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Synthesis, Characterization and Anthelmentic Activity of Pyridazine Derivative

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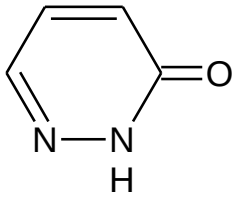
Abstract:
A series of novel pyridazinone and pyridazine chloride derivatives were synthesized and characterized using spectroscopic techniques including infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. All synthesized compounds displayed characteristic peaks consistent with their proposed structures. The compounds were subsequently evaluated for anthelmintic activity using standardized in vitro methods. The results demonstrated that all title compounds exhibited significant anthelmintic activity, with compounds C2 and C6, bearing electron-rich chloro substituents at the 4th position of the pyridazinone and pyridazine chloride nuclei, showing relatively marked anthelmintic potency compared to other analogues. Structure-activity relationship analysis indicated that electron-donating groups, particularly chloro groups, enhanced the anthelmintic efficacy of these compounds. These findings underscore the potential for further structural optimization through the introduction of diverse electron-withdrawing and electron-donating substituents at the 4th position of the pyridazinone and pyridazine chloride scaffolds. The present work suggests that rational molecular design incorporating such substituents may yield significantly more potent anthelmintic and antioxidant agents with improved therapeutic profiles. Future investigations should focus on synthesizing additional analogues with varied electronic properties to identify lead compounds for preclinical and clinical development.

Keywords: Pyridazinone derivatives, pyridazine chloride derivatives, synthesis, IR spectroscopy, NMR spectroscopy, anthelmintic activity, structure–activity relationship, chloro substitution, electron-donating groups, lead optimization.

Introduction

Medicinal chemistry involves the identification, synthesis and development of new chemical entities suitable for therapeutic use. It also includes the study of existing drugs, their biological properties, and their quantitative structure-activity relationships (QSAR). Pharmaceutical chemistry is focused on quality aspects of medicines and aims to assure fitness for the purpose of medicinal products[1]During the early stages of medicinal chemistry development, scientists were primarily concerned with the isolation of medicinal agents found in plants. Today, scientists in this field are also equally concerned with creation of new synthetic drug compounds[3]. Pyridazinones are the heterocyclic molecules having 2 nitrogen atoms in their structure. It is a grey powder with faint vanilla odour and is insoluble in water. The general structure and properties of pyridazinones are as follows:



pyridazin-3(2*H*)-one

Table 1: Physicochemical properties of Pyridazinone

Molecular Formula	$C_4H_4N_2O$
Density	1.28 ± 0.1 g/cm^3
CAS No.	96489-71-3
Formula Weight	96.08736
Surface Tension	50.7 ± 7.0 dyne/cm
Melting point	112
Boiling point	430

Anti-Oxidant activity: Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions.

Anthelmintic activity:

Anthelmintic, any drug that acts against helminthic infections, *i.e.*, those caused by parasitic worms. The term vermifuge is often applied to remedies used to remove intestinal worms; only rarely do the agents directly kill the parasites. No anthelmintic is completely effective, completely without toxic effect upon the host, or equally active against all worms.

Materials and Methods

The following experimental methods were used for the characterization of the synthesized compounds.

- Melting points of the synthesized compounds were determined in open capillary tube and are uncorrected.
- Infrared spectra (υ-cm^{-1}) were recorded on a SHIMADZU FT-IR 4000; using KBr disks.
- NMR spectra were taken on 200 MHz FX – 909 JEOL spectrophotometer using TMS as internal standard and the solvent used as $CDCl_3$.

Instruments

- Single pan electronic balance.

- Hot air oven.
- Heating mantle.
- Hot plate.
- Digital Melting point apparatus.
- Dessicator.
- IR Spectrometer.

Procedure for the synthesis of Pyridazinones and Pyridazine chlorides: Synthesis of substituted aryl butanoic acid:

It was synthesized by Friedel Craft's acylation of substituted benzene with succinic anhydride in presence of aluminium chloride. The mixture was refluxed for 5 hrs. A solution of Conc. HCl (2.5%) was then added to the mixture and concentrated to a small volume by heating on water bath. On cooling the crystalline compound was separated out. The acid gave effervescence test with sodium bicarbonate.

Synthesis of substituted phenyl tetrahydropyridazinones:

The formed compound was condensed with Hydrazine hydrate in Alcoholic solution for 5.2 hrs. After completion of reaction the mixture was poured into crushed ice.

Synthesis of chloro substituted phenyl pyridazine:

The formed pyridazinone was allowed to react with Thionyl chloride. The mixture was condensed for 3.5 hrs. After completion of reaction, mixture was poured into the crushed ice.

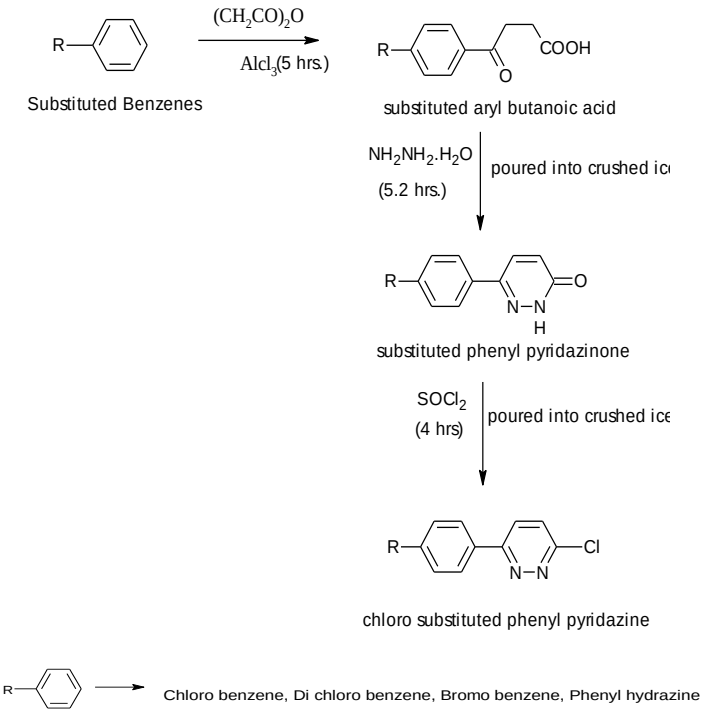
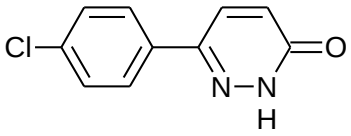
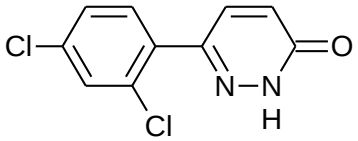
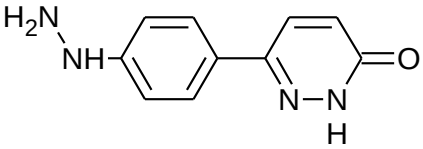
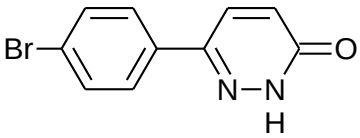
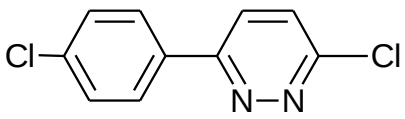
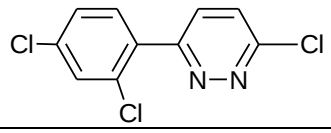
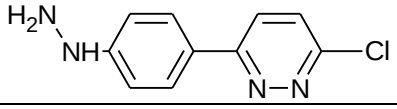
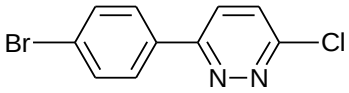


Table 2: Chemicals Used

Succinic anhydride	Lobachemie Laboratory reagents & Fine chemicals
Chloro benzene	Thomas Baker (Chemicals) Limited
Dichloro benzene	S d fine- chem. Limited
Phenyl hydrazine	S d fine- chem. Limited
Hydrochloric acid	Himedia Laboratories Pvt. Ltd.
Ethanol	O R Distilleries, Gajulamandyam
Aluminium chloride	Himedia Laboratories Pvt. Ltd.
Bromo benzene	S d fine- chem. Limited
Thionyl chloride	Qualigens fine chemicals
Hydrazine hydrate	S d fine- chem. Limited

Table 3: Structures and IUPAC Names of Synthesized Compounds

COMPOUND CODE	STRUCTURE	IUPAC NAME
C1		6-(4-chlorophenyl)pyridazin-3(2*H*)-one
C2		6-(2,4-dichlorophenyl)pyridazin-3(2*H*)-one
C3		6-(4-hydrazinylphenyl)pyridazin-3(2*H*)-one
C4		6-(4-bromophenyl)pyridazin-3(2*H*)-one
C5		3-chloro-6-(4-chlorophenyl)pyridazine

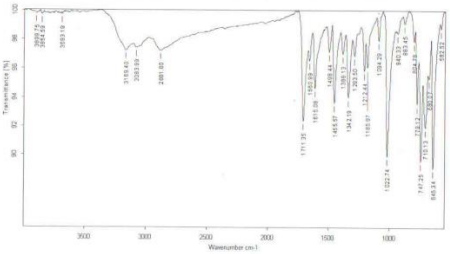
C6		3-chloro-6-(2,4-dichlorophenyl)pyridazine
C7		3-chloro-6-(4-hydrazinylphenyl)pyridazine
C8		3-(4-bromophenyl)-6-chloropyridazine

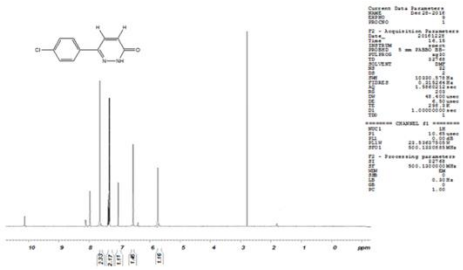
Spectral Analysis: The structure of the synthesized compounds was characterized by MB 8000 series Fourier transform – Infrared Spectrophotometer by KBr - pellet method. IR values and spectras are measured in cm^{-1} and results are shown below.

Table 4: IR interpretation of synthesized compounds

Compound	Position of absorption band (cm^{-1})
C1	3083(C-H stretch in aromatic), 883(=C-N stretch), 1615(N-H stretch in Amides), 1660(C=O stretch in Amides).
C2	3043(C-H stretch in aromatic), 1666.91(C=O stretch in Amides), 850 (=C-N stretch in aromatic).
C3	3036(C-H stretch in aromatic), 852(=C-N stretch), 1544(N-H stretch in Amides),16272(C=O stretch in Amides),
C4	3277(C-H stretch in aromatic), 820(=C-N stretch), 1664(C=O stretch in Amides).
C5	3174(C-H stretch in aromatic), 855(=C-N stretch), 782(C-Cl stretch).
C6	3333 (C-H stretch in aromatic), 872(=C-N stretch), 741, 755(C-Cl stretch).
C7	3340(C-H stretch in aromatic), 832(=C-N stretch), 742(C-Cl stretch).
C8	3142(C-H stretch in aromatic), 784(=C-N stretch)

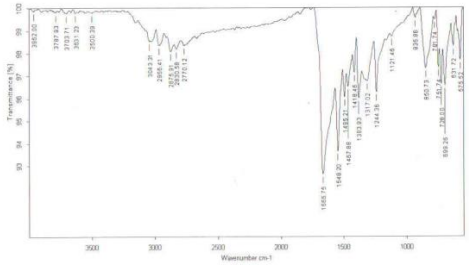
IR,[1] H NMR spectral data (400MHz) of 6-(4-chlorophenyl)pyridazin-3(2*H*) one

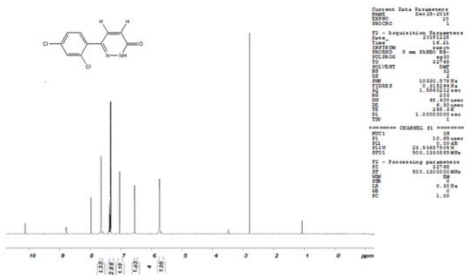




Chemical shift: 7.3 – 7.6 (4H, -CH protons in Benzene ring); 5.7 – 6.5 (2H, -CH protons in Pyridazinone ring), 7.0 (1H, -NH proton in Pyridazinone ring)

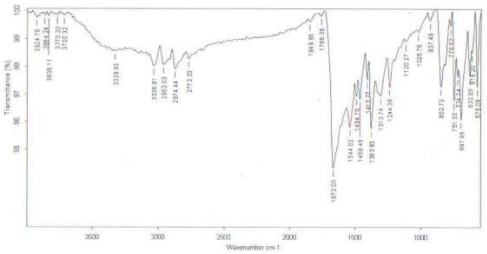
[1]H NMR spectral data (400MHz) of 6-(2,4-dichlorophenyl)pyridazin-3(2H)-one

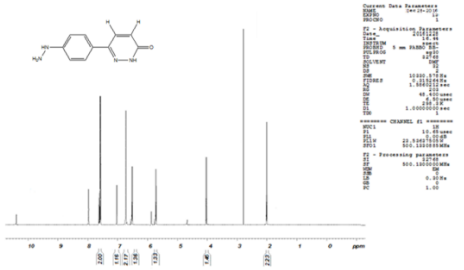




Chemical shift: 7.2 – 7.5 (3H, -CH protons in Benzene ring); 5.7 – 6.5 (2H, -CH protons in Pyridazinone ring); 7.0 (1H, -NH proton in Pyridazinone ring)

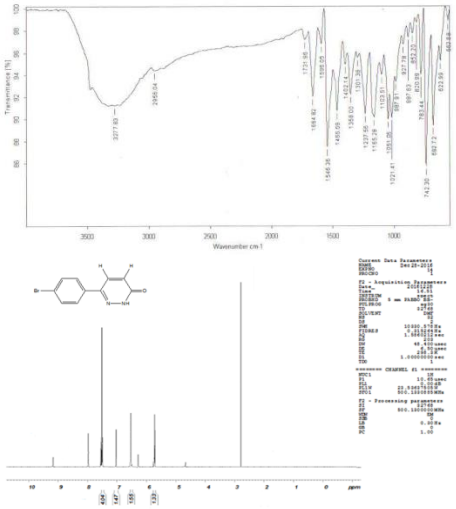
[1]H NMR spectral data (400MHz) of 6-(4-hydrazinylphenyl)pyridazin-3(2H)-one





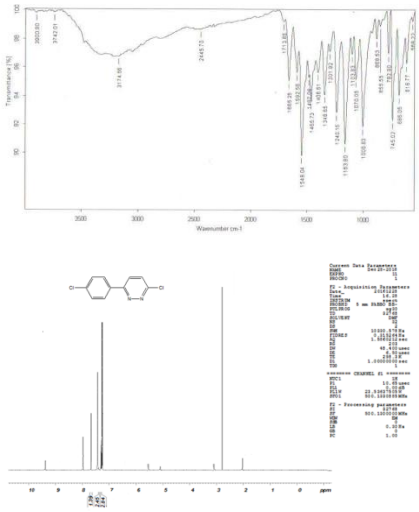
Chemical shift: 6.7–7.5 (4H, -CH protons in Benzene ring); 5.7–6.5 (2H, -CH protons in Pyridazinone ring); 7.0 (1H, -NH proton in Pyridazinone ring); 2.0–4.0 (3H, hydrazine {-$NHNH_2$} protons)

^{1}H NMR spectral data (400MHz) of 6-(4-bromophenyl) pyridazin-3(2H)-one:



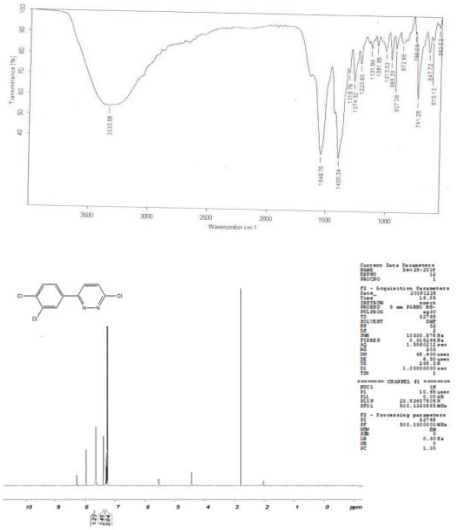
Chemical shift: 7.5 (4H, -CH protons in Benzene ring); 5.7 – 6.5 (2H, -CH protons in Pyridazinone ring); 7.0 (1H, -NH proton in Pyridazinone ring)

^{1}H NMR spectral data (400MHz) of 3-chloro-6-(4-chlorophenyl)pyridazine



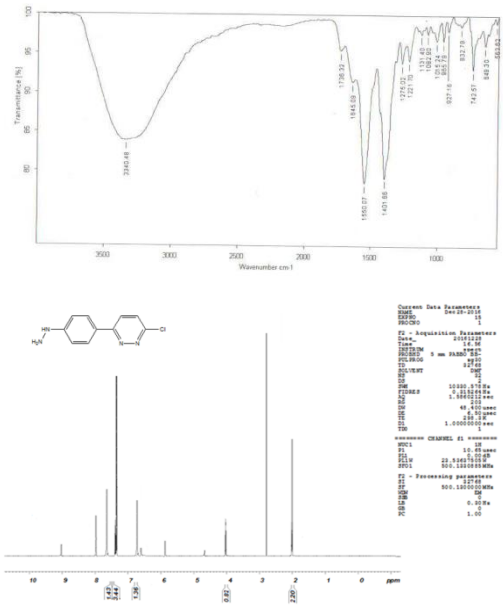
Chemical shift: 7.33 – 7.42 (4H, -CH protons in Benzene ring); 7.31 & 7.71 (2H, -CH protons in Pyridazinone ring)

^{1}H NMR spectral data (400MHz) of 3-chloro-6-(2,4-dichlorophenyl)pyridazine



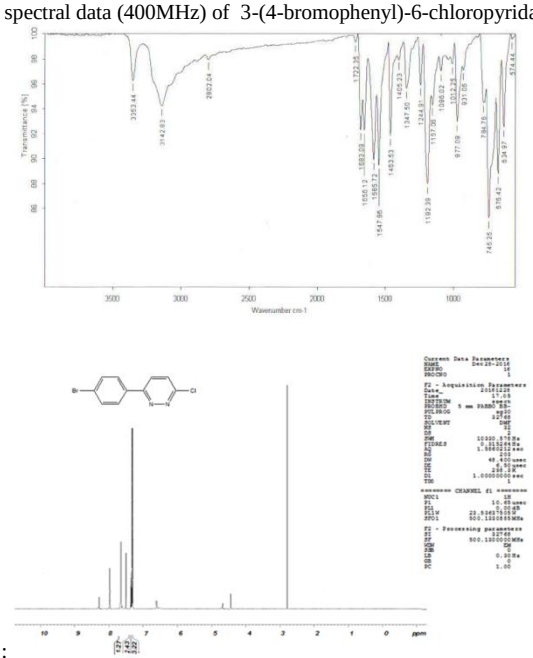
Chemical shift: 7.27 – 7.43 (3H, -CH protons in Benzene ring); 7.31 & 7.71 (2H, -CH protons in Pyridazinone ring)

^{1}H NMR spectral data (400MHz) of 3-chloro-6-(4-hydrazinylphenyl) pyridazine:



Chemical shift: 6.72 – 7.40 (4H, -CH protons in Benzene ring); 7.31 & 7.71 (2H, -CH protons in Pyridazinone ring); 2.0 – 4.0 (3H, hydrazine {-NHNH$_2$} protons)

^{1}H NMR spectral data (400MHz) of 3-(4-bromophenyl)-6-chloropyridazine:



:

Chemical shift: 7.37 – 7.49 (4H, -CH protons in Benzene ring); 7.31 & 7.71 (2H, -CH protons in Pyridazinone ring)

Evaluation of biological activity

Anthelminthic activity:

Healthy adult Indian earthworms, *Pheretimaposthuma*, were used in the study. All earthworms were collected from vermin compost, Kurnool, washed with normal saline to remove faecal matter. All earthworms of equal size 8cm in length were used for all experimental protocol due to its anatomical and physiological resemblance with the human intestinal parasites (Vidyarathi et al., 1977, 1984).

Drug: Albendazole (Standard).

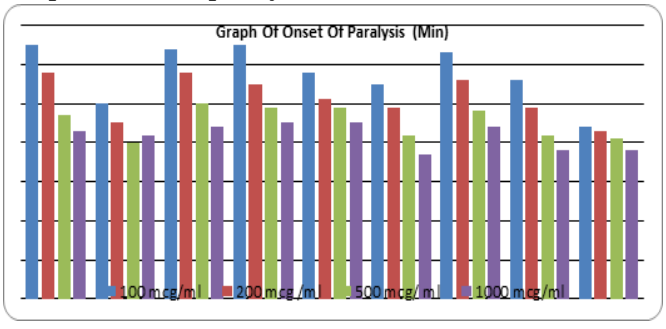
Chemicals: Normal saline.

Procedure: The anthelminthic assay was carried as per the method of Ajaiyeoba et al. (Bate smith, 1962) with minor modifications (Deore et al., 2009). The assay was performed on the adult earthworm, *Pheretima posthuma.* All the test solutions and standard drug solutions were prepared freshly before starting the experiment. Samples of standard drug and test drug was prepared at the concentrations 100,200,500 and 1000µg/ml in distilled water and eight earthworms of approximately 8 cm in length were placed in each nine centimeter petridish containing 25ml of above test solutions of drugs. Albendazole was used as standard drug and normal saline as control. Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was noted after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^0C) followed with fading away of their body colors.

Results and Discussion

A facile method has been devised to synthesize the substituted pyridazinones and pyridazine chlorides. The methods include mild conditions and the yields were satisfactory. The proposed reaction led to the expected products and in all cases the products were obtained in pure form. However, they were purified by recrystallization from ethanol. The yields of the synthesized compounds were found to be in the range from 70%-90%. Structures of synthesized compounds were characterized and confirmed with the help of analytical data such as IR and H^1 NMR. All the title molecules were predicted for biological activity by using PASS Software. The title compounds were predicted to be safe and non-toxic. Among all the synthesized compounds C_2 and C_6 exhibited significant anthelmintic activity remaining all compounds exhibited mild to moderate activity using albendazole as standard results shown in following table

Graph of onset of paralysis:



Graph of Onset of Death

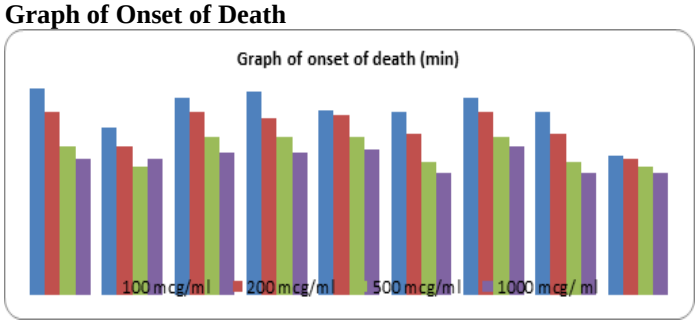


Table 8: Paralysis and onset of death some substituted pyridazine derivatives

Conclusion

All the title compounds were synthesized, characterized and screened for their anthelmintic activity. All the title compounds show characteristic peaks in IR and NMR spectra. The results of anthelmintic activity revealed that all title compounds exhibited significant activity. Title compounds C_2 and C_6 having electron rich chloro groups exhibited relatively marked anthelmintic activity. The study revealed the importance of synthesizing many more compounds with substitution at 4^{th} position of pyridazinone and pyridazine chloride nucleus having electron withdrawing as well as electron donating groups. Such compounds may emerge as much more potent anthelmintic and antioxidant agents.

References

[1] 6^{th} Ed. New York, Lippincott Williams and Wilkins publication, 2002: 1-9.
[2] Foye WO, Williams DA, Lemke TL. Foye's principles of Medicinal Chemistry,
[3] New York, Wiley-interscience, 1995: 1-5.
[4] Graham Patrick L, An Introduction to Medicinal Chemistry, 2^{nd} ed. New York,
[5] M.S.Y. Khan and A.A. Siddiqui, *Indian J. Chem.*,39B, 614(2000).
[6] J.G. Longo, I. Verde and M.E. Castro, *J.Pharm. Sci.*,82,3, 286(1993).
[7] A.A. Siddiqui and S.M. Wani, *Indian J.Chem.*,43B, 1574(2004).
[8] I. Sircar, R.P. Stephen, G. Bobowshki, *J. Med. Chem.*,32,342(1989).
[9] S. Demirayak, A.C. Karaburun and R. Besi, *Eur. J. Med. Chem.*,39,1089(2004).
[10] A. Monge, P. Parrado and M. Font, *J. Med. Chem.*,30,2910(1987).
[11] R.S. Leonard, N. Anbalagan, and V. Gunasekran, *Bio. Pharm. Bull.*,26,1407(2003).
[12] A. Akahane, H. Katayama, T. Mitsunaga, *J. Med. Chem.*,42,5,781(1999).
[13] D.G.H. Livermone, R.C. Bethell, and N.Cammack*J. Med. Chem.*,36,3784(1993).
[14] Almansa, C.; de Arriba, A. F.; Cavalcanti, F. L.; Go´mez, L. A.; Miralles, A.; Merlos, M.; Garcı´a-Rafanell, J.; Forn, J. *J. Med.Chem.*, 44, 35(2001).
[15] Sies, Helmut. "Oxidative stress: Oxidants and antioxidants". *Experimental physiology* 82 (2): 291–5, (1997).

[16] Davies, KJ. "Oxidative stress: The paradox of aerobic life". *Biochemical Society Symposia* 61: 1–31, (1995).

[17] Vertuani, Silvia; Angusti, Angela; Manfredini, Stefano , "The Antioxidants and Pro-Antioxidants Network: An Overview". *Current Pharmaceutical Design* 10 (14): 1677–1694,(2004).

[18] Valko, M; Leibfritz, D; Moncol, J; Cronin, M; Mazur, M; Telser, J "Free radicals and antioxidants in normal physiological functions and human disease". *The International Journal of Biochemistry & Cell Biology* 39 (1): 44–84, (2007).

[19] Stohs, S; Bagchi, D "Oxidative mechanisms in the toxicity of metal ions". *Free Radical Biology and Medicine* 18(2): 321–36, (1995).

[20] Nakabeppu, Yusaku; Sakumi, Kunihiko; Sakamoto, Katsumi; Tsuchimoto, Daisuke; Tsuzuki, Teruhisa; Nakatsu, Yoshimichi. "Mutagenesis and carcinogenesis caused by the oxidation of nucleic acids". *Biological Chemistry* 387 (4): 373–379, (2006).

[21] Valko, Marian; Izakovic, Mario; Mazur, Milan; Rhodes, Christopher J.; Telser, Joshua. "Role of oxygen radicals in DNA damage and cancer incidence". *Molecular& Cellular Biochemistry* 266 (1–2): 37–56, (2004).

[22] Stadtman, E. "Protein oxidation and aging". *Science* 257 (5074): 1220–4, (1992).

[23] Miller, RA; Britigan, BE "Role of oxidants in microbial pathophysiology". *Clinical Microbiology Reviews*10 (1): 1–18, (1997).

[24] Chaudiere, J; Ferrari-Iliou, R "Intracellular Antioxidants: From Chemical to Biochemical Mechanisms". *Food and Chemical Toxicology* 37 (9–10): 949–62, (1999).

[25] Sies, Helmut "Strategies of antioxidant defense". *European Journal of Biochemistry* 215 (2): 213–219, (1993).

[26] Imlay, James A. "Pathways of Oxidative Damage". *Annual Review of Microbiology* 57: 395–418, (2003).

[27] Stohs, S; Bagchi, D "Oxidative mechanisms in the toxicity of metal ions". *Free Radical Biology and Medicine* 18(2): 321–36, (1995).

[28] Ames B, Cathcart R, Schwiers E, Hochstein P, "Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis". *Proc Natl Acad Sci USA* 78 (11): 6858–6862, (1981).

[29] Khaw, Kay-Tee; Woodhouse, Peter, "Interrelation of vitamin C, infection, haemostatic factors, and cardiovascular disease". *BMJ* 310 (6994): 1559–1563, (1995).

[30] , P; Travacio, M; Repetto, M; Escobar, J; Llesuy, S; Lissi, EA, "Evaluation of Total Reactive Antioxidant Potential (TRAP) of Tissue Homogenates and Their Cytosols". *Archives of Biochemistry and Biophysics*388 (2): 261–266, (2001).

[31] Morrison, John A.; Jacobsen, Donald W.; Sprecher, Dennis L.; Robinson, Killian; Khoury, Philip; Daniels, Stephen R. "Serum glutathione in adolescent males predicts parental coronary heart disease". *Circulation* 100 (22): 2244–2247, (1999)..

[32] Akiba, S; Matsugo, S; Packer, L; Konishi, T" Assay of Protein-Bound Lipoic Acid in Tissues by a New Enzymatic Method". *Analytical Biochemistry* 258 (2): 299–304, (1998).

[33] Glantzounis, G. K.; Tsimoyiannis, E. C.; Kappas, A. M.; Galaris, D. A., "Uric Acid and Oxidative Stress". *Current Pharmaceutical Design* 11 (32): 4145–4151, (2005).

[34] El-Sohemy, Ahmed; Baylin, Ana; Kabagambe, Edmond; Ascherio, Alberto; Spiegelman, Donna; Campos, Hannia, "Individual carotenoid concentrations in adipose tissue and plasma as biomarkers of dietary intake". *The American journal of clinical nutrition* 76 (1): 172–179, (2002).

[35] Sowell, Anne L.; Huff, Daniel L.; Yeager, Patricia R.; Caudill, Samuel P.; Gunter, Elaine W, "Retinol, alpha-tocopherol, lutein/zeaxanthin, beta-cryptoxanthin, lycopene, alpha-carotene, trans-beta-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multi wavelength detection". *Clinical chemistry* 40 (3): 411–416, (1994).

[36] Stahl, W; Schwarz, W; Sundquist, AR; Sies, H "cis-trans isomers of lycopene and β-carotene in human serum and tissues". *Archives of Biochemistry and Biophysics* 294(1): 173–177, (1992).

[37] Zita, ČEstmír; Overvad, Kim; Mortensen, SvendAage; Sindberg, Christian Dan; Moesgaard, Sven; Hunter, Douglas A., "Serum coenzyme Q10 concentrations in healthy men supplemented with 30 mg or 100 mg coenzyme Q10 for two months in a randomised controlled study". *BioFactors* 18 (1–4): 185–93, (2003).

[38] Beris, H., Antioxidant effects a basis of drug selection, *Drugs*, 42,1991, 569-605.

[39] Jacob,V. and Michael, A., Nutritional antioxidants: mechanism of action, analyses of activities and medical applications, *Nutrition*, 49, 1999,1-7.

[40] Ashok, K.J., Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidant therapy, *Current Science*, 2001, 1179-1186.

[41] David, G.B., Erik, E.A., Rohini, S. and Alfins, Antioxidant enzyme expression and ROS damage in prostatic intraepithelial neoplasia and cancer, *Cancer*,89, 2000, 124-134.

[42] Vani, T., Rajani, M., Sarkar, S. and Shishoo, C.J., Antioxidant properties of the ayurvedic formulation triphala and its constituents, *Inter. J. Pharmacognosy*,35, 1997, 313-317.

[43] Sanchez-Moreno, C., Larrauri, J. and Saura-Calixto, F., Free radical scavenging capacity of selected red and white wine, *J. Sci. Food. Agric.*, 79, 1999, 1301-1304.

[44] Navarro, M.C…. et al., Free radical scavenger and anti hepatotoxic activity of rosmarinus tomentosus, *Plantamedica*, 59, 1993, 312-314.

[45] Babu, B.H., Shylesh, B.S. and Padikkala, J., Antioxidant and hepatoprotective effect of Alanthus icicifocus, *Fitoterapia*, 72, 2001, 272-277.

[46] Robak, J. and Gryglewski, R.J, Flavonoids are scavengers of superoxide anions, *Biochem.Pharmcol.*, 37, 1998, 837-841.

[47] Jayaprakash, G.K., Singh, R.P. and Sakariah, K.K., Antioxidant activity of grape seed extracts on peroxidation models in-vitro, *J.Agric Food Chem.,* 55, 2001, 1018-1022.

[48] HyeRhi Cho et al., Peroxynitrite Scavenging Activity of Herb extracts, *Phytother. Res.,* 16, 2002, 364-367.

[49] Rice-Evans, C and Miller, N.J., Total antioxidant status in plasma and body fluids, *Methods Enzymol,* 243, 1994, 279-293.

[50] Kanner, J et al., Natural antioxidants in grapes and wines, *J. Agric. Food. Chem.,* 42, 1994, 64-69.

[51] Simonetti, P., Pietta, P. and Testolin, G., Polyphenol content and total antioxidant potential selected Italian wines, *J. Agric. Food.Chem.,* 45, 1997, 1152-1155.

[52] Vinson, J.A and Hontz, B,A., Phenol antioxidant index: comparative antioxidant effectiveness of red and white wines, *J. Agri. Food. Chem.,* 43, 1995, 401-403.

[53] Frei, B., Stocker, R., England, L. and Ames, B.N., Ascorbate the most effective antioxidant in human blood plasma, *Advances in medicinal experiments and biology*, 264, 1990, 155-63.

[54] Cao, G., Alessio, H.M. and Culter, R.G., Oxygen radical absorbance capacity assay for antioxidant free radicals, *Bio. Med.,*14, 1993, 303-311.

[55] Joseph, K… et al, Natural antioxidants in grapes & wines, *J. Agri food chem.,* 42, 1994.

[56] Pieroni, A… et al, In vitro anti-oxidant activity of ethnic Albanians in southern Italy, *Phytother. Res.,* 16, 2002, 467-473.

[57] Opoku, A.R., Maseko, N.F. and Terblanche, S.E., The in vitro antioxidative activity of some traditional Zulu medicinal plants, *Phytother. Res.,* 16, 2002, S51-S56.

[58] Benzie, I.F.F. and Strain, J.J.,The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP Assay, *Anal. Biochem.,* 239, 1996, 70-76.

[59] Ho, K.Y., Huang, J.S., Tsai, C.C., Lin, T.C and Lin, C.C (1999) "Antioxidantactivity of tannin component from Vacciniumvitisidaea" *Pharm.Pharmcol*:51:1075-78

[60] Chiaki, S and Naomi, O (1998) "Antioxidative polyphenols isolated from Theobromacocoa" *J.Agric Food Chem:* 46: 454-57.

[61] Kimura, Y., Okuda, H and Okuda, T (1984) "Studies on activities of tanninsand related compounds" *Plantamed*:50: 473-76

[62] Gutteridge, J.M.C and Wilkins, S (1986) "Copper salt dependent hydroxylradical formation. Damage to proteins acting as antioxidants" *Biochem biophys Acta:* 754: 38-41

[63] Weiqiang Xing, Yan Fu, Zhang Shi, Dong Lu, Haiyan Zhang, Youhong Hu. "Discovery of novel 2,6-disubstituted pyridazinone derivatives as acetylcholine esterase inhibitors." *Eur. J. Med. Chem.,* pp 95-103, May 2013.

[64] Makarem M. Saeed, Naida A. Khalil, Eman M. Ahmed, and Kholoud I. Eissa. "Synthesis and Anti-inflammatory Activity of Novel Pyridazine and Pyridazine Derivatives as Non- ulcerogenic Agents". *Arch. Pharm. Res., Vol. 35, No. 12,pp 2077-2092, 2012.*

[65] Asif Husain, Aftab Ahmad, Anil Bhandari, Veerma Ram. "Synthesis and Antitubercular activity of Pyridazinone derivatives". *J. Chil. Chem. Soc., Vol. 56, No. 3, pp. 778-780, 2011.*

[66] Ravinesh Mishra, Anees A, Siddiqui, Asif Husain, Mohd. Rashid, AtishPrakash, MukulTailang, Muneesh Kumar, Neeti Srivastava. "Synthesis, Characterization and Anti-Hypertensive activity of some New Substituted Pyridazine derivatives". *J.Chil.Chem. Soc., 2011, 56(4): 856-859.*

[67] Sridhar Thota, Ranju Bansal. "Synthesis of new Pyridazinone derivatives as Platelet aggregation inhibitors" *Med. Chem. Res., 19, pp 808-816, 2010.*

[68] Pooja S. Banerjee, P.K. Sharma and Rajesh Nema. "Synthesis and Anticonvulsant activity of Pyridazinone Derivatives". *International j. of Chem Tech Research, Vol. 1, pp 522-525, July- Sept 2009.*

[69] Said Ahmed Soliman Ghozlan, Ismail Abdelshafy Abdelhamid, Mohamed HilmyElnagdi. "Functionally substituted arylhydrazones as building blocks in heterocyclic synthesis: routes to pyridazines and pyridazinoquinazolines" *Arkivoc, (xiii),pp 147-157, 2006.*

[70] Eddy SOTELO, Alberto COELHO, and Enrique RAVIÑA. "Stille-Based Approaches in the Synthesis of5-Substituted-6-phenyl-3(2*H*)-pyridazinones". *Chem. Pharm. Bull. Vol.* 51, Issue 4, pp 427—430, 2003.

[71] Richard Hoogenboom, Guido Kickelbick, and Ulrich S. Schubert. "Synthesis and Characterization of Novel Substituted 3,6-di(2-pyridyl)pyridazine Metal-Coordinating Ligands". *Eur. J. Org. Chem.,* 4887-4896, 2003.

[72] Chun Sing Li, Christine Brideau, Chi Chung Chan, Stella Charleson, Elizabeth wong. "Pyridazinones as selective cyclooxygenase-2 inhibitors". *Bioorganic and medicinal chemistry letters, Vol. 13, Issue 4, pp 597-600, 2003.*

[73] Deniz S. Dogruer, M. FethiSahin. "Synthesis and Analgesic and Anti- inflammatory activity of New Pyridazinones". *Turk. J. Chem. Vol. 27, pp 727-738, 2003.*

[74] David J.Katz, Dean S. Wise and Leroy B. Townsend. "Synthesis and Chemistry of some Pyridazine Nuclosides Related to certain 5- Substituted Pyrimidine Nucleosides". n*J. Heterocyclic chem., 20, 369 (1983).*

[75] Jack G. Samaritoni, George Babbitt. "Homolytic alkylations of substituted pyridazines".n*J. Heterocyclic chem., Vol. 28., Issue 3, pp- 583-587,1991.*

[76] The Conjugate Addition of tert-Butylmagnesium Chloride to 3,6- Disubstituted Pyridazines.*Acta Chem. Scand. Vol. 16, No. 8, 1962.*

[77] Addition of Grignard reagents to Pyridazines. *ActaChem. Scand. Vol. 21, No. 8, 1967.*

[78] Ingolf Crossland and Hans Kofod. "Dimethylamination of Chloropyridazines". *Acta Chem. Scand. Vol. 21, No. 8, 1967.*

[79] William V. Curran, Adma Ross. "6- Phenyl-4,5-dihydro-3(2H)-Pyridazinones. Series of Hypotensive

agents". *J. Med. Chem., Vol. 17, Issue 3, pp 273-281, 1974.*

[80] Peater Maatyus. "3(2H)-Pyridazinones: Some recent aspects of synthetic and Medicinal chemistry". *J. of heterocyclic chem., 1998, 35(5): 1075-10891*

[81] F. A. Yassin, A. F. EL- Farargy, M.M. El- Mobayed, M.Y. El- Kady, and M. R. ABD El- Maksoud. "Synthesis and Reaction of 6(9'-Anthracenyl)-3(2H)-Pyridazinone Derivatives and study of their Microbiological Activity". *Jour. Chem. Soc. Pak., Vol. 13, No. 4, 1991.*

[82] Kumar, K. A., Mohanakrishna, A., Sudheer, M., Rajesh, K. S., & Ramalingam, P. (2011). UV-Spectrophotometric method for the estimation of Alprazolam in tablet dosage Form. *Int. J. Chem. Tech. Res*, *3*(1), 161-164.

[83] Sudheer, M., Rao, A. N., Theja, D. H. H., Prakash, M. S., Ramalingam, P., & Mohan, A. M. (2012). Development of stability indicating RP-HPLC method for simultaneous determination of azithromycin and ambroxol HCl (SR) in the tablet formulation. *Der Pharmacia Lettre*, *4*(3), 803-810.

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