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Pharmacological Evaluation of *Thevetia neriifolia*

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Mini Review

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ABSTRACT

In my previous paper determination and estimations of different glycosides, proteins analysis, spectroscopic characteristics, and chromatographic techniques, etc. were performed and found the plant toxic in nature. This paper is based on the pharmacological activities which were performed in the lab and found that the plant possess anti-microbial and anti-termite activity as well. A small dose of plant part certainly helps in possessing both of the activities and gave desired results.

INTRODUCTION

Thevetia neriifolia is commonly known as Yellow Oleander. Scientifically it is also known as *Thevetia peruviana*. This plant contains different glycosides in every part of it esp. cardiac glycosides [1-5]. Heart failure in today's era has become common cause of death. In spite of having such famous medicines and techniques one cannot control over it and the mortality rate is same from the past several decades [6-10].

CHEMISTRY OF T. NERIFOLIA

T. neriifolia is found to be most toxic plant among all and it has been noticed that a small dose if given to a human being may cause fatal death. This plant contains cardiac glycosides in it named Thevetin A and B and other glycosides too [11-16]. Cardiac glycosides have been used for centuries as therapeutic agent. Compounds containing steroid nucleus having unsaturated lactone at C-17 position and one or may be more residues at C-3 position too which are found in most of the plants and toad species which acts as venoms and toxins that serves as protection against any predator. Glycoside in this plant also contains digoxin and its related compounds which is a cardiac glycoside and used in the treatment of several cardiac diseases [17-25].

In 1900s digoxin was prescribed for most of the cardiac diseases because of its expedient pharmacokinetic nature and has different routes of administration. A number of cardiac glycosides have been isolated from *Thevetia neriifolia*, one of them is peruvoside is found to be most potent compound as compare to digoxin [26-30].

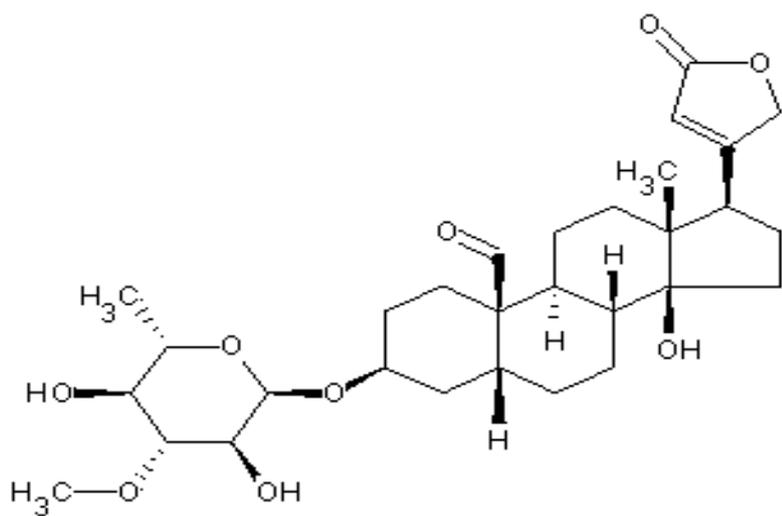


Figure 1: Structure of Peruovside

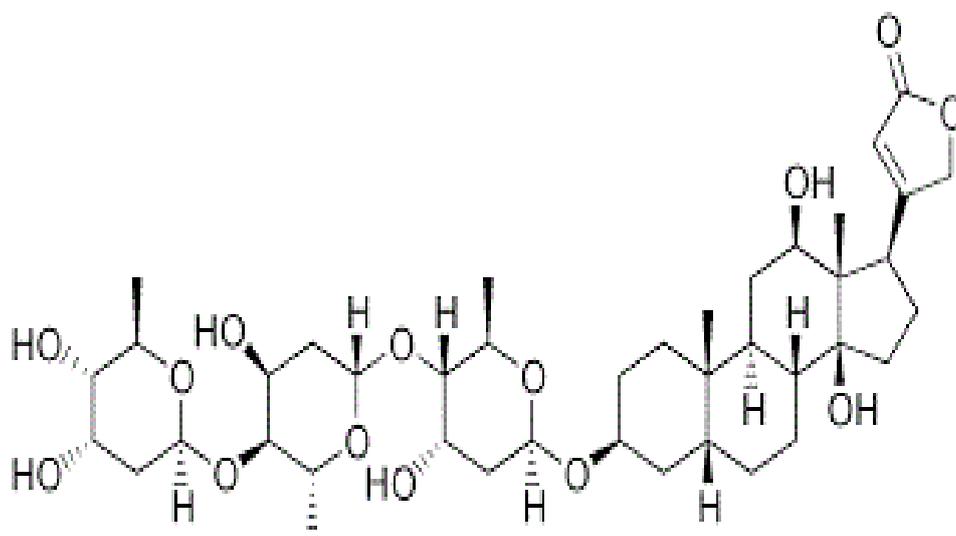


Figure 2: Structure of Digoxin

Thevetian glycosides are chemically closely related to each other (Chart 1). The aglycones as well as glycosides undergo isomeric changes in the presence of bases. A number of glycosides are also present in each and every part of this plant like thevetin B & digitoxigenin- β -gentiobiosyl (1 \rightarrow 4)- α -L-acoprioside: 19-carboxy digitoxigenin- β -gentiobiosyl-(1 \rightarrow 4)- α -L-thevetoside, thevetin A, cannogenin - β -gentiobiosyl-(1 \rightarrow 4)- α -L-acofrioside, & cannogenin - α -L-rhamnoside, uzarigenin- β -gentiobiosyl-(1 \rightarrow 4)- α -L-thevetoside & thevetogenin- β -gentiobiosyl-(1 \rightarrow 4)- α -L-thevetoside, & thevetogenin- β -gentiobiosyl-(1 \rightarrow 4)-2-O-acetyl- α -L-thevetoside, thevetogenin- β -gentiobiosyl-(1 \rightarrow 4)- α -L-acofrioside, thevetogenin- β -glucoside (1 \rightarrow 4)- α -thevetoside which were identified in the polar fraction of the frozen leaves. Among all these theviridoside was the first chemical which was isolated and characterized in *T.neriifolia*. It is completely destroyed in yellow senescent leaves due to blocked chromogenic group [31-40].

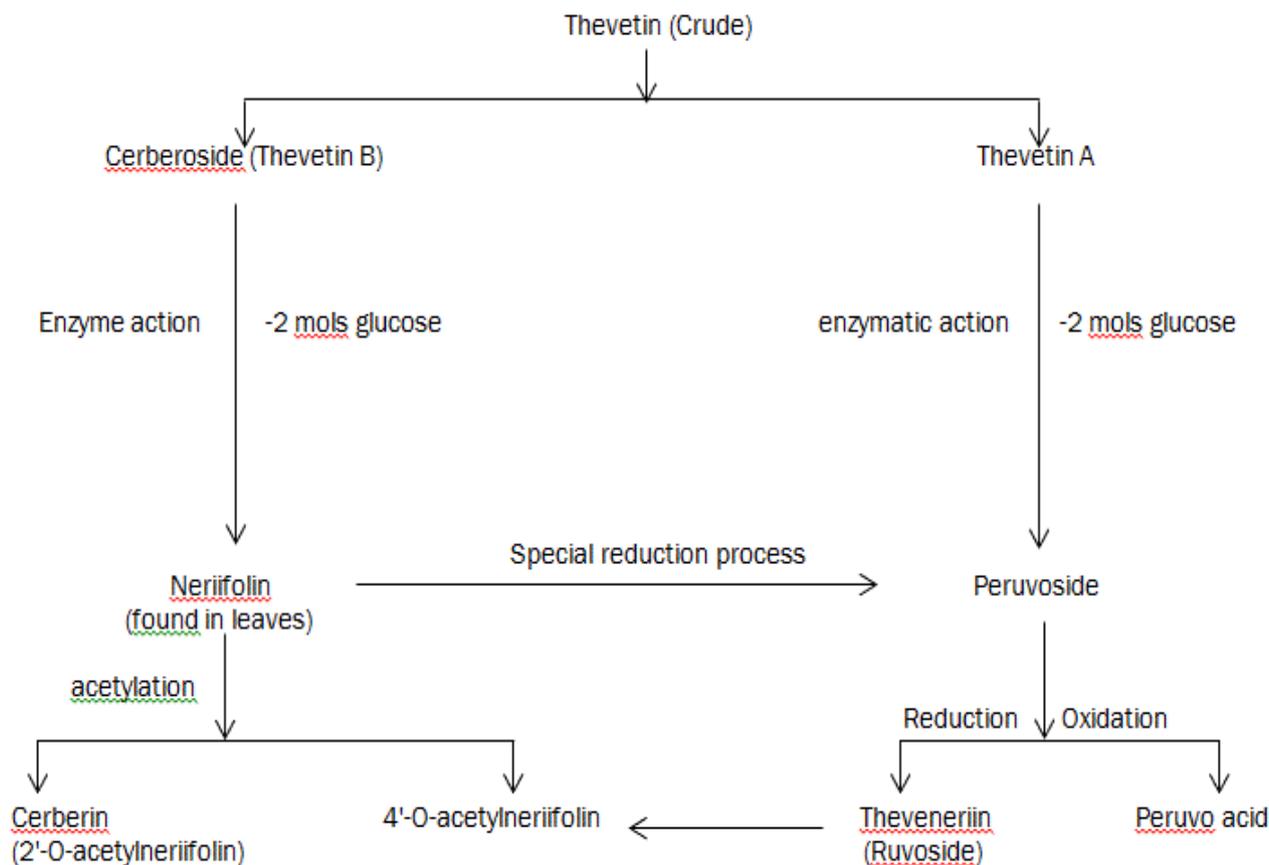


Chart 1: Representation of Thevetian glycosides chemically

PHARMACOLOGICAL EVALUATION

Since ancient times (1000 BC) *T.neriifolia* has been used in curing different skin diseases by Charak. It was classified under toxicity by Sushruta in 1000 BC and was known as horse poison, hence named as Ashwamarak-the horse killer in Sanskrit language. In 1863 its cardio tonic activity was discovered and since then its plant parts was lightened as toxic plant. If ingested then this will produce numbness in tongue when chewed. The seeds cause fatal death to the animals if ingested [41-45].

During our research we have reviewed that this plant is pharmacologically very useful in nature besides its toxicity. Anti-termite and anti-microbial activity was performed on the leaves of this plant [46-53].

ANTI-MICROBIAL ACTIVITY

Preparation of microbes

The test microbes were collected and maintained in freshly prepared Nutrient agar Slants. Then it was incubated at 37 °C for overnight. A loopful of bacterial strain was added to a 50ml of L. B Broth in a conical flask and it was incubated at 37 °C for overnight [54-60].

Agar-well diffusion method

Agar well diffusion method was used to detect the antimicrobial activity of leaf extract against various organisms (*E.coli*, *S.typhi*, *Staphylococcus*, *V.cholerae* and *Shigella*). The culture of bacteria was spread on to the agar plates using L-rod. The wells were cut using gel puncture and to it, 100µl of extract was added. The plates were then incubated at 37 °C for 24 hours. After incubation the zone of bacterial inhibition was measured [61-65].

The extract obtained from the leaves of *Thevetia peruviana* were analyzed for the presence of phytochemical components. The results show that the presence of phenols and proteins in the leaf extract. Purple or pink colour change indicates the presence of protein. The dark yellow colour was observed in the TLC plate and it shows the presence of phenols. Glycosides were confirmed by performing Borntrager's test. The purified compound was analyzed for the presence of phytochemical components using GC/MS. The results show that eleven different compounds were present [67-70]. They are:

(i) 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4HPyran-4-one.

(ii) 3-methyl 1- heptanol

(iii) 3,6-Dimethylundecane

(iv) Tetradecane

(v) Lauric acid

(vi) Mome inositol

(vii) Palmitic acid

(viii) Ethyl palmitate

(ix) Phytol

(x) Ethyl(9z,12z)-9,12- octadecadienoate

(xi) Ethyl linolenate

RESULTS AND DISCUSSION

Among all compounds, the three other compounds resulted in high peaks (mome inositol, Ethyl palmitate, Ethyl linolenate). Apart from these 2,3-Dihydro-3,5- Dihydroxy-6-Methyl-4H-Pyran-4-one compound has an anti-cancerous, anti-microbial, anti-inflammatory property. It is a Millard reaction product of glucose and glycine.

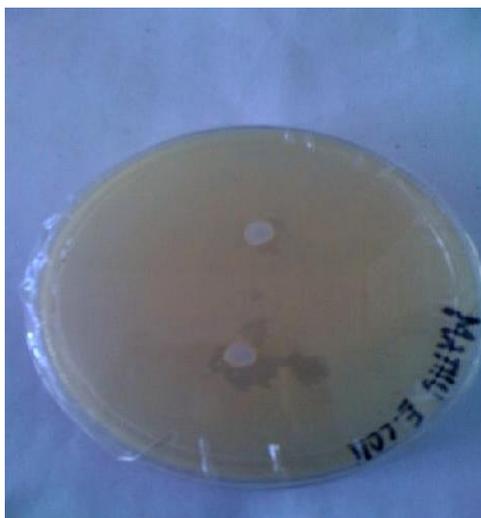


Figure 3a: Antibacterial activity (E.coli)

Momo inositol - use of a compound that specifically inhibits the lipolytic activity of HSL, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment of a disorder where a decreased level of plasma FFA is desired. The amount of glycosides compounds present in the sample was quantified using spectrophotometer. The results show 2.89% of glycosides present in 3ml of extract.

The 100µl of leaf extract was tested against *E.coli*, *Shigella*, *Staphylococcus*, *Salmonella typhi*, and *Vibrio cholera*. The result showed little activity against *E.coli* and *Shigella*. Good results can be obtained by increasing the concentration of the leaf extract.

The seed extract of *Thevetia peruviana* was done previously by Omolara (2007), and the amount of cardiac glycosides was compared in dry seed meal and in extraction. The result showed that the protein content was increased in seed after treated with different quantities of alcohol and this protein amount was compared with untreated seed sample. The protein obtained from the treated sample can therefore serve as a protein source for

animal feed formulation. The compound 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4-one and mome inositol analyzed by GC/MS has an anti-cancerous and anti-proliferate property. This anti-proliferate and pro-apoptotic effects of 2,3 dihydro-3,5-dihydroxy- 6-methyl-4H-pyranone through inactivation of NFkappaB in human colon cancer cells was previously done and proved by Ban et al. (2007). These compounds can be isolated and used for further studies using MTT assay.

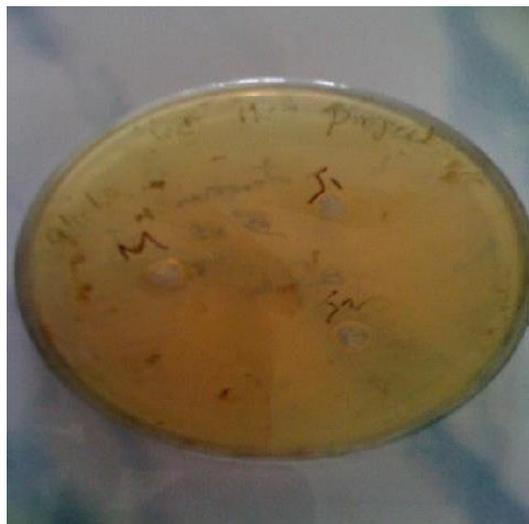


Figure 3b: Antibacterial activity (Shigella)

Present investigation suggests that leaf extract of *Thevetia peruvaina* contain higher amount of protein and lesser amount of Glycosides. GC mass analysis shows 11 novel compounds were present in leaf extract. Leaf extract showed antimicrobial activity against *E.Coli* and *Shigella*. Presence of 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4H-Pyran-4 proves its antibacterial activity. Among the 11 compound 2,3-Dihydro-3,5-Dihydroxy-6-Methyl- 4H-Pyran-4-one and mome inositol has an anticancerous and anti-proliferate property.

ANTI-TERMITE ACTIVITY

Toxicity and repellent effects of medicinal plant extracts on subterranean termites (*Isoptera: Rhinotermitidae*) have also been demonstrated. The presence of unsaturated linoleic acid in Yellow oleander oil), which has drying properties, makes Yellow oleander oil suitable for making a surface coating such as paint [71-75].

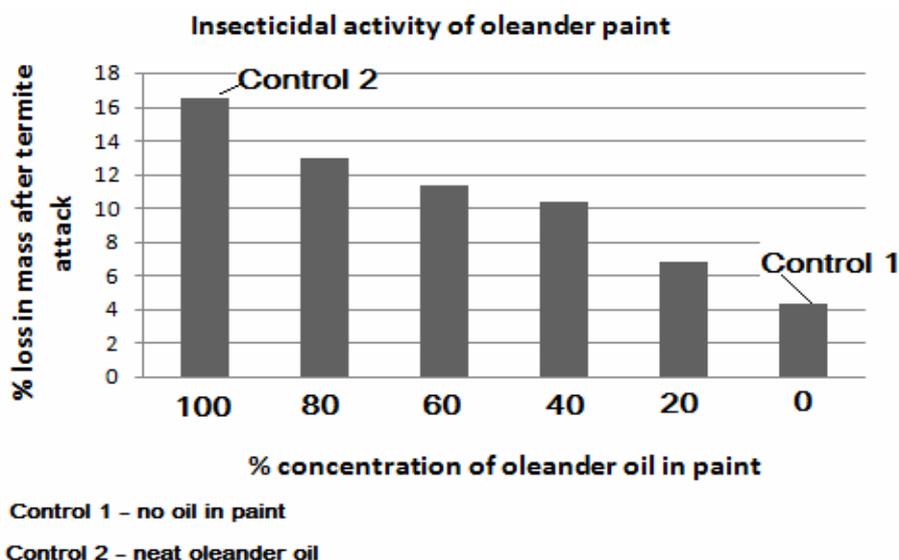


Figure 4: Repellent activity of Oleander paint towards *Microtermes* spp.

Labeled dry plywood plates (6 x 6 inches) were painted on both sides, (in triplicate) with the formulated paints. One set of control plates was painted with neat oleander oil, while the other was painted with a paint in which Yellow oleander oil was not added. After drying to constant weight in the laboratory environment, each plate weight was determined. The wooden plates were then placed side by side and covered with foliage under a termite (*Microtermes* spp) nest and left for a period of one month. Moisture was constantly maintained by pouring water on the foliage within the exposure period, so as to maintain appropriate environmental conditions favourable to termites [76-78].

After the exposure period, the wooden plates were washed with clean water to remove soil and debris, and dried in the oven at 50 °C to a constant weight. The mass of each plate was then determined and the average weight loss calculated [78-81].

RESULTS AND DISCUSSION

The Oleander paint inhibited the tested microbes in a concentration dependent manner. The control paint (containing 0.0% oil) did not inhibit the test bacteria and fungus. From these results, it was concluded that oleander paint was self-preserving against bacterial and fungal attack. Antibacterial and antifungal activity of *T. peruviana* plant extracts had been earlier established (Obasi and Igboechi, 1991; Gata et al., 2003) and collaborates with the present findings. From above figure it was evident that the oleander paint repelled *Microtermes* spp. The repellent action was highest when pure oleander oil was used. However, no termite deaths were reported in this study. Insecticidal and toxicity of Yellow oleander oil has been reported (McLaughlin et al., 1980; Panigrahi and Raut, 1994; Langford and Boor, 1966). Also anti-termite activity of medicinal plant extracts has been documented (Verena-Ulrike and Boor, 2001). The present findings demonstrate that paint made from *T. peruviana* plant oil extract could substantially protect timber from termite attack.

POPULARITY OF THEVETIA PERUVIANA WITH TIME

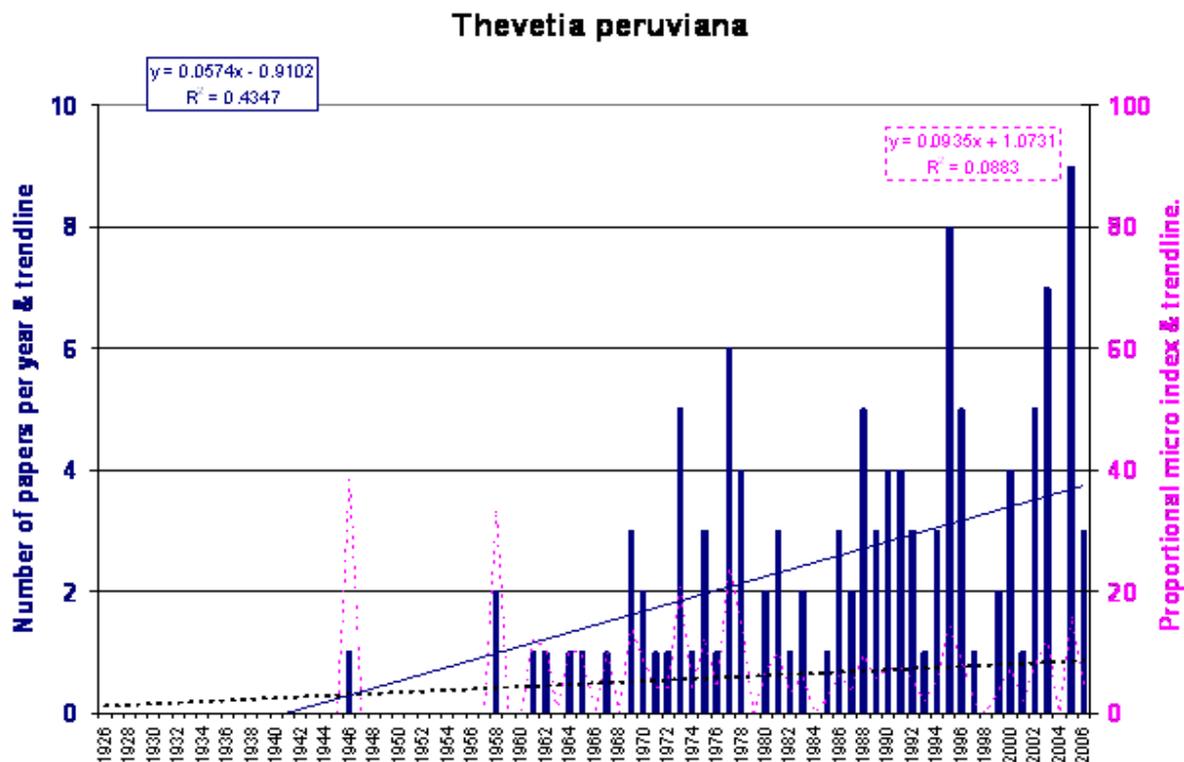


Figure 5: . [Plots of numbers of papers mentioning *Thevetia peruviana* (filled column histogram and left hand axis scale) and line of best fit, 1926 to 2006 (complete line, with equation and % variation accounted for, in box on the left hand side); Plots of a proportional micro index, derived from numbers of papers mentioning *Thevetia peruviana* as a proportion (scaled by multiplying by one million) of the total number of papers published for that year (broken

line frequency polygon and right hand scale) and line of best fit, 1926 to 2006 (broken line, with equation and % variation accounted for, in broken line box on the right hand side)]

CONCLUSION

From all this it has been concluded that the plant *T. neriifolia* showed most potent activity on anti-microbial and anti-termite. Yellow oleander paint possesses antimicrobial and anti-termite activities. *T. peruviana* oil extract would serve as an environmentally friendly bactericide and fungicide for oil based paints. Both of the activity gave significant results.

REFERENCES

1. Odhiambo PO et al. Phyto-Chemical Screening of Wild Types and Tissue Cultured Yellow Oleander *Thevetia peruviana* Pers.K.Schum in Kenya. *Adv Pharmacoeconom Drug Safety*. 2012;1:120.
2. Ravalli R et al. A Review on Antimicrobial Resistance in Developing Countries. *Biochem Pharmacol*. 2015;4:r001.
3. Huang H et al. Ozone-Oxidation Products of Ibuprofen and Toxicity Analysis in Simulated Drinking Water. *J Drug Metab Toxicol*. 2015;6:181.
4. Saganuwan SA. Arithmetic-Geometric-Harmonic (AGH) Method of Rough Estimation of Median Lethal Dose (Ld50) Using Up- and - Down Procedure. *J Drug Metab Toxicol*. 2015;6:180.
5. Jen HC et al. Al. Effect of Different Treatments on Excretion of Tetrodotoxin after Oral Administration in Rat. *J Drug Metab Toxicol*. 2015;6:179.
6. Charfi R et al. PRES Induced By Cyclosporin with Normal Blood Concentrations in a Bone Marrow Recipient. *J Drug Metab Toxicol*. 2015;6:178.
7. Gandhimathi C et al. Controlled Release of Dexamethasone in PCL/Silk Fibroin/ Ascorbic Acid Nanoparticles for the Initiation of Adipose Derived Stem Cells into Osteogenesis. *J Drug Metab Toxicol*. 2015;5:177.
8. Ghani A et al. Response of Wheat (*Triticum aestivum*) to Exogenously Applied Chromium: Effect on Growth, Chlorophyll and Mineral Composition. *J Environ Anal Toxicol*. 2015;5:273.
9. Saito K et al. Anticoagulant Managements of Left Ventricular Assist Device Implantation in Two Patients with Heparin-Induced Thrombocytopenia (HIT): Use of Argatroban as an Anticoagulant for Cardiopulmonary Bypass. *J Anesth Clin Res*. 2015;6:525.
10. Andolina A. Bone Marrow Transplantation and Mesenchymal Stem Cells in Niemann Pick A Disease. *J Bone Marrow Res*. 2015;3:155.
11. Lozano UP et al. Autotransplant in Multiple Myeloma with Oral Melphalan, without Cryopreservation. *J Bone Marrow Res*. 2015;3:154.
12. Elgemeie GH et al. Design, Synthesis and Cytotoxic Evaluation of Novel Heterocyclic Thioglycosides. *Med chem*. 2014;4:814-820.
13. Elgemeie GH et al. Design, Synthesis and In vitro Anti-tumor Evaluation of Novel Acrylohydrazide Thioglycosides. *Med chem*. 2014;4:400-406.
14. Uchiumi F et al. Effect of Lignin Glycosides Extracted from Pine Cones on the Human SIRT1 Promoter. *Pharm Anal Acta*. 2013;4: 266.
15. Odhiambo PO et al. Phyto-Chemical Screening of Wild Types and Tissue Cultured Yellow Oleander *Thevetia peruviana* Pers.K.Schum in Kenya. *Adv Pharmacoeconom Drug Safety*. 2012;1:120.
16. Harry-O'kuru RE et al. Medicinal Components Recoverable From Sicklepod (*Senna obtusifolia*) Seed: Analysis of Components by HPLC-MSn. *J Chromatograph Separat Techniq*. 2012;S2:001.
17. Steele J et al. Heparin Induced Thrombocytopenia and Cardiac Surgery: A Comprehensive Review. *J Blood Disord Transfus*. 2011;S2:003.
18. Bamigboye-Taiwo OT et al. Cardiac Dimensions and Functional Parameters in Nigerian Children with Homozygous Sick Cell Anaemia Using Echocardiography. *J Clin Exp Cardiol*. 2015;6:368.
19. Hwang J et al. Spinnaker Sail Sign Accompanied with Pneumopericardium and Pneumoperitoneum. *J Neonatal Biol*. 2015;4:177.
20. Divekar A et al. Non-Restrictive Ductal Patency in Management of Cardiac Failure in Congenital Diaphragmatic Hernia - Non-Invasive Biventricular Assist. *J Neonatal Biol*. 2015;4:171.

21. Hasan R et al. Apical Thrombus Mimicking Cardiac Myxoma: Application of Cardiovascular Magnetic Resonance. *OMICS J Radiol.* 2015;4:181.
22. Seki Y et al. Landiolol Hydrochloride Normalizes Diminished Levels of Cardiac Vascular Endothelial Growth Factor (VEGF) Signaling System Components in Lipopolysaccharide-Induced Sepsis Independent of Inflammatory Markers. *J Vasc Med Surg.* 2015;3:193.
23. Bignami E et al. Endogenous Ouabain Changes Rapidly During Cardiac Pulmonary by Pass. *J Steroids Hormon Sci.* 2011;S3:002.
24. Mitra S et al. Mesoporous Nano-carbon particle Loaded Fisetin has a Positive Therapeutic Effect in a Murine Preclinical Model of Ovalbumin Induced Acute Allergic Asthma. *J Nanomedicine Biotherapeutic Discov.* 2015;5:132.
25. Charfi R et al. PRES Induced By Cyclosporin with Normal Blood Concentrations in a Bone Marrow Recipient. *J Drug Metab Toxicol.* 2015;6:178.
26. Siniscalco D, Luongo C. Research Hypothesis in Autism: The Role of Therapeutical Ozone. *Autism Open Access.* 2015;5:e129.
27. Huang Z. The Activity of Hyaluronan and Hyaluronidase PH20 in Inflammation-A Role by Reagent Contaminants?. *J Clin Cell Immunol.* 2015;6:314.
28. Altamimi A et al. Successful Outcome Following a Loading Dose of NAcetylcysteine to Treat Hepatotoxicity after Repeated Therapeutic Doses of Paracetamol. *J Clin Toxicol.* 2015;5:245.
29. <http://esciencecentral.org/journals/2329-6836/2329-6836.S1.004-009.pdf>
30. http://omicsgroup.org/journals/2167-7689/2167-7689_S1.008_009.pdf
31. Rubio F et al. Survey of Glyphosate Residues in Honey, Corn and Soy Products. *J Environ Anal Toxicol.* 2014;5: 249.
32. Akan JC et al. Determination of Organochlorine, Organophosphorus and Pyrethroid Pesticide Residues in Water and Sediment Samples by High Performance Liquid Chromatography (HPLC) with UV/visible Detector. *J Anal Bioanal Tech.* 2014;5:226.
33. Barganska Z et al. Determination of Pesticide Residues in Honey using the GC-MS/MS Technique. *J Bioprocess Biotech.* 2014;4:182.
34. Mahesh MS and Mohini M. Crop Residues for Sustainable Livestock Production. *J Adv Dairy Res.* 2014;2: e108.
35. Wang SW et al. Simultaneous Quantitative Determination of Nine Bufadienolides in Traditional Chinese Medicinal Toad Skin from Different Regions of China by High-Performance Liquid Chromatography-Photodiode Array Detection. *Pharm Anal Acta.* 2015;6:345.
36. Huckabee M. New Diagnoses Cover Venomous Toads and Exploding Spacecrafts . *Primary Health Care.* 2014;4:172.
37. Essawy AE et al. Hepatotoxicity Induced by Antifungal Drug Fluconazole in the Toads (*Bufo Regularis*). *J Drug Metab Toxicol.* 2010;1:106.
38. Ragab AR et al. Clinical Utility of Serum Digoxin Level in Cardiac Patients for Diagnosis of Chronic Digitalis Toxicity. *J Clin Toxicol.* 2012;2:150.
39. Chadha S et al. A Case of 2:1 Atrio-Ventricular Block in Digoxin Toxicity. *J Clin Experiment Cardiol.* 2011;2:154.
40. Fortuna A et al. In vitro and In vivo Relevance of the P-glycoprotein Probe Substrates in Drug Discovery and Development: Focus on Rhodamine 123, Digoxin and Talinolol. *J Bioequiv Availab.* 2011;S2.
41. <http://omicsonline.org/0975-0851/0975-0851.S1.017-028.pdf>
42. Kumar V et al. Taguchi and Quadratic via Chromogenic Design Methodology: A Better to Best Estimation Process (Tizanidine Hcl) Bulk/Pharmaceutical. *Pharm Anal Acta.* 2014;5:319.
43. Rathee P et al. Statistical Design for Optimization and Determination of Tizanidine Hcl using Folin-Ciocalteu (Fc) as Chromogenic Reagent. *Pharm Anal Acta.* 2014;5:307.
44. Tsiambas E et al. HER2/neu Expression and Gene Alterations in Pancreatic Ductal Adenocarcinoma: A Comparative Immunohistochemistry and Chromogenic in Situ Hybridization Study Based on Tissue Microarrays and Computerized Image Analysis. *J. Pancreas.* 2006;7:283-294.
45. Rustamov NK and Abbasova GG. Determination of Manganese in Tap Water by a New Extraction-photometric Method. *J Environ Anal Toxicol.* 2014;4:205.

46. Verma N et al. Development of "Field Level" Chromogenic Assay for Aflatoxin M1 Detection in Milk. *Adv Dairy Res.* 2013;1:108.
47. dos Santos JL and Chin CM. Pan-Assay Interference Compounds (PAINS): Warning Signs in Biochemical-Pharmacological Evaluations. *Biochem Pharmacol (Los Angel).* 2015;4:e173.
48. Baraka AM and Guemei A. Can Pharmacological Targeting of Advanced Glycation End Products Provide Protection Against Experimentally Induced Liver Fibrosis? *Biochem Pharmacol (Los Angel).* 2015;4:167.
49. Tomar GS et al. A Comparative Study of Two Different Doses of Fentanyl Added to Bupivacaine for Intermittent Epidural Labor Analgesia: A Prospective Randomized Double Blind Study. *J Anesthe Clinic Res.* 2011;2:145.
50. Tomar GS et al. Role of Clonidine in Fascia Iliaca Compartment Block for Preoperative Analgesia in Post Hip Fracture Patients: A Comparative Study. *J Anesthe Clinic Res.* 2011;2:121.
51. <http://esciencecentral.org/journals/2327-5162/2327-5162.S1.009-051.pdf>
52. <http://esciencecentral.org/journals/2327-5162/2327-5162-S1.004-037.pdf>
53. <http://esciencecentral.org/journals/2327-5162/2327-5162-S1.004-031.pdf>
54. <http://esciencecentral.org/journals/2327-5162/2327-5162-S1.003-072.pdf>
55. <http://esciencecentral.org/journals/2327-5162/2327-5162-S1.002-060.pdf>
56. <http://omicsgroup.org/journals/2167-0501/2167-0501-S1.002-077.pdf>
57. Kollberg H. Avian Antibodies (IgY) to Fight Antibiotic Resistance. *Clin Microbiol.* 2015;4:194.
58. Mishra N and Sundari SK. Native PGPM Consortium: A Beneficial Solution to Support Plant Growth in the Presence of Phytopathogens and Residual Organophosphate Pesticides. *J Bioprocess Biotech.* 2015;5:202.
59. http://omicsonline.org/2157-7110/2157-7110_S1.009_046.pdf
60. http://omicsonline.org/2157-7110/2157-7110_S1.009_042.pdf
61. Hetland G et al. Andosan™-An Anti-Allergic and Anti-Inflammatory Ingredient Prepared from *Agaricus blazei* Mushroom. *J Clin Cell Immunol.* 2015;6:320.
62. Agarwal P et al. Pulmonary Hemorrhage in an Infant with Coronavirus Infection. *J Neonatal Biol.* 2015;4:175.
63. Kedar S et al. Giant Cell Reparative Granuloma of the Maxilla Presenting as A Pulsatile Mass: A Rare Case Report. *Otolaryngol (Sunnyvale).* 2015;5:184.
64. Da Costa G et al. Cheek Plumper with a Salivary Reservoir: An Esthetic and Functional Treatment Option for a Xerostomic Patient with Sunken Cheeks. *Dentistry.* 2015;5:293.
65. Maia Jr H et al. The Inhibitory Effects of *Pinus pinaster* Extract and Resveratrol on Aromatase Expression in the Eutopic Endometrium of Endometriosis Patients using Oral Contraceptives. *Gynecol Obstet (Sunnyvale).* 2015;5:285.
66. Nicolini C et al. SpADS and SNAP-NAPPA Microarrays towards Biomarkers Identification in Humans: Background Subtraction in Mass Spectrometry with *E.coli* Cell Free Expression System. *J Mol Biomark Diagn.* 2015;6:214.
67. Agulles TM. How Egg Quality Impacts the Health of Day-One-Chicks? *Poult Fish Wildl Sci.* 2014;2:124.
68. <http://omicsonline.org/2155-9597/2155-9597.S1.008-053.pdf>
69. <http://omicsonline.org/2161-1165/2161-1165-S1.004-021.pdf>
70. <http://omicsonline.org/2157-7560/2157-7560-S1.017-018.pdf>
71. <http://omicsonline.org/2155-952X/2155-952X-S1.020-015.pdf>
72. <http://omicsonline.org/2161-0681/2161-0681-S1.006-009.pdf>
73. <http://omicsonline.org/2153-2435/2153-2435.S1.10-032.pdf>
74. Mamuye Y et al. Isolation and Antibiotic Susceptibility Patterns of *Shigella* and *Salmonella* among Under 5 Children with Acute Diarrhoea: A Cross-Sectional Study at Selected Public Health Facilities in Addis Ababa, Ethiopia. *Clin Microbiol.* 2015;4:186.
75. Mengistu G et al. Prevalence and Antimicrobial Susceptibility Patterns of *Salmonella* serovars and *Shigella* species. *J Microb Biochem Technol.* 2014;S2:006.
76. Dagnew M et al. Bacterial Profile and Antimicrobial Susceptibility Pattern among Food Handlers at Gondar University Cafeteria, Northwest Ethiopia. *J Infect Dis Ther.* 2013;1:105.
77. <http://omicsonline.org/1948-5956/1948-5956.S1.035-029.pdf>

78. Ab Majid AH et al. Colony Genetic Organization and Breeding Pattern of Subterranean Termite (*Reticulitermes flavipes*) over the Three Field Seasons in Nebraska, U.S.A. *Entomol Ornithol Herpetol.* 2014;3:133.
79. Sattar A et al. Efficacy of Plant Extracts Against Subterranean Termites i.e., *Microtermes obesi* and *Odontotermes lokanandi* (Blattodea:Termitidae). *J Biodivers Biopros Dev.* 2014;1:122.
80. Li T et al. New Insights into Microbes in the Midgut of Termite *Coptotermes formosanus*. *J Bioremed Biodeg.* 2014;5:220.
81. Igwe CU et al. Chemical Analysis of an Edible African Termite, *Macrotermes nigeriensis*; a Potential Antidote to Food Security Problem. *Biochem & Anal Biochem.* 2011;1:105.