µg/ml. The identified compound is to proceed for the structural analysis and can be recommended in the pharmaceutical industry

Acknowledgements

We thank Tamil Nadu State Council for Science and Technology, DOTE Campus, Chennai – 600 021, India under Students Project Scheme – 2014 BS-17 for granting the fund and supporting this project.

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