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INVESTIGATION OF THE EFFECT OF BIOACTIVE COMPOUND ISOLATED FROM SEEDS OF *PSORALEA CORYLIFOLIA* L. ON DNA OF PATHOGENIC BACTERIA

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ABSTRACT

Four species of bacteria viz., *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Xanthomonas* sp was treated with 250, 500, 1000 and 1500ppm concentration of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one isolated from the seeds of *Psoralea corylifolia* for 24 hours and DNA bands was observed in all the treated bacteria by agarose gel electrophoresis. In control without any treatment of bioactive compound, *E. coli, S. aureus, B. subtilis* and *Xanthomonas* sp. recorded 12, 14, 14 and 13 DNA bands respectively. In treated, *E. coli,* recorded 14, 15, 16 and 16 at 250, 500, 1000 and 1500ppm concentration, *S. aureus* recorded 14, 15, 16 and 16, *B. subtilis* recorded 15, 16, 17, 17 and *Xanthomonas* sp. recorded 13, 14, 15 and 16 at 250, 500, 1000 and 1500ppm concentration of the bioactive compound respectively.

KEYWORDS: *Psoralea corylifolia*, bioactive compound, Bacteria, DNA.

INTRODUCTION: Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases (Mothana et al., 2010). Approximately 60%-80% of the world's population still relies on traditional medicine for the treatment of common illnesses (WHO, 2007; Dev, 2010; Schuster and Wolber., 2010). Emergence of multiple drug resistant strains of microorganisms due to indiscriminate use of antibiotics to treat infectious diseases has generated a renewed interest in herbal medicine. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectra of untreatable bacterial infections and adds urgency to the search for new infection combating strategies and new effective therapeutic agents (Penchala, 2013). The world has been facing a big challenge of antimicrobial resistance that affects the efforts under taken to prevent and control infectious diseases caused by bacteria (Elibariki et al., 2016, WHO, 2014). Medicinal plants are used on a large scale in medicine against drug-resistant bacteria, which are considered one of the most important reasons for the lack of success of treatment in infectious diseases (Ayman Al-Mariri and Mazen et al., 2014). Herbal medicine has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources as a good choice, because these natural resources have ordinarily fewer side effects (Zargari, 1996). The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new antimicrobial compounds from higher plants particularly medicinal plants (Duraipandiyan, 2006). Considering the effect of synthetic antibiotics, in the present study, bioactive compound isolated from the seeds of *Psoralea corylifolia* was tested against four bacterial species to identify the mode of action DNA sequence of the bacteria.

MATERIALS AND METHODS

Plant Material

Fresh, dried, healthy seeds of *P.corylifolia* were collected from seed market, Mysore. The seeds were washed thoroughly with running tap water for 2-3 times and once in sterile distilled water. Then the seeds were air dried at room temperature on a sterile blotter under shade (Verma and Dohroo, 2003).

Isolation of the Bioactive compound

Twenty five grams of the powdered seeds of *P. corylifolia* were extracted from petroleum ether and methanol mixture in the ratio 9:1(v/v) by refluxing for eight hours at 50- 60^0 C in a Soxhlet apparatus. The excess of solvent was removed by distillation under

reduced pressure. The concentrated extract was cooled for 48hours at 5° C to obtain pure compound as crystals. The crystals was dissolved in chloroform and eluted by silica gel (6x120 mesh size) column chromatography (2x40 cms) using n-hexane: chloroform (9:1) (v/v). The purity of the bioactive compound was confirmed by TLC and R_f value was determined. Yield and Melting point of the bioactive compound was determined following the procedure of Al-Fatimi et al., 2006. For structural elucidation, the bioactive compound was subjected to IR (Infra red) spectrometer analysis,^{1}H- NMR, ^{13}C- NMR (Al-Fatimi et al., 2006) and Gas Chromatograph- Mass Spectrum (GC- MS) analysis(Yanez et al.,2005).

Test organisms

Four different bacteria namely *Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram Positive), *Bacillus subtilis* (Gram Positive) and *Xanthomonas* sp (Gram negative). isolated from soil were subcultured on nutrient agar medium. After 24 hours of incubation at 37°C the cultures were preserved aseptically in lower temperature until further use.

Preparation of Inoculum and treatment of bacteria with bioactive compound

A loopful of all the test bacteria were taken and sub-cultured in test tube containing 10 ml of nutrient broth. The test tubes were incubated at 37°C for 24 hours. The broth was standardized using sterile normal saline to obtain a population of 10 cfu/ml. One ml of each species of bacteria (*E. coli*, *S.aureus*, *B. subtilis* and *Xanthomonas* sp.) was added to eight conical flask filled with 150ml of nutrient broth aseptically. Different concentration of the bioactive compound (2H-Furo[2,3-H]-1-benzopyran-2-one) 250, 500, 1000 and 1500ppm was added to four conical flask and one control will be maintained for each concentration without bioactive active compound. This was repeated for all the test bacteria. The treated and control conical flask was incubated for 24 hours at 37°C. After incubation, the inoculated broth with bacteria and bioactive compound, control broth with only bacteria and without bioactive compound was subjected for centrifugation. The residue which contains only bacteria both in treatment and control was collected and subjected for DNA analysis.

Extraction of DNA and agarose gel electrophoresis

2 ml overnight culture of bacteria was taken and the cells are harvested by centrifugation for 10 minutes. 875 µl of TE buffer is added to the cell pellet and the cells are resuspended in the buffer by gentle mixing. 100 µl of 10% SDS and 5 µl of Proteinase K are added to the cells. The above mixture is mixed well and incubated at 37º C for an hour in an incubator. 1 ml of phenol-chloroform mixture is added to the contents, mixed well by inverting and incubated at room temperature for 5 minutes. The contents are centrifuged at 10,000 rpm for 10 minutes at 4ºC. The highly viscous jelly like supernatant is collected using cut tips and is transferred to a fresh tube. The process is repeated once again with phenol-chloroform mixture and the supernatant is collected in a fresh tube. 100 µl of 5M sodium acetate is added to the contents and is mixed gently. 2 ml of isopropanol is added and mixed gently by inversion till white strands of DNA precipitates out. The contents are centrifuged at 5,000 rpm for 10 minutes. The supernatant is removed and 1ml of 70% ethanol is added. The above contents are centrifuged at 5,000 rpm for 10 minutes. After air drying for 5 minutes, 200µl of TE buffer or distilled water is added. 10 µl of DNA sample is taken and is diluted to 1 or 2 ml with distilled water. The concentration of DNA is determined using a spectrophotometer at 260/280 nm. The remaining samples are stored for further experiments. This protocol was repeated for all the test bacteria (Sadasivam, 1996). The obtained DNA from all the test bacteria was subjected for Agarose gel electrophoresis for observations of number of bands obtained comparing with control.

RESULT AND DISCUSSION

Isolation of Bioactive Compound: This was obtained as white crystalline flakes. Recrystallisation was done using methanol, M.P.138^{0}C, R_f 0.47 in the UV maximum of 242,248,297nm. Yield, 5mg/250gms of seed. The IR spectrum of 2H-Furo[2,3-H]-1-benzopyran-2-one showed absorption band in the region of 1652.9 cm^{-1} for-C-O- stretching. Further absorption bands at 1550.7cm^{-1} and 1454cm^{-1} were due to the presence of coumarin ring oxygen and furan ring oxygen respectively. In ^{1}H- NMR spectra, the signal due to C_3 -H and C_4-H of coumarin appeared at δ 6.41 as doublet and at δ 7.81 as singlet. The aromatic protons (C_7 + C_8) are mingled together and appeared at δ 7.7 as multiplet. The signal due to the $C_{3'}$ and $C_{2'}$ protons appeared at 6.84 and 7.49 as doublet. ^{13}C- NMR data of the bioactive compound 2H-Furo [2,3-H]-1-benzopyran-2-one are recorded as solvent $CDCl_3$, δ 146.85(C_2), δ 143.8 (C_4), δ 119.77(C_3) , δ 115.5(C_7) , δ 114.76(C_8) , δ_2 106.3($C_{3'}$) and δ 99.86 ($C_{2'}$) atom. In GC-MS, the molecular ion peak at M/Z 186.17 consistent of molecular formula $C_{11}H_6O_3$. The peak at M/Z 158 was due to the formation of coumarin cation. The recorded chromatogram of plot matches with chromatogram of known compound 2H-Furo[2,3-H]-1-benzopyran-2-one(Figure 1).

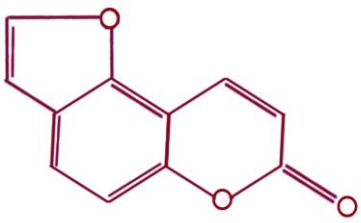

Figure 1: Molecular structure of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one isolated from the seeds of *Psoralea corylifolia*

Extraction of DNA and agarose gel electrophoresis

Among the four bacteria tested at 250, 500, 1000 and 1500ppm concentration of the bioactive compound for 24 hours of duration , *E. coli* showed 12 DNA bands in

control and at 250ppm concentration, it was recorded 14 bands. At 500ppm, it was recorded 15 bands, at 1000ppm it was recorded 16 bands and at 1500ppm concentration, it was recorded 16 bands. *S.aureus* recorded 14 DNA bands in control and at 250, 500, 1000 and 1500ppm concentration, it was recorded 14, 15, 16 and 16 band respectively. In *B. subtilis* at 250ppm, it showed 15 bands and at 500ppm and 1000ppm concentration, it was recorded 16 and 17 bands and at 1000ppm concentration, it was recorded 17 DNA bands. *Xanthomonas* sp recorded 13 bands in control and at 250ppm and 500ppm, it was recorded 13 and 14 bands and at 1000 and 1500ppm concentration, the number of DNA bands was 15 and 15 respectively (Figure 2).

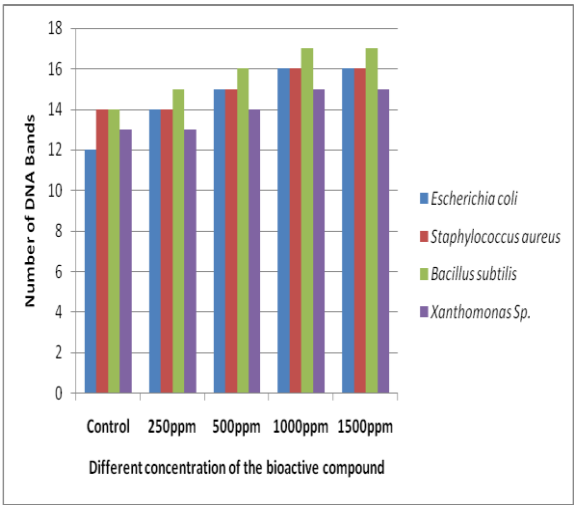

Figure 2: Comparative analysis of DNA bands treated with bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one isolated from the seeds of *Psoralea corylifolia*

From the above experiment it was observed that, in all the species of test bacteria when treated with different concentration of the bioactive compound, there was an increase in number of DNA bands in treated compared to control. Hence, it can be predicted that, the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one breaks the DNA sequence. Hence if DNA sequence was fragmented, the disease causing ability of the pathogenic bacteria may be lost. Hence, the bioactive compound isolated from *P. corylifolia* which is eco-friendly can be used for different microorganisms which cause different diseases.

CONCLUSION

From the above observation, it can be concluded that, a further investigation is needed to identify the mode of action and to determine which functional gene sequence in pathogenic bacteria is effected by bioactive compound.

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REFERENCE

1. Mothana RAA, Abdo SAA, Hasson SS, Althawab FMN, Alaghbari SAZ, Lindequist U. (Antimicrobial, antioxidant and cytotoxic activities and phytochemical screening of some Yemeni medicinal plants). Evidence Based Complementary Alternative Medicine, 2010; 7(3): 323-330.
2. WHO. WHO country cooperation strategy 2006-2011. India 2007. Supplement on traditional medicine. New Delhi: WHO, 2007.
3. DEV S. (Impact of natural products in modern drug development). Indian Journal of Experimental Biology, 2010; 48(3): 191-198
4. Schuster D, Wolber G. (Identification of bioactive natural products by pharmacophore-based virtual screening). Current Pharmaceutical Design, 2010; 16(15): 1666-1681.
5. Penchala S, Talari S, Guguloth V, Azmeera R, Nanna RS. (Phytochemical Screening and Antimicrobial Activity of Leaf Extract of Wrightia tomentosa). International Research Journal of Biological Sciences, 2013; 2(3): 23-27.
6. Elibariki EK, Cecilia L, Musa C.(Evaluation of Antibacterial Activity of Five Selected Medicinal Plants in Tanzania against Gram Negative Bacteria). European Journal of Medicinal Plants, 2016; 12(2): 1-7.
7. WHO. (Antimicrobial resistance: Global report on surveillance). World Health Organization; 2014.
8. Ayman Al-Mariri, Mazen S. (In Vitro Antibacterial Activity of Several Plant Extracts and Oils against Some Gram-Negative Bacteria). Iran J Med Sci., 2014; 39(1): 36–43.
9. Zargari A. (Medicinal Plants). 6th ed. Tehran: Tehran University Publication, 1997; 1: 249–265.
10. Duraipandiyan V, Ayyanar M, Ignacimuthu S. (Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamilnadu, India.).BMC Complimentary and alternative medicine, 2006; 6: 35.
11. Verma S ,Dohroo NP. (Evaluation of botanicals in vitro against Fusarium oxysporum f. sp. Pisi causing wilt of pea). Plant Disease Research, 2003; 18(2): 131-134.
12. Al-Fatimi MAA, Julich WD, Jansen R, Lindequist U. (Bioactive components of the Traditionally used Mushrooms Podaxis pistillaris). Evid. Based Complement Alternat. Med., 2006; 3(1): 87-92.
13. Yanez C, Pezoa J, Rodriguez M, Nunez-Vergara LJ, Squella JA.(Voltammeric Behavior of a 4-Nitroimidazole derivative Nitro radical Anion formation and stability). Journal of Electrochem. Soc, 2005; 152(6): 46-51.
14. Sadasivam S: Biochemistry - New Age International, 1996; 256.