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Computer-Aided Interpretation of Vero Cell Line Enhancement and Classification Using Image Processing Methods

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Abstract- Vero cell line classification using computer based automated analysis is proposed in this paper. The method aims to help cancer research and can reduce the burden of manual labelling. The impact of metal complexes when applied on cancer cell lines is the aim of this study. A combination of methyl, ethyl, phenyl groups of Ruthenium and thiosemicarbazone (TSC) is used as the metal complex. The structure variations of cultured Vero cell lines when treated with TSC metal complexes were analysed. Hybrid Switching Filter is used as a pre processing method for enhancing the synthesised cell line images . The filter is used to remove impulse noises which degrade the image during image acquisition due to poor lighting conditions or faulty switching elements. A hybrid approach is used in the filter which combines linear and nonlinear concepts. Using boundary detectors, these pre-processed images are then segmented. For edge detection, Canny's algorithm is used which is based on abrupt pixel value variations. As a reliable decision maker, this method can be used to replace the costly short tandem repeat (STR) analysis which is used for laboratory studies because of the advantageous factors like accuracy, cost effectiveness and time consumption.

Index Terms- Thiosemicarbazones; Vero cell lines; Ruthenium; Digital image enhancement; Canny's algorithm.

1. INTRODUCTION

Cancer is a collection of related diseases by which the body cells begin to divide and spread into surrounding tissues without stopping. This results in mass of uncontrolled abnormal cells. Around 16 million new cases were recorded in 2015 with a cancer related worldwide death report of 8 million deaths. Cancer is classified into five main types namely carcinoma, sarcoma, lymphoma, leukaemia and adenoma. Factors influencing cancer are gene mutation(Sau Har Lee *et al*., 2011), carcinogens (free radical formation), hereditary**,** chemicals, viruses, radiation or spontaneous mutations, different lifestyle like cigarette smoking, dietary factors (Murugan *et al*., 1999).

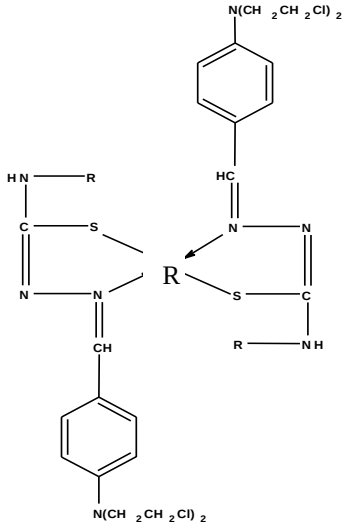
According to David Karnofsky (2008) it is the type, age, health status, the stage of the cancer and additional personal characteristics that depends the treatment of cancer. It includes surgical removal of the cancer from the body (Parekh and Chanda 2007), Chemotherapy administered (Teitz, *et al*., 1994), radiations (Kinzler, *et al*., 2002), Gene therapy which replaces damaged genes (Schneider, 2005), Immunotherapy (Prinessa Chellan, *et al*., 2001) and Hormone therapy (Manjul Tiwari, 2012). Cancer specific immune system cells (induced pluripotent stem cells) help to enhance the immune system. Knowledge of a biological target is very essential in finding new medications in drug designing. The drug is mainly an organic small molecule which can activate the function of a bio molecule like a protein, which can results in a therapeutic advantage to the patient. Ligand-based design of drugs is the commonly used one which is based on the information of other molecules that binds to the biological target. The activity of new analogs is predicted by a quantitative structure-activity relationship (QSAR). The study aims to explore the anticancer properties of thiosemicarbazone (TSC) and its complex along with Ruthenium metal.

Thiosemicarbazone (TSC) belongs to thiourea derivatives that are derivatives of parent aldehydes or ketones. According to Kizilcikli, *et al*. (2006), the versatile ligand properties of TSC and their metal complexes have gained significant attention in coordination chemistry. The studies by Floyd *et al*. (2012), developed Triapine® (3-aminopyridine-2-carboxaldehyde TSC) as an anticancer drug and is now in clinical phase II on several cancer types. Thiosemicarbazone's Metal Complexes.

1.1. *Thiosemicarbazone's Metal Complexes*

From the metallic state, when metals lose electrons, positively charged ions are formed which are soluble in biological fluids. Under relevant physiological conditions, Ruthenium can have a wide range of oxidation states (II, III and IV). Between

those oxidation states, the energy barrier to inter conversion is relatively low, results in oxidation state changes inside the cell. Due to these metal complexes, ligand exchange rates are slowed and for the remainder of that cell's lifetime, Ruthenium ion binds tightly to the cell.



R: -H, -CH_3,-C_2H_5, -C_6H5

Fig. 1. Structure of Ruthenium thiosemicarbazone complex

Because of the higher effective nuclear charge (Zeff), Ruthenium complexes have fewer side effects (Prabhakaran *et al.*, 2011). The cell line structural variations because of the biochemical reactions on treating with stimulating agents are analyzed using an image enhancement method followed by a segmentation method. The chance of image degradation by impulse noise can be surmounted by the use of the Hybrid Switching Filter (Sreejith L Das and Alamelu Nachiappan, 2012). The filter removes the impulse noise (Krishnan Nallaperumal *et al.*, 2006) by substituting the noisy pixel values with a new one. The entire image is scanned by using a 3x3 mask. Each step, nine pixel values will be obtained and are sorted. The mean of middle three values is taken for the replacement of noisy pixel. This process repeats for all the noisy pixels in the image. This enhanced image is then given for segmentation. Such automated cell line classification facilitates in improving and speed up various areas of cancer research. Several types of cell lines can be tagged and detected with this easy-to-use tool. Authentication of human cell lines by STR analysis is a time consuming and tedious process which can be replaced by this automated approach. The structure variations of the synthesized cell line images have to be analyzed by considering the features extracted after edge detection. The amount of variation from the base image gives the measure of impact on metal complexes. The entire process is shown in the block diagram given in figure 2.

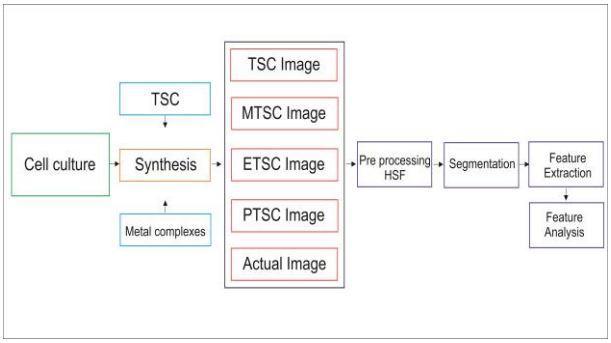


Fig. 2 Block diagram of the proposed method

2. MATERIALS AND METHODS

2.1. *Cell Culture*

2.1.1. *Vero cells*

Vero cell lines are derived from the kidney tissue of a well differentiated renal carcinoma. The morphology of these cells are epithelial.

The condensation of p-[N, N-Bis (2-chloroethyl) aminobenzaldehyde and ethylthiosemicarbazide in ethanol at ambient temperature resulted in the synthesis of the Schiff base compound, p-[N, N-Bis (2-chloroethyl) aminobenzaldehyde-methyl/ ethyl/ phenyl TSC. The yellow crystalline precipitate of p-[N, N-Bis (2-chloroethyl) aminobenzaldehyde-methyl/ ethyl/ phenyl Thiosemicarbazone is used to study the structural and spectral properties.

2.1.2. *Complexes synthesized and used*

1. Complex : Ruthenium + TSC
2. Complex : Ruthenium + Methyl TSC
3. Complex : Ruthenium + Ethyl TSC
4. Complex : Ruthenium + Phenyl TSC

2.1.3. *Synthesis of the ligands*

Ligand: Methyl/Ethyl/Phenyl TSC

2.1.4. *Synthesis of the Metal TSC Complexes*

A mixture of Ruthenium chloride (1 mmol) and CEAB-TSC/MTSC/ETSC/PTSC (2 mmol) in ethanol was stirred using magnetic stirrer for 4 hours at room temperature. It was set aside overnight, a crystalline product separated out, which was filtered, washed with cold ethanol and dried in vacuum.

2.1.5. Solution Making

All the TSC complexes of Ruthenium are soluble in DMSO. According to the molecular weight, the strength of all the drug solutions was set up as 1 µ molar. The Vero carcinoma cell lines were obtained from NCCS Pune India. The cell lines were cultured at 37^0C under 5% CO_2 in standard Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 1% Non- essential amino acid and 1% penicillin/streptomycin(GIBCO Invitrogen) up to 70% confluency.

Media and glassware were sterilized in an autoclave at 15 lbs pressure/inch^2 for 20 min.

2.1.6. Cytotoxicity assay

The cytotoxicity test for the various TSC compounds and complexes were evaluated using, MTT method. Vero cells were seeded (3×10^4/well) in 96-well plates in 10µl of growth medium (MEM) containing 10% FCS mixture in each well incubate at 37°C in a 5% CO2 incubator. After 24 h of monolayer cell cultivation, the medium was removed and replaced by a 100 µl of varying concentrations (25-1000 µg/ml) of the compounds and complexes of TSC polysaccharides in MEM medium containing 2% FCS in respective wells, control cell contain MEM medium containing 2% FCS incubate at 37°C in a 5% CO2. 20 µl of MTT in (5mg/ml) PBS solution/well were added after 72 h incubation to incubate at above condition for 4 h. Crystal formation was observed, after which the medium was replaced by 100 ml of DMSO solution in each well, An Elisa reader at 620 nm was used to measure the optical density of each well. All experiments were carried out in triplicate, and data in the form of mean ± SD are recorded **(**Kizilcikli *et al*., 2006 and Manjul Tiwari, 2012**)**.

2.2. *Feature Extraction*

Images were acquired with Olympus CKX41 inverted microscope using Olympus DP72 camera with 20X objective. STR profiling is used to check the authentication of the cell lines regularly. The various parameters **(**Ravi *et al*., 2012) used for feature analysis after applying Canny's algorithm (Canny, 1986) along with the equations used are given below.

Mean Squared Error

$$MSE = \frac{1}{MN}\sum_{i=1}^{M}\sum_{j=1}^{N}(x(i,j)-y(i,j))^2 \quad (1)$$

Peak Signal to Noise Ratio

$$PSNR = 10\log_{10}\frac{(2^n-1)^2}{\sqrt{MSE}} \quad (2)$$

Average Difference

$$AD = \frac{1}{MN}\sum_{i=1}^{M}\sum_{j=1}^{N}(x(i,j)-y(i,j)) \quad (3)$$

Mean Absolute Error

$$MAE = \frac{1}{MN}\sum_{i=1}^{M}\sum_{j=1}^{N}|x(i,j)-y(i,j)| \quad (4)$$

Normalized Cross-Correlation

$$NK = \frac{\sum_{i=1}^{M}\sum_{j=1}^{N}(x(i,j)\times y(i,j))}{\sum_{i=1}^{M}\sum_{j=1}^{N}(x(i,j))^2} \quad (5)$$

Structural Content

$$SC = \frac{\sum_{i=1}^{M}\sum_{j=1}^{N}y(i,j)^2}{\sum_{i=1}^{M}\sum_{j=1}^{N}(x(i,j))^2} \quad (6)$$

Image Fidelity

$$IF = 1 - \frac{\sum_{i=1}^{M}\sum_{j=1}^{N}(x(i,j)-y(i,j))^2}{\sum_{i=1}^{M}\sum_{j=1}^{N}(x(i,j))} \quad (7)$$

Peak Mean Square Error

$$PMSE = \frac{1}{MN} \times \frac{\sum_{i=1}^{M}\sum_{j=1}^{N}(x(i,j)-y(i,j))^2}{MAX(x(i,j))^2} \quad (8)$$

Structural Similarity Index Metric

$$SSIM = \frac{(2\times\bar{x}\times\bar{y}+C1)(2\times\sigma_{xy}+C2)}{(\sigma_x^2+\sigma_y^2+C2)\times((\bar{x})^2+(\bar{y})^2+C1)} \quad (9)$$

Where $x(i, j)$ and $y(i, j)$ represents the input image and distorted image respectively and i and j are the pixel position of the M×N image. C1 and C2 are constants, $\bar{x}$ and $\bar{y}$ are averages of x and y respectively, σ_x^2 and σ_y^2 are the variances of x and y, and σ_{xy} is the covariance of x and y.

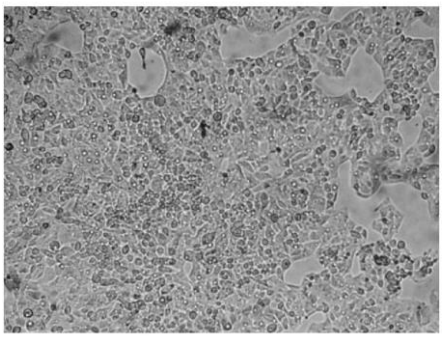
3. RESULTS AND DISCUSSION

The proposed automated cell line classification system for Vero cell line images can aid the biologist as a second reader and the need for time-consuming, costly and biochemical tests can be avoided. Thiosemicarbazone metal complexes were taken for cell culture. Cell structure analysis requires metal complexes and its importance in biological activities. The use of lipophilic nature of the Thiosemicarbazone metal complex than free ligand enhanced the interaction of metal ions with the organism. The biological activity is increased due to the chelate complexes of metal ion. The Ruthenium complexes having bulky terminal group exhibit higher biological activity. Phenyl group is considered as more stable with highest biological activity when compare to other ligands in the order Ph>Et>Me>H. D block metals shows less biological toxicity compared to others (Kalaivani, *et al*., 2012, David Hecht, *et al*., 2012). The ability of Ruthenium to form octahedral complexes and higher electro negativity results in stronger bonding with bio molecules gave chance to select Ruthenium (Prabhakaran, *et al*., 2011). Due to the higher IC_{50} value of Ruthenium as shown in table.1, Ruthenium Phenyl l Thiosemicarbazone is showing higher biological activity.

Table -1: IC_{50} Values of the synthesised complexes

Name of the complexes	IC_{50} Values of vero cells in µ molar
RuTSC	0.19
RuMTSC	0.31
RuETSC	0.44
RuPTSC	0.62

The cell line images acquired after the synthesis of cell lines with metal complexes were then given to preprocessing. The hybrid switching filter restores the degraded images and is applied to Segmentation block. Canny's edge detection algorithm is used for further analysis. The resultant images are shown in figure 3. In his paper, (Furkan Keskin et al, 2013) used a similar approach for the classification of Human Carcinoma Cell images Using Complex Wavelet-Based Covariance Descriptors.



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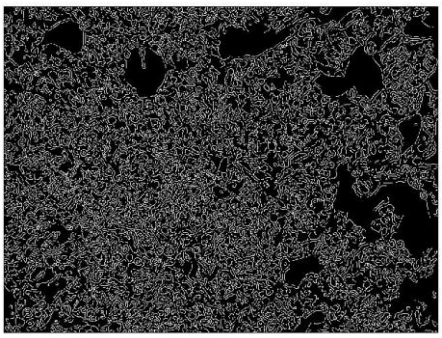

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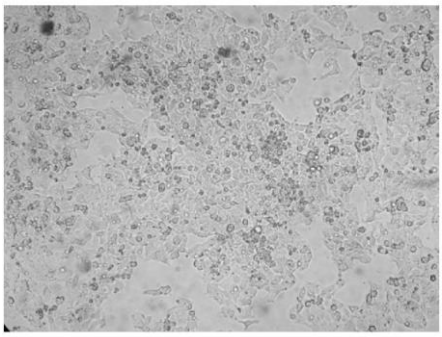

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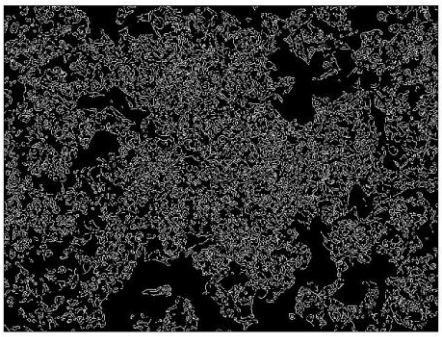

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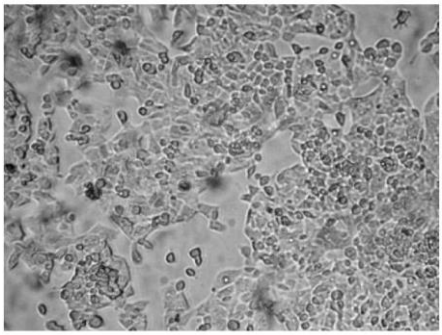

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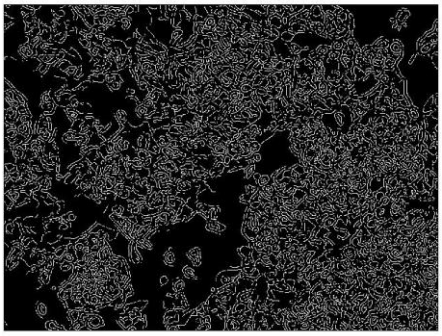

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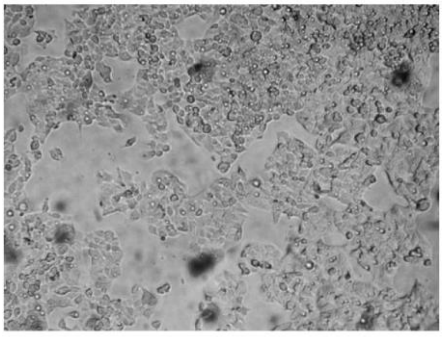

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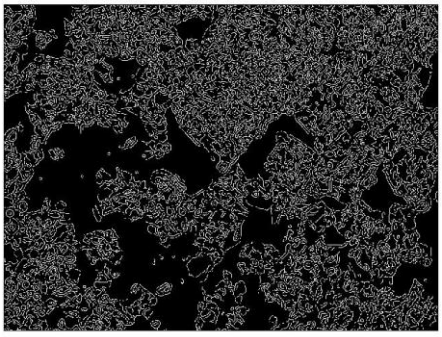

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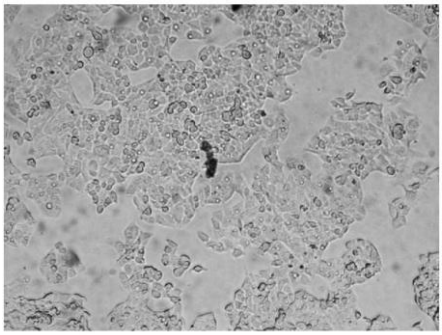

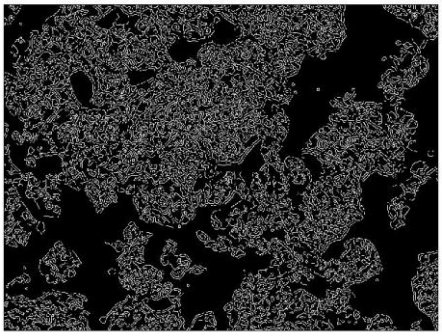

i **j**

Fig. 3 (a) Actual Vero cell line image (c) Ruthenium Thiosemicarbazone cell line image (e) Ruthenium Methyl Thiosemicarbazone cell line image (g) Ruthenium Ethyl Thiosemicarbazone cell line image (l) Ruthenium Phenyl Thiosemicarbazone cell line image (b,d,f,h,j) corresponding segmented images

Table 2 shows the statistical arrangement of the extracted parameters. It can be seen that there is a decrease in the values of error metrics such as MAE, AD, MSE, NK, and PMSE from RuPTSC to RuTSC. Similarly the values of SSIM, SC, IF and PSNR increases from RuPTSC to RuTSC. On the whole, the investigation shows that RuPTSC is less and RuTSC is more similar when compared with actual cell line images. The comparison bear out the impact of RuPTSC complex, which shows more influence on comparing with its methyl and ethyl counterparts.

Table – 2: Parameters analyzed for various quantities of Ruthenium (Ru) complexes for 1 µ molar strength solution

Parameters	RuPTSC	RuETSC	RuMTSC	RuTSC
MSE	0.58	0.54	0.50	0.48
PSNR	41.52	44.42	46.53	49.35
AD	0.05	0.05	-0.04	-0.01
MAE	0.33	0.31	0.28	0.28
NK	0.23	0.20	0.14	0.15
SC	0.79	0.89	1.25	1.26
IF	-0.64	-0.57	-0.40	-0.40
PMSE	0.00	0.00	0.00	0.00
SSIM	0.41	0.59	0.65	0.69

4. CONCLUSION

The day by day increase in the number of cancer cases show the importance of proposed method. As a result, by using the automated detection and classification, the researchers may get benefitted by alleviating their tedious time consuming STR analysis for large number of images. The study can be concluded by proving the efficiency of the proposed method in automating the cell line structure classification with the help of the extracted parameters for analysing the impact of metal synthesised Thiosemicarbazone in cultured cancer cell lines. The importance of cancer studies are there at all times because of the large increase in cancer cases. The resistance shown by certain cancers to traditional chemotherapeutics and made effective drug development a challenging factors. In this contest, the development of potential anticancer complexes received considerable attention.

The accuracy of obtained parameters shows the effectiveness in the selection of pre-processing and segmentation methods. The overall results show that the HSF and Canny's algorithm exhibited better performance in its class. All the parametric output seems to be supportive to the final result. The result shows the superiority of Phenyl Thiosemicarbazone as a ligand on comparing its ethyl and methyl counterparts.

REFERENCES

[1] Canny, J. F. (1986): A computational approach to edge detection. IEEE Transaction and Pattern Analysis, **8**(6), pp. 679-697.

[2] David, Karnofsky (2008): A. Review on anticancer drugs. Cancer journal for clinicians, **18**, pp. 232-234.

[3] Floyd, A. Beckford; Amon, Holt, T. (2015): Prediction of Optical responses of Organic Compounds. Journal of the Arkansas academy of sciences, pp. 27-31.

[4] Furkan, Keskin; Alexander, Suhre; Kivanc, Kose; Tulin, Ersahin, A.; Enis, Cetin; Rengul, Cetin-Atalay (2013): Image Classification of Human Carcinoma Cells Using Complex Wavelet-Based Covariance Descriptors. Plos One, **8** (1), pp. 1-10.

[5] Kalaivani, P.; Prabhakaran, R.; Dallemer, F.; Poornima, P.; Vaishnavi, E.; Ramachandran, E.; Vijaya, Padma, V.; Renganathan, R.; Natrajan, K. (2012): Synthesis, characterization and biological activity of copper and cobalt thiosemicarbazone complexes. Metallomics, **4**, pp. 101–113.

[6] Kinzler; Kenneth, W.; Vogelstein (2002): Thiosemicarbazone metal complexes: From Structure to activity. Bert. McGraw-Hill Medical Pub. Division, pp. 2301-2318.

[7] Kizilcikli, I.; Kurt, Y. D.; Akkurt, B.; Genel, A. Y.; Birteksoz, S.; Otuk, G.; Ulkuseven, B. (2006): Synthesis, characterization and biological activity of copper and thiosemicarbazone complexes. Folia Microbiological Reviews, **52**(1), pp. 15-25.

[8] Krishnan, Nallaperumal; Justin, Varghese (2006): Selective switching median filter for the removal of salt and pepper impulse noise. Proc. of IEEE WOCN 2006, Bangalore.

[9] Parekh and Chanda (2007): Antimicrobial activity of thiosemicarbazone. International Journal of Pharmaceutical Sciences and Research, **3**(6), pp. 1867-1871.

[10] Prabhakaran, R.; Sivasamy, R.; Angayarkanni, J.; Huang, R; Kalaivani, P; Karvembu, R; Dallemer, F; Natarajan, K. (2011): Synthesis on ruthenium chalcone thiosemicarbazone complexes. Inorganica Chimica Acta, **374**, pp. 647–653.

[11]Prinessa, Chellan; Kirkwood, M. Land; Ajit, Soker; Aarun, Au; Seung, Hwan (2001): Novel tetranuclear orthometalated complexes of Pd and Pt. Journal on Organometallics, **31**, pp. 5791-5799.

[12]Manjul, Tiwari (2012): "DNA, Protein binding, cytotoxocity of Palladium thiosemicarbazone complexes. Antimetabolites: established Cancer therapy, **8**, pp. 510-519.

[13]Murugan, A.; Unnikrishnan, B.; Pandhye, S.; Bhonde, R.; Teat, S.; Triantafillou, E.; Sinn, E (1999): Metal Based drugs. Journal on medicinal chemistry, **62**, pp. 177-182.

[14]Ravi, Kumar; Munish, Rattan (2012): Analysis of Various Quality Metrics for Medical Image Processing. International Journal of Advanced Research in Computer Science and Software Engineering, **2**(11), pp. 137-144.

[15]Sau, Har, Lee (2007): Med reviews, **9**, S29-S38

[16]Sreejith, L. Das and Alamelu, Nachiappan (2012): Role Of Hybrid Switching Filter In Image Denoising – A Comparative Study. Proc. of IEEE INDICON-2012, pp. 1180-1183.

[17]Teitz, Y; Barko, N; Abramoff, M.; Ronen, D. (1994): Crystal structure of 3-Phenylpropenal thiosemicarbazone and its Zinc chelates. Journal on Chemotherapy, **40**, pp. 195-201.