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DRUG DISCOVERY

Analyze the efficacy of Opuntia fragilis against bacteria and its effects on diabetic protein butyryl cholinesterase

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ABSTRACT

Opuntia commonly known as prickly pears is used by humans as therapeutic medicine for past centuries. However, there is no report on the anti-microbial activity of the species *Opuntia fragilis*. This study concentrates on the anti-microbial activity against Gram positive and negative bacteria by disc diffusion assay method. The methanolic, butanolic and water extracts of the plant were screened against *E.coli, Shigella spp., P.vulgaris* and *S. aureus*. The results showed that methanolic extract exhibit remarkable inhibition than butanol. No activity was observed with water fractions.

Keywords: *Opuntia fragilis*; Disc diffusion assay; Butyryl cholinesterase; Anti-microbial activity; Diabetics.

Abbreviations: PDB - Protein data bank; OD - Optical density; 3, 4-DMPEA - 3, 4-Dimethoxyphenethylamine. .

1. INTRODUCTION

Natural products perform various functions, and many of them have interesting and useful biological activities. There are more than 35,000 plant species being used in various human cultures around the world for the medicinal purpose (Joy et al., 2001). There are about 45,000 plant species in India, with concentrated hot-spots in the region of Eastern-Himalayas, Western Ghats and Andaman-Nicobar islands. The officially documented plants with medicinal potential are 3,000, but traditional practitioners use more than 6,000. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world. In rural India, 70% of the population is dependent on traditional system of medicine, the Ayurveda (Rice Evans et al., 1997).

Researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against cancer as well as viral and microbial infections (Ames et al., 1993). This study reports a screening program of methanolic, ethanolic, butanolic and water extracts of *Opuntia fragilis* for their anti-microbial properties. There was no previous reports anti-bacterial study on Cactus plant especially in *Opuntia fragilis* species. Opuntia, also known as nopales or paddle cactus is a genus in the cactus family, Cactaceae. Various species of *Opuntia* have yielded flavanoids, lactones, terpenoids and alkaloids. Betalain pigments act as anti-oxidants (Peterson & Dwyer, 1998). Opuntia products are used in cosmetics, lotions, soaps and shampoos (Hegwood, 1990). The objective of this study is to analyze the efficacy of *Opuntia fragilis* against bacteria also against the diabetic protein by in-silico method.

Betalain:
Betalains are a class of red and yellow indole-derived pigments found in plants of the Caryophyllales, where they replace anthocyanin pigments. Betalains also occur in some higher order fungi.

2. MATERIALS AND METHODS

2.1. Collection of plant material

The fresh leaves of *Opuntia fragilis* were collected from Puthur, Villupuram District of Tamil Nadu.

2.2. Extraction of plant material

1 gm of fresh leaves were washed and ground to fine paste using various solvents such as methanol, butanol and water by using a mortar and pestle. The ground samples were kept for 1 day at room temperature. The solvent containing extracts were centrifuged at 8,000 rpm for 5 minutes. The filter was collected and used for further experiments.

2.3. Microbial culture collection

Bacterial stains used in this study (*E.coli, Shigella spp., P.vulgaris and S. aureus*) were collected from microbiological laboratories, Chennai. The collected bacterial stains were cultivated in nutrient agar medium and maintained on nutrient agar slant.

2.4. Anti-bacterial activity assay

Anti-bacterial activity was checked by agar disc diffusion assay method by using methanolic, butanolic and water extracts of *Opuntia fragilis* leaf. 18 petri dishes containing 20 ml of sterile nutrient agar media was prepared. Among that 15 plates were used for tests and 3 for control. The 15 petri dishes were marked for each bacterium (5 plates) from 100 µl to 500 µl respectively. The microbes were swapped on each petri plate and wait for few minutes. Using the gel punch 3 wells was cut on the surface of the agar plates. The extracts were load on the well with the concentration ranging from 100 µl to 500 µl. The control plate was loaded with

BUTYRYLCHOLINESTERASE

Butyrylcholinesterase (also known as pseudocholinesterase, plasma cholinesterase, BCHE, or BuChE) is a non-specific cholinesterase enzyme that hydrolyses many different choline esters. In humans, it is found primarily in the liver and is encoded by the *BCHE* gene. It is very similar to the neuronal acetylcholinesterase, which is also known as RBC or erythrocyte cholinesterase. The term "serum cholinesterase" is generally used in reference to a clinical test that reflects levels of both of these enzymes in the blood. Butyrylcholine is a synthetic compound and does not occur in the body naturally. It is used as a tool to distinguish between acetyl- and butyrylcholinesterase.

John Varghese et al.
Analyze the efficacy of Opuntia fragilis against bacteria and its effects on diabetic protein butyryl cholinesterase,
Drug discovery, 2012, 1(2), 33-35,

Figure 1

Minimal inhibitory concentration

Figure 2

Plate showing antimicrobial activity with various concentrations of plant extracts

Figure 3

The zone of inhibition against bacteria using methanol, ethanol and water extract

Lincomycin:
Lincomycin is a lincosamide antibiotic that comes from the Opuntia fragilis. It has been structurally modified by thionyl chloride to its more commonly known 7-chloro-7-deoxy derivative, clindamycin.

Minimal inhibitory concentration:

In microbiology, minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents.

standard methanol, butanol and water. The plates are kept for incubation overnight to observe the results. The above step is repeated for 3 times to get accurate result.

2.5. Minimal inhibitory concentration assay

The ethanol, methanol, butanol and water extracts of *Opuntia fragilis* plant were tested with 5 different concentrations against the microbes. 20 boiling tubes containing 5 ml of sterilized nutrient broth is taken and was inoculated with microbes, each in 5 tubes. The appropriate concentration of the extract was pipette on to the test tubes from 100 µl to 500 µl (Fig 1). The tubes then incubated at 37°C for 16 to 18 hrs. The activity was evaluated by the OD (optical density) value taken using the UV

Figure 4

No inhibition against bacteria using water extract

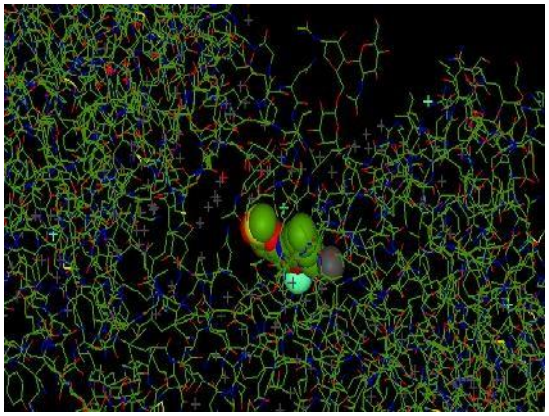

Figure 5

The docking of the ligand with the receptor molecule

spectrophotometer. All tests were repeated 3 times to minimize test error.

2.6. In-silico analysis

The molecule butyryl cholinesterase is responsible for causing diabetics in human. The crystal structure of fully glycosylated human butyryl cholinesterase retived from protein data bank (PDB) and the ID is 4AQD. The compound Lincomycin from Opuntia fragilis has the capability to treat the diabetes. The structure of the compound Lincomycin was collected from PUPCHEM, and the molecular weight is 406.537. The molecular formula is $C_{18}H_{34}N_2O_6S$. The docking experiments were performed to check the efficacy of the ligand (Lincomycin) to the receptor (butyryl cholinesterase).

3. RESULTS AND DISCUSSION

This study reports the anti-bacterial activity of Cactus plants (*Opuntia fragilis*). The extracts were checked against *E.coli, Shigella sp., P. vulgaris* and *S.aureus* (Fig.2). The extracts showeds activity against the all the organisms tested in the study. Among the investigated extracts the methanol fractions exhibited the highest anti-bacterial effect followed by butanol extracts (Fig.3). Water extracts was showed no inhibition. The most pronounced activity was shown (Table 1-3) by methanol extract (inhibition zone 12.8 mm against *E.coli* at the concentration of 500 µl). The butanol extract shows inhibition zone 11.3 mm against *E.coli* at the concentration of 500 µl. The methanol extract of *Opuntia fragilis* had a remarked sensitivity towards *S. aureus* and *Shigella* with inhibition zones 11.8 and 12.8 mm at the concentration of 500 µl respectively.

The butanol fraction of *Opuntia fragilis* showed significant anti-microbial activity against *S. aureus* and *Shigella* with inhibition zones 11.0 and 11.9 mm at the concentration of 500 µl respectively. The water fraction showed no inhibition against any of organisms tested in this

John Varghese et al.
Analyze the efficacy of Opuntia fragilis against bacteria and its effects on diabetic protein butyryl cholinesterase,
Drug discovery, 2012, 1(2), 33-35,

Table 1 Effect of plant extracts against *E.coli*

Plant Extracts	Concentration of plant extracts in µl (Zone of inhibition in mm)				
	100 µl	200 µl	300 µl	400 µl	500 µl
Methanol	11.2mm	11.3mm	11.7 mm	12.3 mm	12.8 mm
Butanol	10.3mm	10.7mm	10.95 mm	11.05 mm	11.30 mm
Water	0	0	0	0	0

Table 2 Effect of plant extracts against *Shigella sp.*

Plant Extracts	Concentration of plant extracts in µl (Zone of inhibition in mm)				
	100 µl	200 µl	300 µl	400 µl	500 µl
Methanol	11.2mm	11.5mm	11.8 mm	12.4 mm	12.8 mm
Butanol	11.0mm	11.15mm	11.3 mm	11.5 mm	11.9 mm
Water	0	0	0	0	0

Table 3 Effect of plant extracts against *Staphylococcus aureus*

Plant Extracts	Concentration of plant extracts in µl (Zone of inhibition in mm)				
	100 µl	200 µl	300 µl	400 µl	500 µl
Methanol	10.8mm	11.03mm	11.2 mm	11.6 mm	11.8 mm
Butanol	2.0mm	10.10mm	10.3 mm	10.5 mm	11.0 mm
Water	0	0	0	0	0

Table 4 Minimal inhibitory concentration

Plant Extracts	Concentration of plant extracts in µl (Zone of inhibition in mm)				
	100 µl	200 µl	300 µl	400 µl	500 µl
Methanol	1.052	0.760	0.256	0.010	0.001
Butanol	1.089	0.914	0.374	0.016	0.010

study even at the maximum concentration (Fig.4). When the concentrations of the extracts were decreased from 500 to 100 µl, slight decrease in inhibition zones was observed. A recent photochemical study of *Opuntia* revealed in the prsense of alkaloids in ample quantities notably substituted phenyl amines like 3-methoxytyramine, candicine, hordenine, N-methyltyramine, betanine and indicaxanthine. It also contains psycho active compounds and derivatives which includes compounds like 3, 4-DMPEA, 4-Hydroxy-3-5-DMPEA and mescaline. It has been reported that it possess strong anti-microbial activity against Gram positive, Gram negative bacteria and pathogenic fungi. It is likely that the presense of this type of compounds may have contributed to the anti microbial activity of *Opuntia fragilis.*

Minimal inhibitory concentration results showed that at the concentration of 500µl, methanol extract shows the maximum activity compare to butanol extract (Table 4). The docking result shows the binding of ligand with the receptor molecule. The scoring function takes a pose as input and returns a number indicating the likelihood that the pose represents a favourable binding interaction. A low (negative) energy indicates a stable system and thus a likely binding interaction (Kitchen et al., 2007). Fig.5 shows the binding of ligand with the receptor molecule and the docking RMS score value is -1.00. As the distance between the target molecule and drug molecule are less than 2.00 Armstrong, it shows high efficiency. The specificity of the drug and the target protein varies depending upon their binding sites.

4. CONCLUSION

Opuntia fragilis exhibit high degree of antibacterial activity. The result shows that this plant has the efficacy against the bacteria *E.coli, Shigella sp., S. aureus* and *P.vulgaris*. The effect may be due to the presence of potentially effective compounds in Opuntia fragilis. The resulting information will contribute to a better understanding of the antimicrobial activity of the plant. The In-silico analysis showed that the compound Lincomycin has higher specificity towards the target protein butyryl cholinesterase, which causes diabetic in human

SUMMARY OF RESEARCH

1. *Opuntia sp.* was collected to check the antimicrobial activity.
2. The extract was collected using various solvents to screen the effect against Gram positive and Gram negative bacteria
3. In-silico analyses were performed by retrieving the diabetic protein from protein data bank.
4. The structure of the compound lincomycin was retrieved from Pubchem.
5. Docking were performed to find the efficacy of ligand to the receptor molecule.

FUTURE ISSUES

1. GC-MS analysis for diverse compounds present in *Opuntia fragilis.*
2. Compounds present in *Opuntia fragilis* can be checked against cancer, ageing, asthma, cardiovascular disease, immune-system decline, brain dysfunction, snake bite etc.

DISCLOSURE STATEMENT

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John Varghese et al.
Analyze the efficacy of Opuntia fragilis against bacteria and its effects on diabetic protein butyryl cholinesterase,
Drug discovery, 2012, 1(2), 33-35,