IOSR Journal Of Pharmacy *www.iosrphr.org*
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 8, Issue 2 Version. 1 (February 2018), PP. 60-67

Physical and Biological Evaluation of Cordiarimide B Isomers as Multidrug Compounds

Jeevan, B. V.[1]; Umashankara, M.[2]; Shankar. J[3]; Kumara, M. N*.[1]; Niranjan Raj, S.[4]

1. Chemistry Department, Yuvaraja's College, University of Mysore, Mysuru-570005.
2. Department of Studies in Chemistry, Karnataka State Open University, Mukthagangothri, Mysuru-570006.
3. P.G. Department of Biotechnology, Teresian College, (Affiliated to University of Mysore), Siddartha Nagar, Mysuru-570011.
4. Department of Studies in Microbiology, Karnataka State Open University, Mukthagangothri, Mysuru-570006.

**Corresponding Author:* kumaramanikya@yahoo.com

ABSTRACT: Multidrug compounds are emerging as a new class of therapeutic agents with potent pharmacological activities for a broad spectrum of diseases. In this study, physical stability, plasma stability, cytotoxicity, superoxide anion radical scavenging activity and antimicrobial activity of four isomers (*S*)-3-amino-1-((*R*)-2-hydroxy-2-phenylethyl)piperidine-2,6-dione(**1),** (*S*)-3-amino-1-((*S*)-2-hydroxy-2-phenylethyl) piperidine-2,6-dione(**2**), (*R*)-3-amino-1-((*R*)-2-hydroxy-2-phenylethyl)piperidine-2,6-dione(**3**), (*R*)-3-amino-1-((*S*)-2-hydroxy-2-phenylethyl) piperidine-2,6-dione**(4)** were comparatively evaluated. The results demonstrated that, the compounds (*S*)-3-amino-1-((*R*)-2-hydroxy-2-phenylethyl) piperidine-2,6-dione(**1)** and (*S*)-3-amino-1-((*S*)-2-hydroxy-2-phenylethyl)piperidine-2,6-dione(**2**) manifested high physical stability and low cytotoxicity. Further, these two compounds also exhibited good antimicrobial and superoxide anion radical scavenging activities. Data showed that, the compound (*R*)-3-amino-1-((*R*)-2-hydroxy-2-phenylethyl) piperidine-2,6-dione(**3**) is not a suitable drug molecule. Taken together, these results indicate that the compounds (*S*)-3-amino-1-((*R*)-2-hydroxy-2-phenylethyl)piperidine-2,6-dione(**1),** (*S*)-3-amino-1-((*S*)-2-hydroxy-2-phenylethyl) piperidine- 2,6-dione (**2**) and (*R*)-3-amino-1-((*S*)-2-hydroxy-2-phenylethyl) piperidine-2,6-dione**(4)** possess multidrug favorable features like solubility, lipophilicity, permeability and plasma stability.

Keywords: Physical stability, plasma stability, cytotoxicity, superoxide anion radical scavenging activity, antimicrobial activity, multidrug compounds.

Date of Submission: 19-02-2018 Date of acceptance: 06-03-2018

I. INTRODUCTION

Natural products including extract from plants, animals, microbes and minerals play a remarkable role in discovery of new drugs.[1-5] Recent technical advances reduce the hurdles in isolation, purification, characterization of natural products from the source. Hence, the natural products become a rich source of compounds for drug discovery.[6-8] They possess enormous structural and chemical diversity, therefore, continue to inspire novel discoveries in chemistry, biology and profoundly impacted in understanding of chemical biology of the drug molecules in order to develop new therapy.[9,10]

Prolonged intake of drug causes faster resistance development and imposes significant burdens on healthcare systems. This development of resistance to clinically used drug has increased the demand for discovery of new chemical scaffolds with multi drug activity.[11,12] Presently, there has been a renewed interest in natural product research to overcome failure of alternative drug discovery methods to deliver many lead compounds in key therapeutic areas.[13] Recently, glutarimide alkaloids, Cordiarimides B is discovered as part of a screening program designed to find new antioxidants from plant genus Cordia.[14] Structurally, Cordiarimide B has piperidone ring system, with attached aromatic compound on nitrogen atom. It has two stereo centers, hence, exists in four stereogenic isomers (figure 1). As a part of our interest in synthesis of new natural product drugs, we have earlier reported mild and efficient enantio selective synthesis of Cordiarimide B isomers.[15] In this paper, we are reporting the comparative physical and biological studies of four Cordiarimide B isomers to evaluate their multidrug potentiality.

Figure 1: Four stereogenic isomers

II. FORCED DEGRADATION ASSAY

Forced degradation process which involves degradation of molecules at more severe conditions than the accelerated conditions is a widely used method for studying the physical stability of drug compounds. Hydrogen peroxide is used for oxidation of drug substances in forced degradation studies. The functional group with labile hydrogen is susceptible to oxidation to form hydro peroxides, hydroxide or ketone. The compounds **1-4** were subjected to forced degradation studies in order to find their physical stability. Each compound was accurately weighed to 10 mg and dissolved in a minimum amount of acetonitrile. The solution was made up to 10 mL by adding 3% hydrogen peroxide as per the requirement of the stress study[16] so as to make the concentration of the drug 1 mg/mL. The solutions were injected in triplicate using water–acetonitrile as mobile phase and keeping the injection volume constant (2 µL). To assess precision, six injections of five different concentrations (10, 20, 30, 40, and 50 µg mL^{-1}) were made on the same day and intra-day precision was determined as relative standard deviation. The forced-degradation study revealed that compound **3** quickly degraded under oxidative stresses (figure 2).

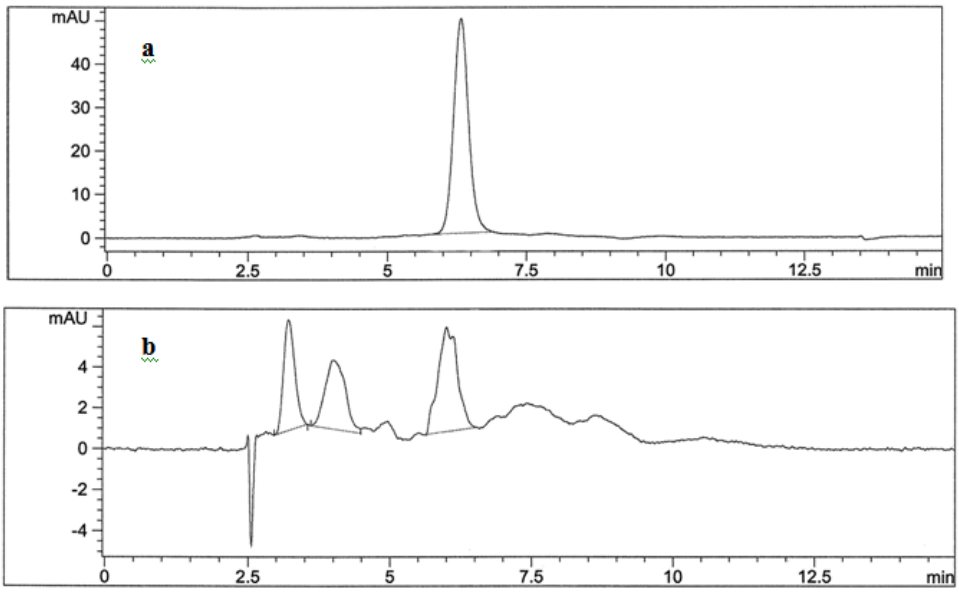


Figure 2: a) Chromatogram of a solution of compound **3**. b) Chromatogram of oxidative hydrolysis compound **3** after 24 hours of incubation at 50µM concentration.

The specificity and selectivity of the method with the samples under these stresses were demonstrated through the evaluation of R_T, and purity data for all peaks in the chromatograms. Compounds **1**, **2** and **4** were not degrading under oxidative stress conditions. The retention times (R_T) and purity of these drugs are given in Table 1. The data reveals that the compound **1** and **2** were recovered with 99% and 98% respectively even after 24 hr exposure to stress condition. The compound **4** was recovered with 80 % purity .This study suggested that compound **1** and 2 have greater physical stability and compound **4** has considerable physical stability in order to be considered as drug molecules.

Table 1: Percentage recovery of compound **1-4** after oxidative degradation study

Compound	Concentration (µM)	Time of exposure (hrs)	% of recovery	Mean (%)
1	10	24	99.4	99.2 ± 0.2
	20	24	99.2	
	30	24	98.9	
	40	24	99.5	
	50	24	99.1	
2	10	24	98.3	98.3 ± 0.8
	20	24	99.0	
	30	24	97.9	
	40	24	98.4	
	50	24	98.3	
3	10	24	2.0	4.8 ± 0.6
	20	24	4.7	
	30	24	3.1	
	40	24	5.4	
	50	24	9.1	
4	10	24	75.9	77.8 ± 0.8
	20	24	79.5	
	30	24	76.3	
	40	24	78.4	
	50	24	79.3	

III. PLASMA STABILITY ASSAY

A drug molecule with a functional group tends to be more susceptible to hydrolysis in blood plasma. Therefore, plasma stability is very useful for screening of pro-drug compounds. Using HPLC method, the *in vitro* stability of the compounds **1-4** was studied in human, and rabbit plasma. The plasma from different species were diluted to 80% with 0.05 M PBS (pH 7.4) at 37°C. The reactions were initiated by the addition of the test compounds to 1 ml of preheated plasma solution to yield a final concentration of 200 µM. The assays were performed in a shaking water bath at 37°C and conducted in triplicate. Samples (50 µl) were taken at 0, 15, 30, 45, 60, 90 min and added to 200 µl acetonitrile in order to deproteinize the plasma. The samples were subjected to vortex mixing for 1 min and then centrifugation at 4°C for 15 min at 14,000 rpm. The clear supernatants were analyzed by HPLC coupled with mass spectrometry. The HPLC and mass spectroscopy profiles of the compounds **1-4** in human plasma after and before incubation is shown in figure 3 and figure 4 respectively.

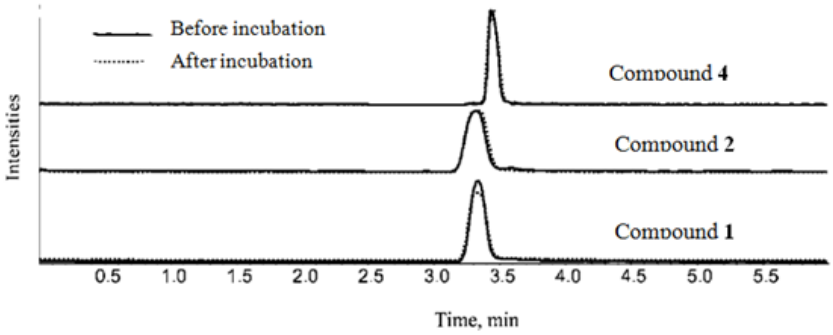


Figure 3: Direct HPLC chromatograms of compound **1**, 2 and **3** human plasma before (solid line) and after (dot line) incubation.

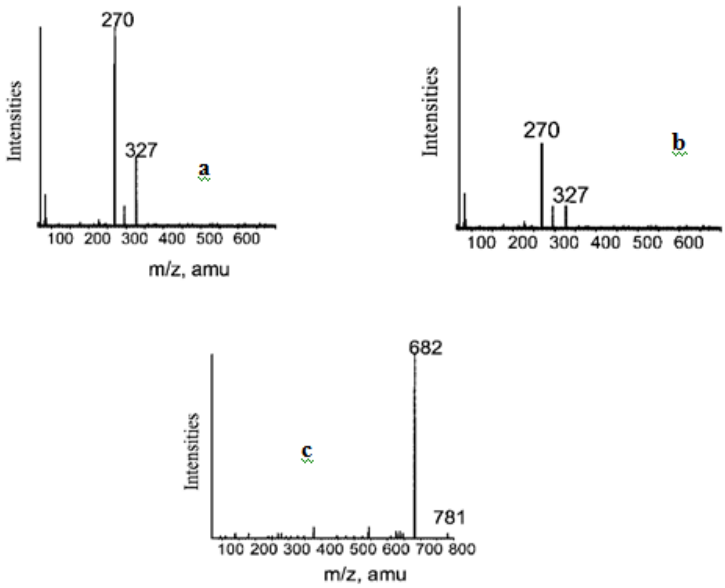


Figure 4: Mass spectra of compounds a) compound1, b) compound 2 and c) compound-3 recorded after 360min incubation in human plasma.

The stability profiles of the compounds **1-4** in human and rabbit plasma are shown in Figure 5. The analysis revealed that, there is a significant difference in the stability of compounds in both human and rabbit plasma. The compound **1** and **2** displayed longer stability in both plasma species. The compound **4** shows longer stability in rabbit plasma. Compound **3** degraded quickly in both human and rabbit plasma thereby showing poor stability.

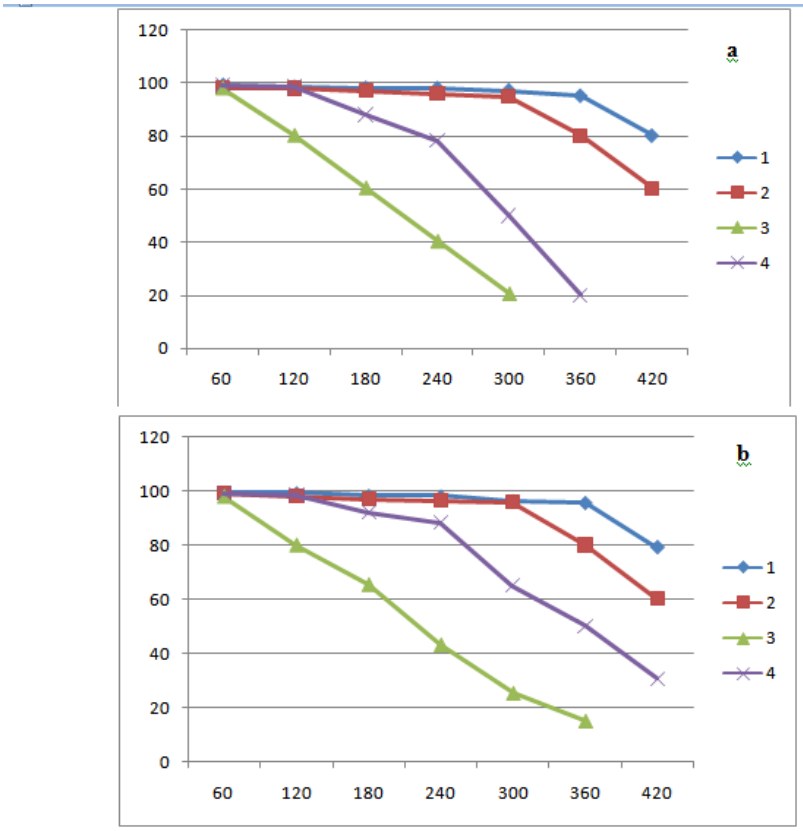


Figure 5: Stability profiles of the compounds **1-4** obtained in a) human and b) rabbit plasma.

IV. *IN VITRO* CYTOTOXICITY ASSAY

Although, high potency is an important factor in pharmacological design, one must also recognize the huge gulf between a tightly bound inhibitor and a bio-available drug. Far too often, promising candidates are abandoned during clinical trials. The compound which is set to become the drug molecule, undergoes safety tests and a series of experiments to prove that it is absorbed in the blood stream, distributed to proper site of action in the body, metabolized sufficiently and demonstrates its non-toxicity. Hence, we conducted the cytotoxicity of compounds **1-4** on MCF7, HepG2 & VERO cells by the MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay. The cell lines were obtained from National Centre for Cell Sciences Pune (NCCS).

To evaluate the cytotoxic activity, cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), (100U) 20µg/ml penicillin, and 100 µg/ml streptomycin. Incubation was carried out at 37 °C with an atmosphere of 5% CO_2. After homogenization, 1 ml of suspension was poured in each well of microtitre plate and kept in desiccator under 5% CO_2 atmosphere. After two days of incubation, the cells were observed in inverted microscope. 0.05 ml of drug was dissolved in 4.95 ml of DMSO to get a working concentration of 1 mg/ml. The working concentration was prepared freshly and filtered through 0.45 micron filter before each assay. Cells were incubated with different doses (1000 to 1.953 µg/ml) of compounds. After 24 hours of incubation, cell viability was determined by the MTT assay.

Compounds **1** and **2** induced cell cytotoxicity in a concentration dependent manner, compound **3** was found to be toxic and failed to show concentration dependent cytotoxicity.. Compound **4** displayed moderate cytotoxic activity compare to compounds **1** and **2**. The results of cytotoxicity assay are presented in table 2.

Table 2: IC_{50}, values of compound 1-4. (IC_{50} is the concentration at which 50% cell death occur).

	HEP G2(IC_{50})	MCF-7(IC_{50})	Normal VERO
Compound	(µg/ml)	(µg/ml)	(µg/ml)
1	31.25	25.26	75.35
2	30.34	23.25	63.34
3	-	-	-
4	15.62	12.33	42.24

From experiential data it is suggested that, the **compounds1**, **2** and **4** are more toxic to cancer cells than normal cells. Cogitating the overall citotoxic activity of tested compounds, it is suggested that, compounds **1** and **2** could be considered as potential anticancer drugs.

V. SUPEROXIDE ANION RADICAL SCAVENGING ACTIVITY

Antioxidants are characterized by their ability to scavenge free radicals. Proton radical scavenging action is an important attribute of antioxidants, which is measured by superoxide anion radical scavenging assay. Superoxide radical is known to be very detrimental to cellular machineries as they are precursor of other reactive oxygen species and they cause lipid peroxidation leading to tissue destruction and several other illnesses. Toxicity of superoxide depends on the capacity to inactivate iron-sulphur clusters containing enzymes that are important in several metabolic pathways. Superoxide anion radical scavenging activity of compounds **1-4** was measured using the NTB reduction assay as described by Nagulendran et al.[16] 3 ml of 16 mM Tris-HCl buffer (pH 7.4), which contained 1 ml of 150 µM NBT and 1 ml of 234 µM reduced nicotinamide adenine dinucleotide (NADH) was added to the test tubes containing various concentrations (10, 50 and100 µg) of compounds **1-4**. The reaction was initiated after addition of 1 ml of 40 µM phenazine methosulfate into the reaction mixture and the tubes were incubated at 28 ± 2^0C for 5 min. After incubation, when the reaction mixture had reached a stable colour, the absorbance was measured at 560 nm against a blank using UV-visible spectrophotometer. Ascorbic acid was used as the standard. The concentration dependent superoxide anion radical scavenging activity of the compound **1-4** is shown in figure 6 and the data is presented in table -3. The result revealed that, compound 1 is a strong antioxidant agent, compounds **2** and **4** show weak antioxidant activity. Comparatively compound **2** is better than compounds exhumed and compound **3** in antioxidant activity even at the concentration of 100 µg

Table-3: Percentage of Superoxide anion radical scavenging activity: of the compounds **1-4** at different concentrations

.

Test of samples	10 µg	50 µg	100 µg
1	0.8	24.0	40.0
2	0.8	18.4	28.0
3	1.6	8.0	16.0
4	0.0	9.6	15.2
Ascorbic acid	37.4	46.9	59.1

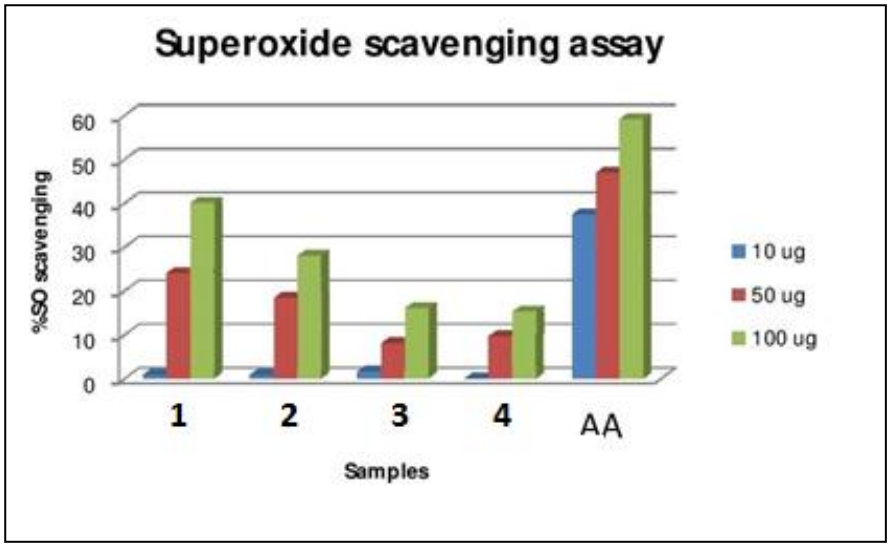
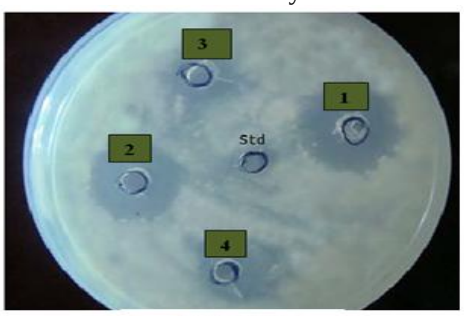


Figure 6: % of superoxide scavenging assay

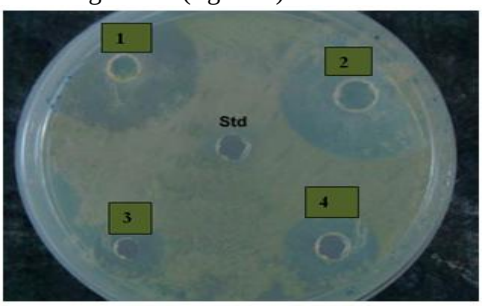
VI. ANTIMICROBIAL ACTIVITY

The growth of the microorganisms depends on the type of the nutrition they utilize. Some chemical nutrients enhance the vegetative growth or reproductive growth. Resistance of pathogenic bacteria to available antibiotics is rapidly becoming a major problem in the community and hospital based healthcare settings. Microbial resistance towards the drug creates a very serious problem. Because of resistance development, many drugs are not active which were very effective before. Moreover, the toxic effects produced by these antibiotics are also reducing their significance. We examined the antimicrobial activity of the compounds **1-4** to explore their potential multi drug nature.

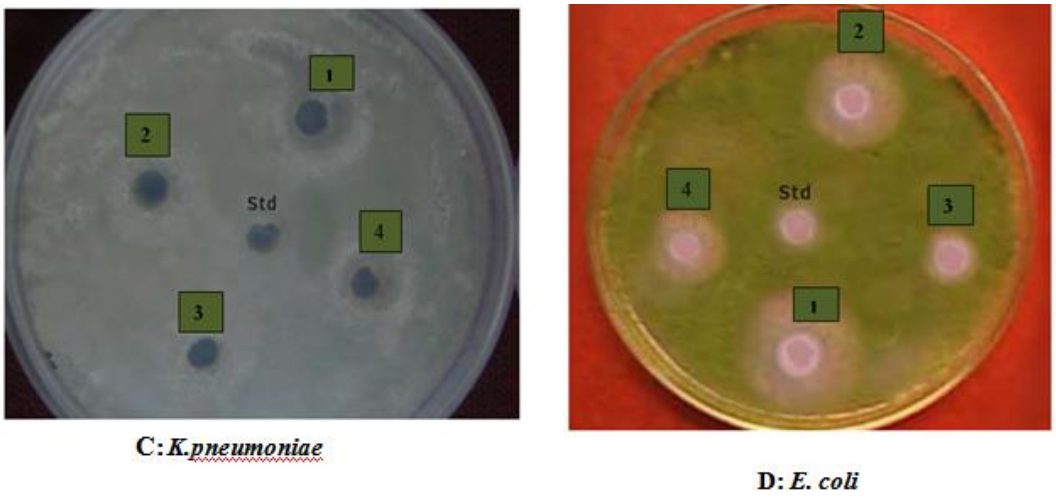
The microbial strains used in present study were obtained from Institute of Microbial Technology Department of Microbiology, Manasagangothri, Mysore. The bacterial cultures were *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumonia* and *Bacillus cereus*. Antimicrobial activity (zone of inhibition) of the compounds **1-4** against the bacterial species was done by paper disc diffusion method. The compounds at the concentration of 50 µg/mL in minimum quantity of methanol and water in the nutrient agar media were screened for their antibacterial activity. The paper discs inoculated with bacteria were incubated for 24 hrs at 37^0C. After the incubation, the zone of inhibition produced by the test compounds was measured in mm. compound **1** showed maximum antibacterial activity for all the tested microorganisms (figure 7).



A: *B.cereus*



B: *E. aerogenes*



C: *K.pneumoniae*

D: *E. coli*

Figure 7: Antimicrobial activity of compound **1-4** with different microorganisms

The screening tests were performed in triplicate and the results were taken as mean of determinations. The results of antibacterial activity of the synthesized compounds is summarized in Table 4. Maximum inhibition was obtained in ***B .cereus*** and ***E. coli*** with 12+++ and 14 +++ respectively for compound **1**. Similarly, compound 2 showed maximum inhibition zone with 12++ for ***E. coli, B. cereus*** and ***E. aerogenes***. The compound **3** and compound **4** showed restrained and minimum activity, respectively. More specifically, compound **4** represented higher susceptibility to all bacterial strain compared to compound **3**.

Table 4: Antimicrobial activity (zone of inhibition) of compound **1-4** against clinical pathogens

Test of samples	***B. cereus***	***E. aerogenes***	***E. coli***	***K. pneumoniae***
1	12+++	10++	14 +++	10++
2	12++	12++	12++	6+
3	6+	8+	6+	8+
4	10++	6+	12+++	12+++
Std**	22	28	22	28

Results are expressed as a mean of the three determinations (n=3)
** Std = chloramphenicol 10µg/disc
+++ = Good activity
++ = moderate
+ = poor activity

VII.RESULTS AND DISCUSSION

Interest in the pharmacological effects of bioactive compounds in treatments and prevention of multi disease has increased dramatically over the past decade. Any new drug must have to possess physical as well as *in vitro* stability and exhibit multi drug activities in various cells through different forms of cytotoxic effects without exhibiting considerable damage to normal cells. Our observations on physical stability of compounds **1-4** reveals that compound **3** has very poor physical and cellular plasma stability and also high cytotoxicity. This study suggested that compound **3** is not a good candidate to become drug molecule. Compound **4** possesses good physical stability, though shows moderate cellular plasma stability and cytotoxicity, hence compound **4** is also not a good candidate to become a drug molecule. Compound **1** and **2** display very good physical as well as cellular plasma stability and low cytotoxicity. Moreover, both the compounds **1** and **2** show good antioxidant, anti microbial and anti cancer activities. Hence, these two compounds have all the potential characteristics to become multi drug molecules. Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths worldwide and about half a million individuals die from this disease annually. Similarly, breast cancer is the second leading cause of cancer-related death in women. Thus, it is imperative to search for new alternatives to breast cancer and HCC prevention agents. The study of inhibitory effect of compound **1**and **2** on breast cancer cells at different concentrations is under progress. The preliminary evidences obtained so far suggest that compound **1** may be a potential chemotherapeutic or a chemo preventive agent based on its ability to induce apoptosis in cancer cells with relatively low toxicity to normal cells. Further, studies with *in vivo* trials need to be conducted to establish compounds **1** and **2** as safe agents for cancer therapy.

VIII. CONCLUSION

In conclusion, the present investigation of Compounds **1-4** for their physical stability, antioxidant, anti microbial and anticancer activity may be of great use for the development of multi drug compound as a therapeutic against various diseases for pharmaceutical industries. Compound **1** and **2** were found to be potent multi drug molecules, as evident by their high physical and cellular plasma stability. A drug is considered to be worthy of further testing if it has a therapeutic index value of 16 or greater. The present study found that the therapeutic index value of 16 in MCF-7 cases of cancer lines for the compound **1** and **2**. The results of the study support the development of new antimicrobial and anti cancer drugs.

Acknowledgements

This study supported by a research grant from the University Grant Commission (UGC) is greatly acknowledged. The authors also thank Institution of Excellence (IOE), University of Mysore for the Mass and HPLC data, and Mr. Jeevan V. B, thanks IOE, University of Mysore, for providing research fellowship.

REFERENCES

[1]. Mishra, B. B.; Tiwari, V.K. Natural products: An evolving role in future drug discovery. Eur. J. Med. Chem. 2011, 46, 4769–4807.
[2]. Rey-Ladino, J.; Ross, A.G.; Cripps, A.W.; McManus, D.P.; Quinn, R. Natural products and the search for novel vaccine adjuvants. Vaccine 2011, 29, 6464–6471.
[3]. Cragg, G.M.; Newman, D.J. Biodiversity: A continuing source of novel drug leads. Pure Appl. Chem. 2005, 77, 7–24.
[4]. Haefner, B. Drugs from the deep: Marine natural products as drug candidates. Drug Discov. Today 2003, 8, 536–544.
[5]. Butler, M.S. The role of natural product in chemistry in drug discovery. J. Nat. Prod. 2004, 67, 2141–2153.
[6]. D. C. Rees, M. Congreve, C. W. Murray and R. Carr, Nat. Rev. Drug Discovery, 2004, 3, 660–672.
[7]. I. Tickle, A. Sharff, M. Vinkovic, J. Yon and H. Jhoti, Chem. Soc. Rev., 2004, 33, 558–565.
[8]. A. M. Davis, S. J. Teague and G. J. Kleywegt, Angew. Chem., Int. Ed., 2003, 42, 2718–2736.
[9]. S. W. Muchmore and P. J. Hajduk, Curr. Opin. Drug Discovery Dev., 2003, 6, 544–549.
[10]. X. Salvatella and E. Giralt, Chem. Soc. Rev., 2003, 32, 365–372.
[11]. Feher, M. and Schmidt, J.M. Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry. J. Chem. Inf. Comput. Sci. 2003, 43, 218–227.
[12]. Grabowski, K. and Schneider, G. Properties and architecture of drugs and natural products revisited. Curr. Chem. Biol. 2007, 1, 115–127.
[13]. Ganesan, A. The impact of natural products upon modern drug discovery. Curr. Opin. Chem. Biol. 2008, 12, 306–317.
[14]. Joshua, P.; Thinley, G.; Vilailak, P.; Chulabhorn, M.; Somsak, R.;Prasat, K. *J. Nat. Prod*, 2010, *73*, 992–994.
[15]. Jeevan, B. V.; Umashankara, M.; Sunil kumar, Y. C; Kumara, M. N; Rangappa, K. S. Mild and efficient Enantioselective Synthesis of Glutarimide Alkaloid Cordiarimide B and its isomers and their antioxidant study. Asian journal of Chemistry, (accepted) id 21170/2017.
[16]. Nagulendran, K. R.; Velavan, S.; Mahesh, R.; Begum, V. H. In vitro antioxidant activity and total polyphenolic content of Cyperus rotundus rhizomes. journal of chem. 2007; 440-449.

IOSR Journal of Pharmacy (IOSR-PHR) is UGC approved Journal with Sl. No. 5012

Jeevan, B. V." Physical and Biological Evaluation of Cordiarimide B Isomers as A Multidrug Compounds"IOSR Journal of Pharmacy (IOSRPHR), vol. 8, no. 2, 2018, pp. 60-67