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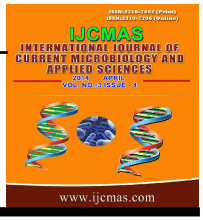

Original Research Article

Influence of flavonoids and glycosides from *Caesalpinia coriaria* (Jacq) wild as bactericidal compound

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A B S T R A C T

The scope of the present study was to identify the bioactive compounds and to investigate the mode of action as antibacterial compound from *Caesalpinia coriaria* (Jacq) Willd. 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 leakage of reducing sugar and proteins through the membrane. Based on the current findings, the action model of these two bioactive compounds results in the leakage of cellular materials. Hence the bioactive compounds enter the inner memebrane and inactivate the enzyme system (respiratory chain dehydrogenase) thus inhibiting the respiration and growth of cells. Thus the result suggests that *Caesalpinia coriaria* was a potential candidate plant for the management of organism for antibacterial activity.

Keywords

Antibacterial activity; *Caesalpinia coriaria;* flavonoids; glycosides; TLC-Thin Layer; Chromatography

Introduction

Plants used for traditional medicine contain wide range of compounds that can be used to treat both chronic and acute diseases. According to Diallo et al (1999), a vast knowledge of how to use the plants is still of great importance. According to World Health Organization (WHO), 80% of the world's population relies mainly on traditional therapies which use the plants extracts o their active substance (WHO, 1993). In India, around 17,000 species of higher plants, 7500 are known for their medicinal uses (Shiva, 1996). 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). A great structural diversity exists among antimicrobial phytocompounds (Cowan, 1999).

Microbial resistance to antibiotic has become a global concern (Westh *et al*., 2004). Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and Caffeic acids are common representatives of a wide group of phenylpropane-derived compounds which are in the highest oxidation state (Duke, 1985). The

mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Mason and Wasserman, 1987). Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection (Dixon *et al*., 1983). "Tannin" is a general descriptive name for a group of polymeric phenolic substance capable of tanning leather or precipitating gelatin from solution, a property known as astringency. Their molecular weights range from 500 to 3000, and they are found in almost every plant part: bark, wood, leaves, fruits, and roots (Scalbert, 1991). Tannins may be formed by condensations of flavones derivatives which have been transported to woody tissues of plants. Alternatively, tannins may be formed by polymerization of quinine units (Gessman, 1963). Condensed tannins have been determined to bind cell walls, of ruminal bacterial, preventing growth and protease activity (Jones *et al*., 1994).

The mechanism of antimicrobial action of plant secondary metabolites is not fully understood but several studies have been conducted in this direction. Flavonoids may act through inhibiting cytoplasmic membrane function as well as by inhibiting of DNA gyrase and β-hydroxyacyl-acyl carrier protein dehydratase activities (Cushnie and Lamb, 2005; Zhang et al., 2008) and inhibited the synthesis of DNA and RNA of *Vibrio harveyi* (Ulanowska et al., 2006). It has been suggested that terpenes promote membrane disruption; coumarins cause reduction in cell respiration and tannins act on microorganism membranes as well as bind to polysaccharides or enzymes promoting inactivation (Ya et L., 1988; Cowan, 1999).

Currently, plant products are considered to be important alternative sources of new antimicrobial drugs against antibiotic-resistant microorganisms (Schuenzal et al., 2002). According to this trend, the usage of natural compounds derived from plants for the prevention of pathogenic and spoilage microorganisms in food have been extensively reported (Rasooli., 2007). Membrane integrity is fundamental for the control of cytoplasmic pH in bacteria, which is essential for many physiological activities (Olson, 1993). The capacity of cells to maintain a pH gradient (higher pH inside than outside the cell) may also supply information about cellular viability (Chitarra et al., 2000).

Materials and Methods

Plant sample collection

The Sample leaves of *Caesalpinia coriaria* were collected form Captain Srinivasa Murti Drug and Ayurveda Research Institute, Chennai, Tamilnadu, India. The samples were allowed to dry under the shade completely. The leaves were ground into powder which was used for further extraction. The ethanol extract powder was defatted with petroleum ether (40-60°C). The extract was then percolated with methanol until exhaustion at 40^0C by rotary evaporator. The condensed was partition using ethylacetate. This ethylacetate extraction contained crude flavonoids (Amal et al., 2009). The ethanol extract powder were extracted three times with methanol at 25°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).

Effect of antimicrobial compound and its leakage in the membrane of pathogenic bacteria

To detect the leakage of reducing sugars and proteins through membrane, tubes containing MH broth with varying concentration of plant extract (10, 20, 30 & 40µl/ml) were inoculated with pathogenic bacteria cells at final concentration of 10^9 cfu/ml. Control experiments were conducted without antibacterial compound. The cultures were incubated at 37± 2°C with shaking at 150 rpm. One millilitre culture was sampled immediately after inoculation and after being treated for 24 h. They were centrifuged at 12,000 rpm, and then the concentrations of reducing sugars and proteins were determined (Miller, 1959; Bradford, 1976).

Assay of enzymatic activity respiratory chain dehydrogenase in pathogenic bacteria

The dehydrogenase activy was determined according to iodonitrotetrazolium chloride method (Iturriaga et al., 2001; Kim et al., 1994; Kim *et al*., 2009). MH medium, different concentration (10, 20, 30 & 40µl/ml) of antibacterial compound and the pathogenic bacteria cells were added into 10 ml cultures separately resulting in final concentrations of 10^8 CFU/ml pathogenic bacteria. Experiments were carried in absence of antibacterial compound, as the control. The pathogenic bacteria cells were boiled for 20 min to inactivate the enzymes completely the control (-), while the cells were not boiled and their enzymes maintained native activity as the control (+). Cultivatations were performed at 37± 2^0C with shaking at 150 rpm. One millilitre culture was sampled separately from the cultures and centrifuges at 12,000 rpm, then the supernatants were discarded and the bacteria washed by phosphate-buffered saline (PBS) twice and 0.9 ml was added to suspend the bacteria. INT solution (0.1 ml 0.5%) was added, the culture was incubated at 37^0C in dark for 15,30,45 & 60 minutes, and then 50 µl formaldehyde was added to terminate the reaction. The culture was centrifuged to collect the bacteria and 250 µl solutions of acetone and ethanol 1:1 in volume was used to distill the INF twice. The dehydrogenase activity was then calculated according to the maximum spectrophotometrically absorbance of INTF at 490 nm by spectrophotometer.

Results and Discussion

Effect of glycosides and flavonoids from *Caesalpinia coriaria* as leakage causing agent in the membrane of pathogenic bacteria

Presence of reducing sugar and protein were estimated in the phytocompounds treated on the bacterial broth cultures and the results predicted the leakage of cell membrane of pathogenic bacteria (*E.coli*, *Staphylococcus aureus, and Klebsiella pneumoniae*). The O.D values obtained by estimation of reducing sugar was referred with the standard graph of glucose. Initially the presence of reducing sugar was absent in the control indicating no leakage in cell membrane, while the leakage of membrane was noticed by estimating the reducing sugar in the

bacterial cultures treated with glycoside and flavonoid compound of *Caesalpinia coriaria.* The values ranged from 22-60 and 32.66 -70 μg/ml of carbohydrates at zero and 24 hours incuabation of *E.coli*, 11- 41.66 and 16.33-54 μg/ml of carbohydrates at zero and 24 hours incuabation of *Staphylococcus aureus*. 22.54 – 31.73 and 31-72 μg/ml of carbohydrates at zero and 24 hours incubation of *Klebsiella pneumoniae* as per the cultures treated with glycoside and flavonoid compound obtained from *Caesalpinia coriaria* (Graph 1,2).

The presence of protein was also detected which in turn revealed the leakage form cell membrane of the pathogenic bacteria. The OD values obtained were referred with standard graph of BSA. Initially the protein estimated was higher in all bacterial cultures when compared to control. It implied that the antibacterial compounds were potent against the pathogen even at initial stage. After 24 hours of treatment the amount of protein estimated was much higher than the initial value. The amount of protein estimated at zero and 24 hours ranged from 22-61.33 and 32.33 -61.66 μg/ml of proteins at zero and 24 hours incubation of *E.coli*, 32.33-62.33 and 43.66- 62 μg/move proteins at zero and 24 hours incubation of *Staphylococcus aureus*. 11.33-47.33 and 26.66 – 54.66μg/ml of proteins at zero and 24 hours incubation of *Klebsiella pneumoniae* as per the cultures treated with glycoside and flavonoid compound obtained from *Caesalpinia coriaria*(Graph 3,4).

This study revealed that higher the concentration of antibacterial compound and longer the duration of inhibition, the higher might be the leakage from the membrane. However, the amount of reducing sugar and protein estimated were comparatively higher in glycosides and flavonoids compounds of *Caesalpinia coriaria* treated *Staphylococcus aureus* cells.

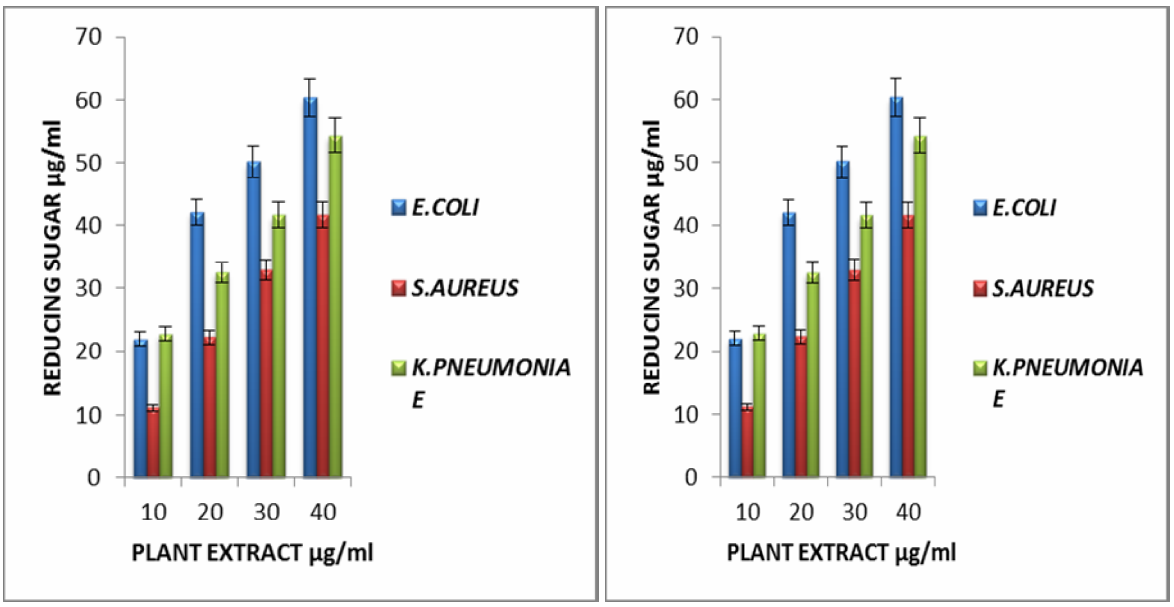
Effect of antibacterials on the respiratory chain debydrogenase of pathogenic bacteria

Effect of glycosides and flavonoids of *C.coriaria* on respiratory chain dehydrogenase of *E.coli*, S.*aureus, K.pneumoniae* were experimented and observed for their inhibitory activity. Accordingly a continuous rise in the enzymatic activity was recorded with increase in incubation time, in the positive control, whereas there was no / less enzymatic activity in the negative control of the experiment. The enzyme activity of *E.coli, S.aureus, K.pneunomiae* treated with 10, 20, 30, 40 μg/ml of glycosides and flavonoid compound of *C.coriaria* showed less variation when compared to positive control (Graph 5, 6, 7, 8, 9, 10) and in contrast it was even higher than the positive control.

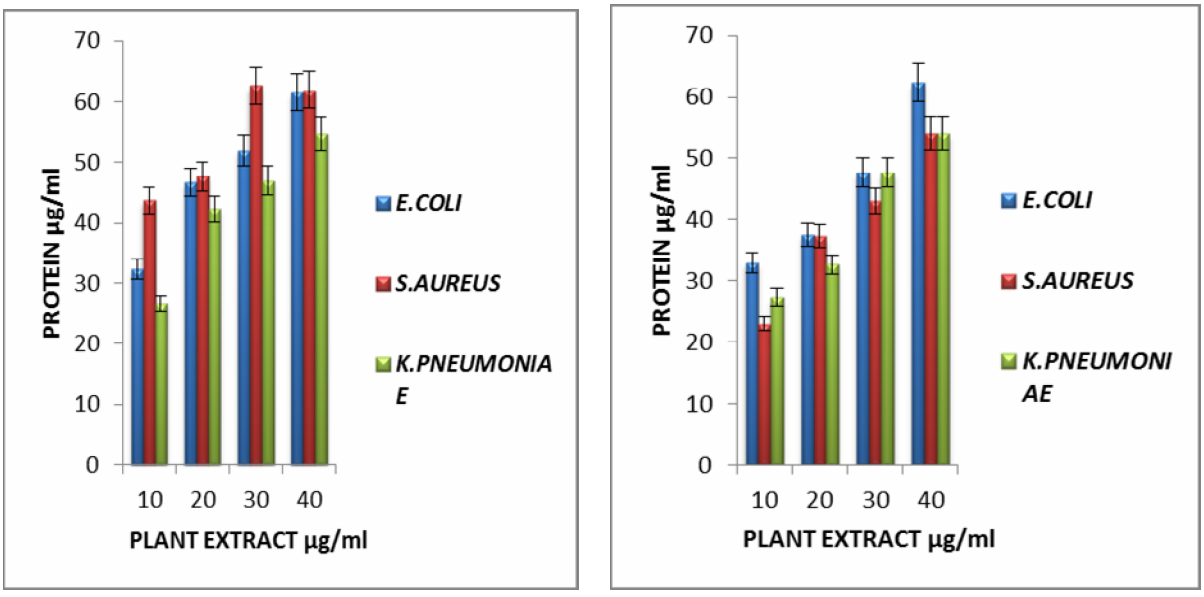
Gradually the activity of the enzyme decreased with increased concentration of the antibacterial. It was also observed that 20 μg/ml concentrations, the enzyme activity was almost increasing in all the bacterial cells and more effective in the degradation of the enzyme. Thus the result that the activity of respiratory chain dehydrogenase of all the pathogenic bacteria was inhibited by the antibacterial obtained from *C.coriara*.

The estimation of reducing sugar and proteins in the bacterial culture medium treated with the varying concentrations

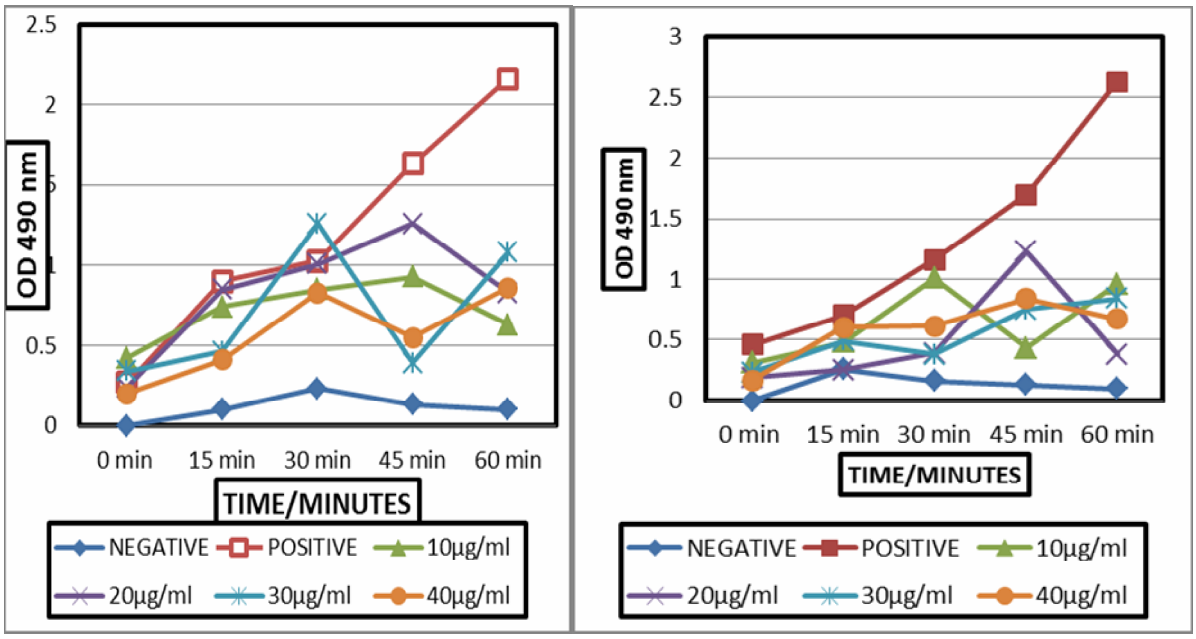
Graph 1, 2 Effect of Glycoside and flavonoids as antibacterial compound on pathogenic organisms at 24 hours (Reducing Sugar)



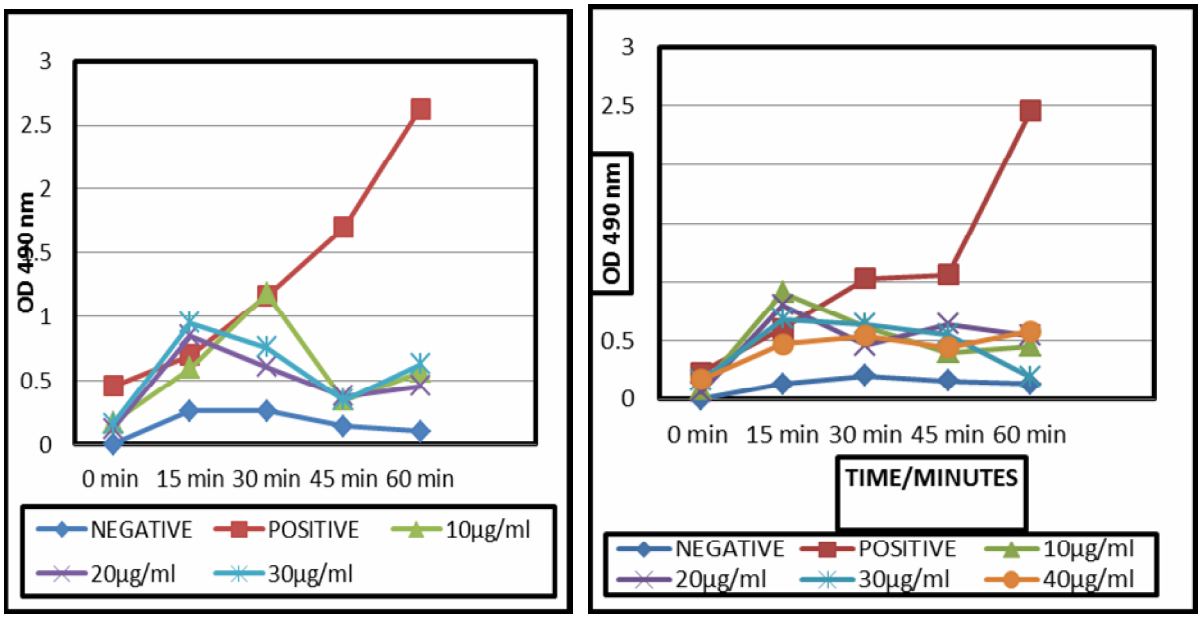
Graph 3, 4 Effect of Glycosides and flavonoids as antibacterial compound on pathogenic organisms at 24 hours (Protein)



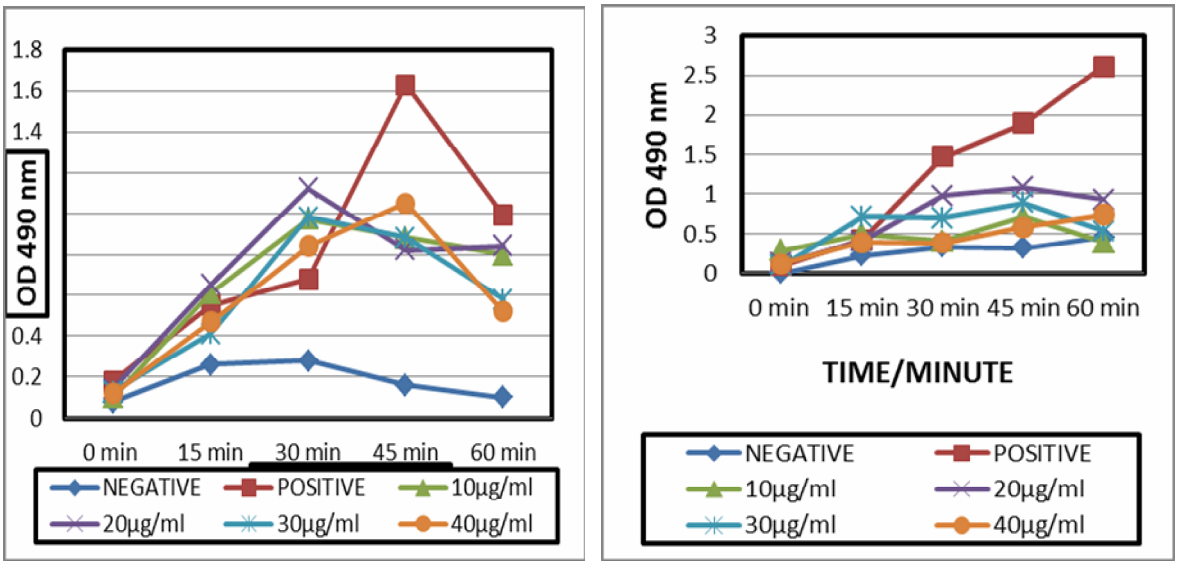
Graph 5, 6 Effect of Glycosides and Flavonoids on respiratory chain d ehydrogenase against *E.coli*



Graph 7, 8 Effect of Glycosides and Flavonoids on respiratory chain dehydrogenase against *Staphylococcus aureus*



Graph 9, 10 Effect of Glycosides and Flavonoids on respiratory chain dehydrogenase against *Klebsiella pneumoniae*



(10, 20, 30, 40µg/ml) of the antibacterial, proved the leakage in the cell membrane of the pathogenic bacteria. This may be due to the inhibitory activity of antibacterial compounds of *Caesalpinia coriaria* that might have induced the release of reducing sugars and proteins to the growth medium. The membrane is essential for the survival of bacteria, loss or damage of this layer destroys the rigidly of the bacterial cell wall, resulting in death. These antibacterial might have caused disorganization in the membrane profile and thereby causing leakage. A research team that had previously found that flavonoids (sophoraflavanone G to have intensive antibacterial activity against *Streptococci* and MRSA (Sato, Tsuchiya, Sakagami.,1995) recently reported attempts to elucidate the mechanism of action of this flavanone (Tsuchiya, 2000). The antibacterial activity was suggested to support the theory that sophoflavanone G demonstrates by reducing membrane fluidity of bacterial cells. Ikigai *et al* discussed that catechins induced leakage of small molecules from the bacterial cells. Hence they concluded that catechins primarily act on and damage bacterial membranes. This damage occurred by two ways. First Catechins may perturb the lipid bilayers by directly penetrating them and disrupting the barrier function. Alternatively, catechins may cause membrane fusion, a process that results in leakage of intramembranous materials. This work constitutes a substantial advance in the development of catechins as antibacterial agents and lends support to Ikigai's work that catechins act on and damage bacterial membrane.

The result of this experiment showed that the activity of respiratory chain dehydrogenase in the pathogenic bacteria might be inhibited by the antibacterial, the lower the enzyme activity was evident. It is assumed that the antibacterial may break through barrier of outer or inner membrane permeability, peptidoglycan or periplasm dehydrogenase and eventually

inhibit the respiratory physiology. The (2010) in their study on the antibacterial activity. Haraguchi et al carried out an investigation into the antibacterial mode of action of two retrochalocones form the roots of *Glycurrhi zainflate* (Li *et al*., 1993). These flavonoids demonstrated inhibitory activity against *S.aureus* and *Micrococcus luteus* but not against *E.coli* and in preliminary studies these flavonoids inhibit the macromolecules (DNA,RNA and protein). The group hypothesized that the licohacones may be interfering with energy metabolism in a similar way to respiratory inhibition, since energy is required for active uptake of various metabolites and for biosynthesis of macromolecules. They also reported that licohacones effectively inhibited NADH-cytochrome C reductase and dehydrogenase (Haraguchi, *et al*., 1998).

In the current findings, the action model of these two bioactive compounds have been described as they break through the permeability of the outer membrane initially resulting in the leakage of cellular materials. In addition to the leakage of cellular materials, the phytochemicals (Glycosides and flavonoids) also enter the inner membrane and inactivate the enzyme system – dehydrogenase thus inhibiting the respiration and growth of pathogenic microorganisms.

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