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Steroidal Ester of *Cassia nigricans* Have Antibacterial Potential

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Plant derived natural products are not only part of traditional medicines but are also essential part of many modern drugs and used to treat various human ailments e.g., cancer, viral, microbial infection, inflammation, metabolic disorders, neurodegenerative diseases etc. (Potterat and Hamburger, 2008; Sohail *et al*., 2011; Karim *et al*., 2011). Although many other organisms (animals and microbes) are also used to derive natural drugs, plants act as a major source for the development of modern drugs. The plant derived drugs have gains global importance due to their multiple therapeutic effects; it is an industry of more than $18 billion (Saklani and Kutty, 2008). But yet only 10-15% of total plant diversity is explored for their medicinal importance and knowledge about them can be increased by studying traditional medicinal plants via modern techniques. Caesalpinioideae is one of the important subfamily of Leguminosae; its members are traditionally used to fulfill nutritional requirements in many areas (Dansi *et al*., 2008). *Cassia* is a medicinally important and genetically diversified genus of Caesalpinioideae, this genetic diversity make its plants adapted to number of environments and is responsible for their different growth habits (Mohanty *et al*., 2010). Its plants have anti-parasitic, antidiabetic, antioxidant, skin protecting etc. effects (Mia *et al*., 2009; Nirmala *et al*., 2008). These activities are due to their phytochemical composition and *Cassia* plants have many flavonoids, phenols etc. which may also responsible for their antibacterial activities (Bahorun *et al*., 2005; Hazni *et al*., 2008). Antibacterial or antiparasitic activities of plants can help in lowering the potential threat caused by bacterial infections. Despite of progress in prevention and inhibition of infectious diseases, bacterial disease are re-emerging in many areas due to their developed resistance against synthetic antimicrobial drugs (Chug, 2008). For example *Staphylococcus aureus* has developed resistance against many antibiotics and now emerged as methicillin-resistant *S. aureus* infections, in United States its infection rate is 31.8 per 100 000 population (Klevens *et al*., 2007). It can be easily transmitted through adjacent environments of community and hospitals, thus bacterial infections needs special attention. Hence plants antimicrobial or antiparasitic activities should be studied as they may hinder the prevalence of bacterial infections.

Cassia nigricans is an important subject of medicinal care, as it is a potent source of flavonoids, anthraquinones and esters; it showed many curative properties (Ayo, 2010). Like antiparasitic, anti-inflammatory, anti-ulcer and it can operate many other digestive system related disorders. Its antibacterial activity was examined by Ayo *et al*. (2009); they tested its leaves extracts against 10 pathogenic bacterial strains. These strains were *S. aureus, Streptococcus pyogenes, Corynebacterium pyogenes, Bacillus subtilis, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Neisseria gonorrhoeae* and *Klebsiella pneumoniae*. They also identified the active compound for antibacterial activity through Gas Chromatography/Mass Spectrometry (GC/MS). According to them, methanolic extracts of *C. nigricans* gave white amorphous powder, which was a steroidal ester. It was 22 carbon (C) compound of ring structure, which contained a hydroxyl, carbonyl and an ester group (-OCH CH). It gave 22 ^{13}C-NMR (Nuclear Magnetic Resonance) signals, out of which 3 were of methyl (CH_3) group. The reminder C atoms showed the grouping depending upon the same peak in ^{13}C-NMR, among which carbonyl and ester group did not make any group; they gave carbon signals at 173.90 and 60.1 ppm, respectively. The founders named the compound as hydroxyestranic acid ethyl ester and found it as potent inhibitor of many bacteria. They grew the bacteria in agar medium and supplied the different concentration of steroidal ester, this compound showed the antibacterial activity in concentration dependant manner. Its antimicrobial activity increased with an increase in extracts concentration, as its 1×10^3 μg mL^{-1} concentration was ineffective against all bacteria while its 5×10^3 μg mL^{-1} maximally inhibited the bacterial growth. These applied concentrations showed different antibacterial tendencies, as its 2×10^3 was only slightly effective against 3 bacterial, while 3×10^3 μg mL^{-1} concentration was slightly/strongly effective against 8 strains. Whereas 4×10^3 strongly inhibited 8 and 5×10^3 μg mL^{-1} strongly inhibited the 10 bacterial strains. Moreover the 4×10^3 μg mL^{-1} showed slight inhibition of *B. subtilis* and *K. pneumoniae* strains. Hence 4×10^3 and 5×10^3 μg mL^{-1} were able to inhibit all studied strains, but strong inhibition of bacteria was the attribute of 5×10^3 μg mL^{-1} concentration. While 4×10^3 μg mL^{-1} could

was the minimum required concentration to inhibit all bacteria. Furthermore, steroidal ester followed a different inhibition zone diameter against bacteria. Its maximum diameter was 48 mm against *S. aureus*, while minimum inhibition diameter was 18 mm against *B. subtilis*. For all other bacterial strains its inhibition zone diameter was more than 20 mm. Thus considering inhibition zone and minimum required concentration parameters, this can be said that steroidal ester extracted from *C. nigricans* was maximum effective against *S. aureus*. Since, its 3×10^{3} μg mL^{-1} concentration strongly inhibited *S. aureus* growth within an inhibition diameter of 48 mm. So *C. nigricans* extracted steroidal ester was effective against many bacterial strains and its most promising activity was obtained at 5×10^{3} μg mL^{-1} concentration.

Nowadays plants are considered as a significant part of drug industry because of their potential therapeutic activities. Their use can inhibit the invasion of infectious agents and their chemical constituent's incorporation may enable the researchers to develop strong antibacterial drugs. Ayo *et al.* (2009) found valuable antibacterial steroidal ester (hydroxyestranic acid ethyl ester) in the methanolic extracts of *C. nigricans*. It inhibited the growth of number of bacterial strains and can be used to form antibacterial drug. Thus more investigation on *C. nigricans* and steroidal ester may provide a competent antibacterial drug.

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