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EVALUATION OF BIOACTIVE POTENTIAL OF 1-BENZYL INDOLE-3-CARBOXY ALDEHYDINE THIOSEMI CARBAZONE SCHIFF BASE LIGAND USING PATHOGENS

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Abstract – In the present study aimed to investigate antimicrobial potential of 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand against human pathogens. A pilot screening of the extracts were carried out by impregnating a 6 mm sterile Whatmann number 1 filter paper discs. The disc was loaded with the 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand to give a final load of 100,150 and 200 mg /disc. The discs were placed on the Petri plate previously seeded with the respective pathogenic strains. The inhibition – zone width was measured and calculated. The whole chemical showed less activity against *P. aeruginosa* (6.7 ± 0.3 mm). The purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand (200 mg /disc) showed a high inhibitory action against *A. hydrophila* (8.1 ± 0.3 mm) and minimum effect on *S. aureus* (5.6±0.2mm). The crude 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand a maximum activity against *P. aeruginosa* (7.2 ± 0.1 mm) and minimum activity against *C. tetani* (3.6 ± 0.1). Of the three samples tested, antibacterial activity was more pronounced in purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand. Antifungal activity of the three samples was evaluated, it was very interesting to observe that all the above three components registered a good antifungal activity. The purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand at a dose level of 200 mg/disc showed the highest inhibitory activity. A dose dependent variations of 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand were observed in the antimicrobial activity. From the results purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand have bioactive potential antimicrobial compounds.

INTRODUCTION

Schiff bases and their metal complexes have been evaluated for a range of bioactivities such as antimicrobial, anti-Alzheimer, anticancer, antiglycation, antileishmanial, antituberculosis, anticonvulsant, anti-inflammatory, antioxidant, antiviral, urease inhibition, pesticidal activity (Abdul Hameeda *et al.,* 2017). The mechanism of Schiff base formation is another variation on the theme of neucleophilic addition to the carbonyl group. In this case, the neucleophile is the amine. In the first part of the mechanism, the amine reacts with the aldehyde or ketone to give an unstable addition compound called carbinolamine. The carbinolamine loses water by either acid or base catalyzed pathways. Since the carbinolamine is an alcohol, it undergoes acid catalyzed dehydration (Serra and Peterson, 2008).

Typically the dehydration of the carbinolamine is the rate-determining step of Schiff base formation and that is why the reaction is catalyzed by acids. Yet the acid concentration cannot be too high because amines are basic compounds. If the amine is protonated and becomes non-neucleophilic, equilibrium is pulled to the left and carbinolamine formation cannot occur. Therefore, many Schiff bases synthesis are best carried out at mildly acidic pH (Carlos Lodeiro *et al.,* 2009). Peoples like in primary health care need 80% ayurvedic or traditional medicine to cure various bacterial diseases as per report of world health organization

approximately (Anil Kumar Dhiman, 2006). The presumption of world health organization synthesized derivatives chemicals empathetically indicates that these items have medicinal values (Basak, 2000). Synthesized and characterized some schiff base analogous of indole-3-carboxaldehyde with aminoacids (histidine, glutamic acid, aspartic acid, leucine and valine) and studied the biological activity (Ramam and Selvan, 2011).

The scientific study of derivation of drugs through bio prospecting and systematic conservation of the concerned chemical base compounds are thus of great important (Hemalatha and Dhasarathan, 2010). Base compounds contain compounds like quercetin, kaemfered, myricetin and lutiolin. Quercitn has anticarcinogenic activity; it also inhibits the growth of several types of cancer celland powerful antioxidants that give them color, flavor, odor and protection against human diseases (Van Duyn and Pivonka, 2000). Hence in the present study attempt has been carried out to find out the antibacterial and antifungal activities of the 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand.

MATERIAL AND METHODS

For the present study the sample of fruit was collected from local market at Courtallam, Tirunelveli district, Tamilnadu, India and brought to the laboratory for the estimation of bioactive potential (Antibacterial and antifungal assay) using pathogenic strains. Fruits sample was extracted with aqueous solution in 1g/mL (stock solution). The disc was loaded with the extracts from stock solution to give a final load of 100, 150 and 200 mg /disc. Pathogens were collected from Arvind hospital lab, Tirunelveli, Tamilnadu, India. All the cultures were cultured in nutrient and Rose Bengal broth (Hi-media) and incubated at 37^0/RT^0 C for 24 hours. Results of fruits antimicrobial activity was compared with standard antibiotic Ciprofloxacin (40µg/mL) for bacterial pathogen and Myconozole (50µg/mL) for fungal pathogens.

Antimicrobial assay: The human bacterial pathogens such as *Staphylococcus aureus, Salmonella pneumonia, Clostridium diphtheria, Bacillus cereus, Clostridium tetani, Escherichia coli, Salmonella typhi, Aeromonas hydrophila, Klebsiella pneumonia, Pseudomonas aerugenosa* and fungal pathogen such as *Aspergillus niger, A. fumigatus, Candida albicans, Phytophthora infestants* and *Trichophyton rubrum* were selected for antimicrobial screening. Ten microlitre of the broth culture was aseptically transferred to the air dried sterile agar plates and spread the culture uniformly with the help of a sterilized spreader made up of glass rod.

The whole chemical, impured and purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand were subjected to pilot study. A pilot screening of the 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand were carried out by impregnating a 6 mm sterile Whatmann number 1 filter paper discs. The discs were allowed to dry completely; the discs were placed on the Petri plate previously seeded with the respective pathogenic strains. Three replicates were used for each treatment. Control discs were kept without any extracts but soaked in respective microlitre of aqueous solvent and dried plates were then kept at 37° C in an incubator for 24hrs. The inhibition – zone width (distance from the edge of the paper disc to the outer edge of the inhibition zone) was measured to the nearest mm, at 24hrs by using Hi-Media antibiotic zone scale and expressed in standard deviation of mean (± SE). The antimicrobial activity of whole chemical, impured and purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand were calculated for different concentrations.

MIC determination

The minimum inhibitory concentration (MIC) of whole chemical, impured and purified 1-Benzyl Indole-3-carboxyaldehydinethiosemicarbazone Schiff base ligand were determined by Kuete *et al.,* (2007) method. The test extract was serially diluted two fold to obtain concentration ranges 2 mg/mL to 122 mg/mL for whole chemical, impured and purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand in titre plates. One hundred microlitres of each concentration was added in a well containing nutrient broth and 5 microlitres of inoculums, the negative control well consisted of 195 microlitres of nutrient broth and 5 microlitres of inoculums. Two hundred microlitres of nutrient broth considered as blank. The plate was covered with sterile plate scale and incubated in 37 °C for 24 hrs. After incubation, the optical density was read at 520nm. The lowest optical density read in minimum inhibitory concentration of whole chemical, impured and purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand.

RESULTS AND DISCUSSION

Results on the antibacterial activity of whole chemical, impured and purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand are presented in the Tables 1 –3. Whole chemical, impured and purified 1-Benzyl Indole-3-carboxy aldehydi nethiosemi carbazone Schiff base ligand showed a good antibacterial activity. Of the three samples tested, antibacterial activity was more pronounced in purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand. Next to purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand, the chemical and impured 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand showed a good antibacterial activity. Of the different bacteria tested antibacterial activity was well expressed against S. *typhi and C. tetani*. The purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand showed less activity against (6.7 ± 0.3 mm) *P. aeruginosa*. The whole chemical (200 mg/disc) showed a high inhibitory action against *A. hydrophila* (8.1 ± 0.3 mm) and minimum effect on *S.aureus* (5.6±0.2mm). The impured 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand registered a maximum activity against *P. aeruginosa* (7.2 ± 0.1 mm) and minimum activity against *C. tetani* (3.6 ± 0.1). In standard antibiotic, Ciprofloxacin (40 µg/mL) showed maximum inhibition in all the tested bacterial pathogens from 14.3 to 26.3 mm. The antibacterial potential in the purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand and whole base chemical suggests that the mite possess antibacterial compounds and this has to be explored in future.

Antifungal activity of the whole chemical, impured and purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand were evaluated. It was very interesting to observe that all the above three components registered a

Table 1. Antibacterial activity of whole base chemical against pathogenic bacteria in Disc plate method.

Organisms	Name	Zone of inhibition (mm) (± S.D) Concentration of whole sample (mg/disc)			Ciprofloxacin (40 µg/mL)
		100	150	200	
	S. aureus	3.3 ±0.1	4.1 ± 0.3	5.6 ± 0.2	14.3
Gram	*S. pneuomoniae*	4.1 ± 0.2	4.6 ± 0.2	6.2 ± 0.3	16.5
Positive	*C. diphtheria*	4.1 ± 0.1	5.1 ± 0.1	6.3 ± 0.2	16.4
Organism	*B. cereus*	4.2 ± 0.3	5.1 ± 0.1	7.2 ± 0.1	18.4
	C. tetani	4.1 ± 0.2	5.6 ± 0.2	7.1 ± 0.3	19.3
	E. coli	4.0 ± 0.3	5.4 ± 0.3	6.3 ± 0.2	16.6
Gram	*S. typhi*	4.5 ± 0.1	5.2 ± 0.1	6.4 ± 0.1	19.4
Negative	*A. hydrophila*	5.4 ± 0.1	5.1± 0.1	8.1 ± 0.3	26.3
Organism	*K. pneuomoniae*	4.1 ± 0.3	5.4 ± 0.3	7.7 ± 0.2	25.4
	P. aerugenosa	4.2 ± 0.2	5.1 ± 0.2	7.2 ± 0.2	24.1

Table 2. Antibacterial activity of impured 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand against pathogenic bacteria in Disc plate method.

Organisms	Name	Zone of inhibition (mm) (± S.D) Concentration of skin extract (mg/disc)			Ciprofloxacin 40 µg/mL
		100	150	200	
	S. aureus	3.2 ±0.2	4.2 ± 0.2	5.1 ± 0.2	14.3
Gram	*S. pneuomoniae*	3.4± 0.1	4.2 ± 0.1	5.4 ± 0.1	16.5
Positive	*C. diptheriae*	3.2 ± 0.3	4.3 ± 0.2	6.6 ± 0.3	16.4
Organism	*B. cereus*	3.2 ± 0.2	3.7 ± 0.3	4.2 ± 0.2	18.4
	C. tetani	3.3 ± 0.1	3.2 ± 0.1	3.6 ± 0.1	19.3
Gram	*E. coli*	4.2 ± 0.2	4.2 ± 0.2	4.6 ± 0.3	16.6
Negative	*S. typhi*	4.4 ± 0.1	5.7 ± 0.3	6.2 ± 0.2	19.4
Organism	*A. hydrophila*	3.4 ± 0.1	5.5 ± 0.1	7.6 ± 0.3	26.3
	K. pneuomoniae	2.6 ± 0.2	3.7 ± 0.2	4.6 ± 0.2	25.4
	P. aerugenosa	3.4 ± 0.2	5.2 ± 0.1	7.2 ± 0.1	24.1

good antifungal activity. For the present investigation, five fungal organisms were selected and the results are presented in the Tables 4 - 6. Of the three products of chemicals tested, the purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand registered the maximum antifungal activity. Next to the purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand, the impured 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand exhibited a high antifungal activity. A dose dependent variation was observed in the antifungal activity. The whole purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand at a dose level of 200 mg/disc showed the highest inhibitory activity. Of the five fungal species tested the dermatophyte, *Trichophyton rubrum* responded much to the extracts of the impured and purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand. Inhibition zone for *T. rubrum* was 8.5 ± 0.1 mm for purified 1-Benzyl Indole-3-carboxyaldehydinethiosemicarbazone Schiff base ligand and 7.3 ± 0.2 for the impured 1-Benzyl Indole-3-carboxyaldehydinethiosemicarbazone Schiff base ligand. This was followed by *Phytophthora infestants*. It showed an inhibition zone of 8.0 ± 0.2 for purified 1-Benzyl Indole-3-carboxy

Table 3. Antibacterial activity of purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand against pathogenic bacteria in Disc plate method.

Organisms	Name	Zone of inhibition (mm) (± S.D) Concentration of hemolymph (mg/disc)			Ciprofloxacin 40 µg/mL
		100	150	200	
Gram	*S. aureus*	4.2 ±0.2	4.6 ± 0.2	7.2 ± 0.3	14.3
Positive	*S. pneuomoniae*	4.6 ± 0.2	5.6 ± 0.3	8.1 ± 0.2	16.5
Organism	*C. diptheriae*	4.2 ± 0.3	5.7 ± 0.1	7.2 ± 0.3	16.4
	B. cereus	3.6 ± 0.2	5.2 ± 0.2	8.3 ± 0.2	18.4
	C. tetani	5.2 ± 0.1	6.6 ± 0.1	8.6 ± 0.1	19.3
Gram	*E. coli*	5.2 ± 0.1	6.2 ± 0.2	7.6 ± 0.2	16.6
Negative	*S. typhi*	6.2 ± 0.2	6.6 ± 0.1	8.4 ± 0.2	19.4
Organism	*A. hydrophila*	3.3 ± 0.1	6.2 ± 0.3	8.3 ± 0.3	26.3
	K. pneuomoniae	4.2 ± 0.3	5.2 ± 0.2	7.4 ± 0.1	25.4
	P. aerugenosa	3.2 ± 0.2	4.2 ± 0.1	6.7 ± 0.3	24.1

Table 4. Antifungal activity of purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand against fungal pathogens in Disc plate method.

Organisms	Zone of inhibition (mm) (± S.D) Concentration of durian fruit sap (mg/disc)			Myconozole 50µg/mL
	100	150	200	
Aspergillus niger	4.3±0.3	5.2 ± 0.3	7.6 ± 0.2	20.3
Aspergillus fumigatus	4.4 ± 0.3	5.5 ± 0.3	8.1 ± 0.2	18.4
Candida albicans	4.5 ± 0.5	5.6 ± 0.1	7.5 ± 0.3	21.6
Phytophthora infestants	3.6 ± 0.4	5.2 ± 0.2	8.0 ± 0.2	20.5
Trichophyton rubrum	5.8 ± 0.2	6.7 ± 0.1	8.5 ± 0.1	23.4

Table 5. Antifungal activity of impured 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand against fungal organisms in Disc plate method

Organisms	Zone of inhibition (mm) (±S.D) Concentration of skin extract (mg/disc)			Myconozole 50 µg/mL
	100	150	200	
Aspergillus niger	3.6 ± 0.2	4.4 ± 0.2	5.5 ± 0.3	20.3
Aspergillus fumigatus	4.1 ± 0.3	4.6 ± 0.3	6.4 ± 0.3	18.4
Candida albicans	4.3 ± 0.2	5.5 ± 0.2	6.7 ± 0.3	21.6
Phytophthora infestants	4.7 ± 0.2	5.2 ± 0.3	7.2 ± 0.2	20.5
Trichophyton Sp	4.6 ± 0.1	5.4 ± 0.2	7.3 ± 0.1	23.4

aldehydine thiosemi carbazone Schiff base ligand and 7.2 ± 0.2 for impured 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand. Antifungal Property was poor in *Aspergillus niger.* The inhibition zone for *Aspergillus niger* was 7.6± 0.2 mm for the haemolyomph of purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand and 5.5 ±o.3 mm for the impured 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand. In standard antibiotic, Myconozole (50 µg/mL) showed maximum inhibition in all the tested fungal pathogens from 18.4 to 23.4 mm.

The whole base chemical had lesser antifungal activity than the durian fruit sap and skin extract. Here also a dose level of 200 mg/disc showed the highest inhibitory activity than 100 mg/disc and 150 mg/disc. The whole base chemical showed a less inhibitory activity (3.8 ± 0.2 mm) against *T. rubrum* at a dose level 200 mg/disc. The response of *Candida albicans* was more to the whole base chemical. Inhibition zone for *C. albicans* was 6.6 ± 0.2 mm at a dose level 200 mg/disc.

In purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand showed lowest minimum inhibitory concentration values against all the test pathogens compared to whole chemical and impured 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand. The ranges of minimum inhibitory concentrations were observed in 16 to 64 ìg/ml of all test chemicals (Table 7). In recent years development of multidrug resistance in the pathogenic bacteria had created major clinical problems in the treatment of infectious disease (Davies, 1994). This and other problems such as toxicity of certain antimicrobial drugs on the host tissues triggered interest in search of new antimicrobial substances/drugs of Schiff bases are synthetically accessible and structurally diverse compounds (Maddux and Barrier, 1980). Microbial resistance and diversity depends on the nature of chemicals (Dhasarathan *et al.,* 2010)

CONCLUSION

Schiff bases are synthetically accessible and

Table 6. Antifungal activity of whole base chemical against fungal organisms in Disc plate method

Organisms	Zone of inhibition (mm) (± S.D) Concentration of whole sample (mg/disc)			Myconozole 50 µg/mL
	100	150	200	
Aspergillus niger	3.6 ± 0.2	4.5 ± 0.1	5.5 ± 0.1	20.3
Aspergillus fumigatus	3.7 ± 0.1	4.3 ± 0.2	5.7 ± 0.3	18.4
Candida albicans	3.8 ± 0.3	4.6 ± 0.3	6.6 ± 0.2	21.6
Phytophthora infestants	3.6 ± 0.2	3.7 ± 0.2	4.6 ± 0.2	20.5
Trichophyton rubrum	3.4 ± 0.1	3.5 ± 0.1	3.8 ± 0.2	23.4

Table 7. Minimum inhibitory concentration (MIC) of test chemicals against pathogens.

Organisms	Name	Test fruit sample (µg/mL)		
		Purified	Impured	Whole
	S. aureus	16	64	32
Gram	*S. pneuomoniae*	32	64	64
Organism	*C. diptheriae*	16	32	32
Positive	*B. cereus*	16	64	32
	C. tetani	32	64	64
Gram	*E. coli*	32	64	64
Negative	*S. typhi*	32	64	64
Organism	*A. hydrophila*	16	32	32
	K. pneuomoniae	32	64	64
	P. aerugenosa	64	64	32
Fungal pathogens	*Aspergillus niger*	32	64	64
	Aspergillus fumigatus	16	64	32
	Candida albicans	32	64	32
	Phytophthora infestants	16	64	32
	Trichophyton rubrum	16	32	16

structurally diverse compounds are the important source in antimicrobial activity. Schiff bases are synthetically accessible and structurally diverse compounds are also very low in zinc which is essential for the function of the immune system, the formation of skin, the healing of wounds, brain function and it is essential for the function of the reproductive organs. In the present study hope this report has given some new insights into whole chemical, impured and purified 1-Benzyl Indole-3-carboxy aldehydine thiosemi carbazone Schiff base ligand.

REFERENCES

Abdul Hameeda, Mariya al-Rashidab, Maliha Uroosc, Syed Abid Ali. 2017. Chemical patterns for therapeutics. Expert Opinion on Therapeutic Patents. 27(1) : 63-79.

Serra, R. and Peterson, S.W. 2008. Multilocus sequence identificationof Penicillium species in cork bark during plank preparation for the manufacture of stoppers. *Research in Microbiology.* 159 : 178–186.

Carlos Lodeiro, Bruno Pedras, Elisabete Oliveira, Hugo Santos, Laura Rodriguez, Ramon Crehuet, Teresa Aviles, Jose Luis Capelo. 2009. Inorganica Chimica Acta. 362 : 2627–2635.

Ramam, N. and Selvan, A. 2011. Studies on DNA binding, electrochemical activation, DNA photocleavage, and biopotency of N and O donor bidentate ligands with Cu(II), Co(II), and Zn(II). *Journal of Coordination Chemistry.* 64 : 534–553.

Maddux, M.S. and Barrere, S.L. 1980. A review of complications of amphotericin-B therapy, recommendations for prevention and management. *Drug Intelligence and Clinical Pharmacy.* 14 : 177- 181.

Van Duyn, M.A. and Pivonka, E. 2000. Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: Selected literature. *J Am Diet Assoc*. 100 : 1511-1521.

Dhasarathan, P., Ashokraja, C. and Theriappan, P. 2010. Microbial diversity in fire work chemical exposed soil and water samples collected in Virduhunagar district, Tamilnadu, India. *Indian Journal of Microbiology*. 50 (1): 46-49.

Anil Kumar Dhiman. 2006. Ayurvedic Drug Plants, Dhaya Publishing House, Delhi, 72-74.

Kuete, V., Kamga, J., Sandjo, P.L., Ngameni, B., Poumale, H.M.P., Ambassa, P. and Ngadjui, B.T. 2011. Antimicrobial activities of the methanol extract, fractions and compounds from Ficus polita Vahl. (Moraceae). *J. Ethnopharmacol.* 6 : 11.

Hemalatha, N. and Dhasarathan, P. 2010. Multi-Drug Resistant Capability of Pseudomonas aeruginosa Isolates from Nasocomal and Non-Nasacomal Sources. *J Biomed Sci. and Res.* 2 (4) : 236-239.

Basak, S.L. 2000. Nutritional and Medicinal Values of plant foods. Paratha Sankar Bara, India, 18-22.