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## PHYTOCHEMICAL SCREENING AND TOXICITY LEVEL OF LEAF AND ROOT PARTS EXTRACTS OF SNAKE PLANT (*SANSEVIERIA TRIFASCIATA*) USING NAUPLII

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### ABSTRACT

**T**he study examines the toxicity level of *Sansevieria trifasciata* (Asparagaceae) leaves and roots extracts. Antimicrobial activity was tested against alkaloid, saponins, tannins, anthraquinones screening by two different methods, the brine shrimp toxicity test and ten-fold serial dilution of the powdered plant material in artificial seawater. Results showed the presence of cytotoxic principles making it effective therapeutic remedy for treating various infections as it possesses effective of its zone of inhibition (lowest concentration of 35.22 µg/ml to the highest concentration of 44.49 µg/ml) using brine shrimp bioassay of *Sansevieria trifasciata* roots and leaves extracts. Thus, the toxic potential of the plant extracts yields greater than the recommended LC<sub>50</sub> value and reveals positive linear relationship between the concentrations of the extract to the mortality rate using nauplii. The more concentrated the treatment the higher mortality had resulted.

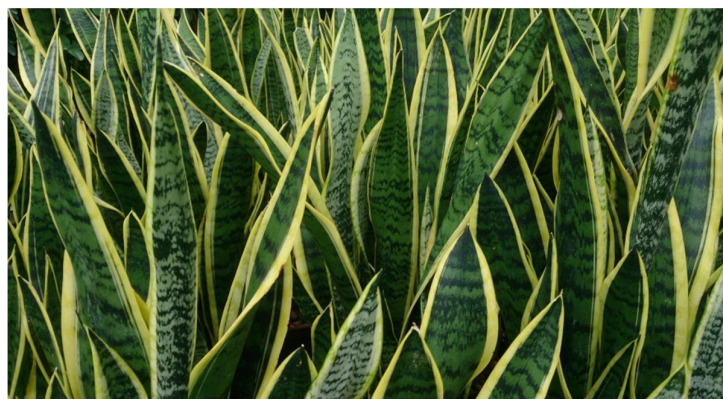
**Keywords:** Phytochemical screening, Brine shrimp, lethality test, secondary metabolites, snake plant

## Introduction

Snake Plant is one of the most recommended plants for improving air quality. The optimal place to keep this is in your bedroom, because it converts CO<sub>2</sub> into oxygen at night it also removes contaminants from air. The NASA conducts a study to the uses of snake plant according to their results they found out that the snake plant absorbs toxins, such as nitrogen oxides and formaldehyde. *Sansevieria trifasciata* is able to absorb 107 types of toxins, including air pollution, cigarette smoke (nicotine), so it would make a great refresher. It also contains phytochemicals such as flavonoids, saponins, and glycosides which reduce the number of bacteria [6]. In China, decoction used for detoxification, as anti-inflammatory, and for treatment of sores and snake bites. It also used to cure boils, cough, bronchitis, traumatic injuries [2].

The search for bioactive compounds for pharmaceutical and industrial applications is still being actively done. Philippine plant species that have been used for indigenous medicinal purpose serves as a reservoir of these compounds. At present, herbal medicines and food supplements are undeniably rampant as an alternative to synthetic drugs. Media advertisements as well as beauty cosmetics brochures and catalogues are flooded with varieties of botanicals and herbals. This is caused by widely accepted through that these products have no side effects, can easily absorbed by the body and can cure almost all of the common ailments; that is in comparison to synthetic drugs [7]. However, labels and packages of these sold herbal medicines states that it has no approved therapeutic claim.

This research would help in the discovery of a new form of medication for the upcoming severe diseases of microbial infection such as the leaves and roots of *Sansevieria trifasciata* are used in traditional medicine for the treatment of asthma, abdominal pains, colic, diarrhea, haemorrhoids, hypertension, menorrhagia, piles, sexual weakness, wounds of the foot, cough, leprosy, rheumatism, glandular enlargement, nutritional deficiencies and treatment of snake bite [10]. The active ingredient from the root is more natural, fewer chemicals so it consists less toxicity, side effects and safe compared to synthetic drugs. The new discovery of this product will create awareness and revolute advantage of the *Sansevieria trifasciata* root usage. This medication well tolerated by patients, with fewer unintended consequences than pharmaceutical drugs because it less toxic and side effects [8]. Thus, this study is important. Its bioactive ingredients were identified and its toxicity level for the effective future formulation of the plant as medicinal plant.



## Materials and Methods

### *Plant materials*

The Fresh leaves and roots of Snake plant were collected from the Engineering Department of Caraga State University along its side pavement and across the Horrizon Bridge on its paved aisle along the its pavement.

The leaves of *Sansevieria liberica* were collected from a single population of the plant from front of the Engineering Department of Caraga State University, Ampayon, Butuan City. The collected plant was authenticated with the biology department.

### *Preparation extract*

The collected plant parts were separated as leaf and roots and were cleaned with deionized water and dried under shade for two weeks at room temperature. Dried leaves and stem were grounded and filtered using 0.3mm mesh. The plant powder was stored in air tight container and maintained at 4°C until use separately.

### *Qualitative Phytochemical Screening*

Phytochemical analysis of each extract has been carried out according to standard protocols [1].

#### *Screening for Alkaloid*

A 0.5 g of the extract was stirred in 5 ml of 1% HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1 ml of the filtrate was treated with a few drops of Wagner's reagent. A reddish brown precipitate indicates the presence of alkaloids. Screening for Flavonoids 2 ml of dilute sodium hydroxide was added to 2 ml of the extract. The appearance of a yellow colour indicates the presence of flavonoids.

#### *Screening for Saponins*

A 1 ml of distilled water was added to 1 ml of the extract and shaken vigorously. A stable persistent froth indicated the presence of saponins. Screening for Phenols Equal volumes (1 ml) of extract and Iron (III) chloride were mixed. A deep bluish green solution gave an indication of the presence of phenols.

#### *Screening for Tannins*

A portion of the extract was dissolved in water, after which the solution was clarified by filtration. 10% ferric chloride solution was then added to the resulting filtrate. The appearance of a bluish black colour indicates the presence of tannins.

#### *Screening for Anthraquinones*

A 0.5 g of the extract was shaken with 10 ml of benzene and filtered. 10% of ammonia solution was added to filtrate and the mixture was shaken. The formation of a pink, red or violet colour on the ammoniacal phase [4].

### *Brine Shrimp Bioassay (Pugulayan et al., 2004)*

The brine shrimp assay is a rapid, reliable and inexpensive general bioassay toxicity test for active plant extracts. The procedure allows the determination of the LC<sub>50</sub> values in parts per million (ppm) of active constituents in the brine medium. This bioassay has been used in the analysis of natural products, pesticides residues and other chemical pollutants in marine environment [9].

#### *Hatching of Brine Shrimp*

Brine Shrimp egg, (*Artemia salina*) obtained from the local pet shop was hatched in a shallow rectangular plastic dish filled with 1 litre of artificial sea water, which was prepared with 35 grams commercial rock salt mixture and 1mL distilled water. Approximately 1 gram of eggs was sprinkled in the compartment. An aquarium aerator was used for *Artemia* cyst suspension to provide an ample supply of oxygen in the medium. A perforated plastic cover was then put over the dish to avoid predatory insects on cysts or nauplii. The container was also well lighted with lamp to maintain the water temperature. After 3 days the hatched cysts have produced fast swimming nauplii which were used in this bioassay. A dropper and a micropipette were used to collect nauplii from the suspension.

#### *Ten-fold Serial Dilution of the Powdered Plant Material in Artificial Seawater*

Three test tubes containing 0.1g or 100mg of each powdered snake leaves and roots, 10mL of artificial water was introduced and the test tube was corked and manually shaken by inverting 5 times. The concentration of the snake plant extract in this tube is 10mg/ml or 10,000µg/mL. Using a permanent marker, this tube was labeled # 1. Five other tubes were labeled 2, 3, 4, 5 & 6. The test tubes were placed in a rack side by side according to their number. To tubes # 2,3,4,5 artificial seawater of 9mL volume was added using 10mL glass pipette and tube # 6 was 10mL artificial seawater and serves as control set-up.

The plant powder in tube # 1(10mg/mL) was allowed to settle down for 1 hour. This should be produced a residue at the bottom and a clear supernatant liquid above it. After the plant powdered has settle to the bottom of tube # 1, 1mL of the supernatant was gently withdrawn using a 1 mL micropipettor and transferred to tube # 2. Tube # 2 was corked and inverted 3 times to mix the content. Tube # 2 was uncorked and return to its slot in the rack between # 1 and # 3. Using again the micropipettor, 1mL was removed from tube # 2 and transferred to tube # 3 to tube # 5. This process performed is a 10-fold serial dilution indicating that in each tube as one move from tube # 1 to # 5, the concentration of the plant extract is being reduced by 10 times of the tube previous to it.

**Table 1.** Ten-fold dilution series of the Three dried powdered specie of *Sansevieria trifasciata* in ASW of various concentrations ranging from 10, 000µg/mL to 1µg/mL.

Tube #	# 1	# 2	# 3	# 4	# 5
Volume of ASW (Diluent)	10 mL	9 mL	9 mL	9 mL	9 mL
Volume of Original Plant Extract (10mg/mL) Serially Diluted	—	1.0 mL	1.0 mL	1.0 mL	1.0 mL
Total volume in tubes # 1 to # 5	10 mL	10 mL	10 mL	10 mL	10 mL
Dilution Series	10-fold	10-fold	10-fold	10-fold	10-fold
Dilution Level of Originated Extract	1	1:10	1:100	1:1,000	1:10,000
Concentration of Plant extract in mg/mL from tubes # 1 to # 5	10	1.0	0.1	0.01	0.001
Concentration of plant extract in µg/mL or ppm from tube # 1 to # 5	10, 000	1, 000	100	10	1

### Bioassay

In each snake plant extract, 18 vials contained with 5 mL ASW were prepared for the application. Approximately 10 shrimps were collected using a wide-mouth plastic medicine dropper. The shrimps were added to each vial. A magnifying lens was used for checking the viability and well-being of the nauplii. The plastic container was closed with a plastic cellophane cover and kept under white light during the 24 hours period. The numbers of viable nauplii (number of surviving) were counted in each vial after 24 hours. A pipette sucked into the vials was used to count the surviving shrimps macroscopically, held against a well-lighted background.

### Statistical Analysis to Determine the Extracts LC<sub>50</sub>

To determine the regression of equation for the data, the Coefficient **b** in the equation of a line **y=a+bx** is computed as

$$b = \frac{n \sum xy - \sum x \sum y}{n \sum x^2 - \sum(x)^2}$$

The Coefficient **a** from the equation of a line above is solved as **a=y-bx** Where y is the mean of y (percent mortality) and **x** is the mean of **x** (log dose).

Assuming that y=50% and substituting it to the regression equation above and then x was solved. And with a scientific calculator, antilog values achieved were converted it back to its dose in ppm to determine the LC<sub>50</sub> of each snake plant extract in ppm. The resulting values were the concentration of the snake plant species that will kill 50% of the *Artemia salina* nauplii at 24 hours. Then correlation coefficient of the results on the data obtained was solved using the equation

$$r = \frac{n \sum xy - \sum x \sum y}{\sqrt{(n \sum x^2 - \sum(x)^2) - (n \sum y^2 - \sum(y)^2)}}$$

Furthermore, the toxicity rating of sample as also analysed according to the (%) mortality group of the test organism [1].

**Table 2.** The toxicity rating of extract based on per cent (%) mortality

Mortality	Toxicity
80-90	Highly toxic
50-79	Moderately toxic
30-49	Slightly toxic
0-29	Non-toxic

Weigh 0.1 gram of the sample then dry it in oven at 50°C for about 4 to 6 hours until constant weight is obtained. Powderized the sample for the application in the tenfold dilution and for brine shrimp bio assay using the nauplii larvae.

#### *Bioassay Procedure*

The procedure described in the literature [8] (Meyer et al., 1982 and McLaghin et. Al., 1990) was adopted for this study. Samples of five different concentrations 1, 10, 100, 1000, 10000 µg/ml were prepared according to the direction given in the literature. Brine shrimp (*Artemia salina* Leach) nauplii were hatched in a specific plastic tank. Fifteen shrimps were transferred to each sample vial and then sea water was added to make the volume of 2ml. The vials were kept for 24 hours, thereafter the active nauplii were counted with the aid of 3x magnifying glass and the per cent death at each dose and control was determined. The data obtained were processed in Probit Statistical Analysis by means of regression to estimate LC<sub>50</sub> values for the significance of death, survival and the mortality of nauplii larvae.

## **Results**

#### *Phytochemical screening of ethanolic extract*

The table below showed the result of the presence of the bioactive compounds found in both the leaves and root parts of the snake plant. It can be seen that both the leaves and roots were highly active with alkaloids, tannins and anthraquinones and two bioactive compounds were absent in the leaves and roots of plants were flavonoids and saponins.

**Table 3.** Phytochemical Screening of the Ethanolic Extract of Snake Plant (*Sansevieria Trifasciata*)

Plant Sample	Test for Alkaloids		Test for Tannins		Test for Flavonoids	Test for Antraquinones	Test for Saponins
	Mayers Reagent	Dragendorffs Reagent	Gelatin Test	Ferric Chloride Test	Smith & Metcalf Method	Borntrager's Test and Modified Borntrager's Test	Froth Test
Snake Plant Leaves Extract	++	++	++	Brownish brown +	-	+	-
Snake Plant Roots Extract	+++	+++	++	++	-	++	-

**Legend:** +++ (Highly present); ++ (Moderately present); + (present) and - (absent)

*Ten-fold dilution test*

**Table 4.** Ten-fold dilution series of the Three dried powdered specie of *Sansevieria trifasciata* in ASW of various concentrations ranging from 10, 000µg/mL to 1 µg/mL.

Tube # microgram/ml	# 1	# 2	# 3	# 4	# 5
Volume of ASW (Diluent)	10 mL	9 mL	9 mL	9 mL	9 mL
Volume of Original Plant Extract (10mg/mL) Serially Diluted	—	1.0 mL	1.0 mL	1.0 mL	1.0 mL
Total volume in tubes # 1 to # 5	10 mL	10 mL	10 mL	10 mL	10 mL
Dilution Series	10-fold	10-fold	10-fold	10-fold	10-fold
Dilution Level of Originated Extract	1	1:10	1:100	1:1,000	1:10,000
Concentration of Plant extract in mg/mL from tubes # 1 to # 5	10	1.0	0.1	0.01	0.001
Concentration of plant extract in µg/mL or ppm from tube # 1 to # 5	10,000	1,000	100	10	1

*Ten-fold Serial Dilution of the Powdered Plant Material in Artificial Seawater*

Three test tubes containing 0.1g or 100mg of each powdered snake leaves and roots, 10mL of artificial water was introduced and the test tube was corked and manually shaken by inverting 5 times. The concentration of the snake plant extract in this tube is 10mg/ml or 10,000µg/mL. Using a permanent marker, this tube was labelled # 1. Five other tubes were labelled 2, 3, 4, 5 & 6. The test tubes were placed in a rack side by side according to their number. To tubes # 2,3,4,5 artificial seawater of 9mL volume was added using 10mL glass pipette and tube # 6 was 10mL artificial seawater and serves as control set-up.

The plant powder in tube # 1(10mg/mL) was allowed to settle down for 1 hour. This should be produced a residue at the bottom and a clear supernatant liquid above it. After the plant powdered has settled to the bottom of tube # 1, 1mL of the supernatant was gently withdrawn using a 1 mL micropipettor and transferred to tube # 2. Tube # 2 was corked and inverted 3 times to mix the content. Tube # 2 was uncorked and return to its slot in the rack between # 1 and # 3. Using again the micropipettor, 1mL was

removed from tube # 2 and transferred to tube # 3 to tube # 5. This process performed is a 10-fold serial dilution indicating that in each tube as one move from tube # 1 to # 5, the concentration of the plant extract is being reduced by 10 times of the tube previous to it.

**Table 5.** Brine shrimp Bioassay for Snake Plant Leaves Extract

Well #	Conc. of Snake Plant Leaves Extract $\mu\text{g/ml}$	Log 10 Conc. Of Snake plant Leaves Extract	Initial nos. of shrimps	Shrimps surviving	Dead shrimps	% Mortality = dead shrimps $\div$ initial no. of shrimps x 100	% Mortality (corrected for control)
1	10,000	4	45	7	38	84	84-11=73
2	1,000	3	45	12	33	73	73-11=62
3	100	2	45	15	30	67	67-11=56
4	10	1	45	20	25	56	56-11=45
5	1	0	45	26	19	42	42-11=31
Control	0 (control)	-----	45	40	5	11	-----
Lethal Concentration @50 (LC <sub>50</sub> ) = <b>44.49 <math>\mu\text{g/ml}</math> (Highly Toxic)</b>							

**Legend:** 0-50 ppm (Highly Toxic); 51-100ppm (Moderately Toxic); 101-200ppm (Slightly Toxic); and 201 and above Not Toxic

#### Brine shrimp bioassay test

Table 5 showed the bioassay using brine shrimp in the leaves extract of snake plant. It showed a high level of cytotoxicity level of 44.49  $\mu\text{g/ml}$ . This means that leaves extract of snake plant is an effective medicinal plant and can be good medicinal plant especially as antibacterial agent due to the high toxicity level as shown in the table 5 above. This only means that the root extract part is more effective herbal medicine compared with leaves extract since the number of surviving shrimps are more than the root extract's number of surviving shrimps as shown in Table 6 below.

**Table 6.** Brine shrimp Bioassay for Snake Plant Roots Extract

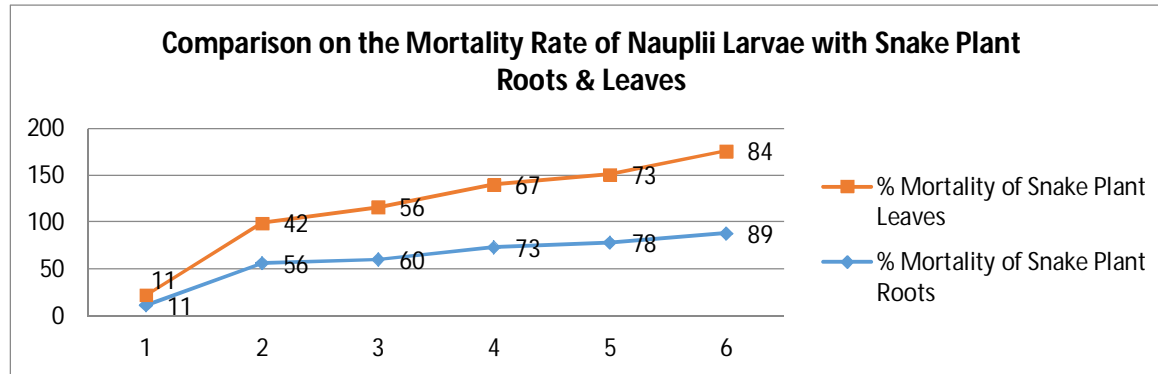
Well #	Conc. of Snake Plant Roots Extract $\mu\text{g/ml}$	Log 10 Conc. of Snake Plant Roots Extract	Initial nos. of shrimps	Shrimps surviving	Dead shrimps	% Mortality = dead shrimps $\div$ initial no. of shrimps x 100	% Mortality (corrected for control)
1	10,000	4	45	5	40	89	89-11=78
2	1,000	3	45	10	35	78	78-11=67
3	100	2	45	12	33	73	73-11=62
4	10	1	45	18	27	60	60-11=49
5	1	0	45	20	25	56	56-11=45
Control	0 (control)	-----	45	40	5	11	-----
Lethal Concentration @50 (LC <sub>50</sub> ) = <b>35.22 <math>\mu\text{g/ml}</math> (Highly Toxic)</b>							

**Legend:** 0-50 ppm (Highly Toxic); 51-100ppm (Moderately Toxic); 101-200ppm (Slightly Toxic); and 201 and above Not Toxic



### Mortality rate of Nauplii Larvae

This table showed that from the values of snake plant for its LC<sub>50</sub> for roots of 35.22 µg/ml and LC<sub>50</sub> of 44.49 µg/ml the brine shrimp bioassay, the root part is less effective when applied as an herbal medicine compared due to the number of less surviving shrimps and that it only proved that it can be less effective when its extracts are applied to these organisms and these events was best observed or shown below as the graph interpretation of comparing Tables 5 and 6 respectively.



**Graph 1.** The Comparison between the Toxicity Level of Snake Plant

This figure showed the comparative status of explaining the toxicity level of both extracts (plants and leaves) and explained why leaves extract is more effective as shown in Table 5 and the roots extract is less effective compared to the leaves extracts as shown in Table 6.

### Discussion / Findings

The screening of the plant material revealed the presence of the alkaloids, tannins, and anthraquinones were summarized in Table 4. Ethanol extract yielded almost all above mentioned phytochemicals. Since our plant extract have revealed the presence of alkaloids, we can say alkaloids also have shown the antibacterial activity.

The evaluated antibacterial activity of alkaloid of *Datura metel* Linn leaves against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi*, *Bacillus subtilis* and *Klebsiella pneumonia* but could not inhibit *Escherichia coli*. Flavonoids are ubiquitous in photosynthesing cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. For centuries, preparations containing these compounds as the principal physiologically active constituents have been used to treat human diseases [17].

Increasingly, this class of natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing antifungal, antiviral and antibacterial activity [6]. The n-butanol purified saponin extract of sorghum bicolor were screened for antibacterial activity against three pathogenic microbes; *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

The extract inhibited the growth of the *S. aureus*. It was found out that saponins have inhibitory effect on gram-positive organism but not on gram negative organism and the fungi [20]. Saponin extract from *Sider* (*Ziziphus spina christi*) were evaluated using antibacterial activity against gram negative bacteria like *E.coli*, *Proteus mirabilis* and gram positive like *Staphylococcus aureus* and *Streptococcus pneumoniae*. The inhibitory effect in in vitro was defined to appear inhibition zone around the paper disc. The study revealed that 30% (w/ v) concentration most active against bacteria [10]. Compounds of pharmacological interest (tannins) were isolated from the plant species, *Solanum trilobatum* Linn and assayed against the bacteria, *Staphylococcus aureus*, *Streptococcus pyrogens*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Escherichia coli* using agar diffusion method.

Tannins exhibited antibacterial activities against all the tested microorganisms [8]. Studies on the antibacterial activity of ethanol, methanol and aqueous extracts of dry flower and ethanol, methanol and acetone extracts of fresh flower of *Cassia auriculata* was conducted using agar disc diffusion method. The microorganisms used include *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Salmonella typhi*, *Salmonella paratyphi A*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Vibrio cholerae* and *Shigella dysenteriae*. The maximum activity was observed against all organisms except *Pseudomonas aeruginosa* and *Klebsiella pneumonia* [14].

The presence of important compounds which were separated by thin layer chromatography and the extract obtained by leaves and roots of the *Sansevieria trifasciata* showed potent antimicrobial activity. That the secondary metabolites in the plant extract was found out through its phytochemical analysis or screening process. Of which extract could be used for the treatment of various infections possessed as it possesses effective zone of inhibition. The toxicity of the extract when diluted with ethanol both the roots and leaves of the ST, its concentration showed that snake plant species killed 50% of the *Artemia salina* nauplii in 24 hours, as a result from its test thru Brine shrimp bioassay of its roots and leaves. As to the degree of lethality aspect of the study, the extractives were found to be directly proportional to the concentration of the extractives ranging from the lowest concentration (35.22 µg/ml) to the highest concentration (44.49 µg/ml). This concentration is a dependent increment in percent mortality of Brine Shrimp nauplii produced by the snake plant and which indicates the presence of cytotoxic principles in these extractives hence, making it as an effective therapeutic remedy for gastric ulcer and will lend the credence to the folkloric use of these plants in treating microbial infection.

## References

1. Anyasor C. & Austria, S. (2010) Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. King Saud University. Elsevier B.V.
2. Bañez, S. (1995) *Phytochemical Screening and Pharmacological Testing of Sanggumay Orchid (Dendrobium superbum Reichb)*. UNP Research Journal
3. Bose, A., Mondal, S., Gupta JK., and Ghosh, T. (2007) *Journal of Natural remedies*, 7, 132-134.
4. Chandrappa C. et al., (2012) Hydrothermal synthesis of amorphous MoS<sub>2</sub>nanofiber bundles via acidification of ammonium heptamolybdate tetrahydrate. National Center for Biotechnology Information, U.S. National Library of Medicine. 8600 Rockville Pike, Bethesda MD, 20894 USA
5. Chukwuka, KS, Ikheloa JO, Okonko IO, Moody JO., and Mankinde TA. (2011) *Adv. Appl. Sci. Res.* (4), 37-48.
6. Cushnie, J. et al., (2008) *How to Prune: Techniques and Tips for Every Plant and Season*. Kyle Cathie; illustrated edition, USA
7. Dhanalakshmi D, Kumar S, Prasad MS, Koli V, Kumar BP., and Harani A. (2011) *Eur. J. Exp. Bio.* 1 (1)
8. Doss, D. et al., (2009) Structural Insight into the Heme-based Redox Sensing from *Mycobacterium tuberculosis*. *ASBMB Journal of Lipid Research*
9. Guevarra, L. (2004) *Clinical Data Mining for Physician Decision Making and Investigating Health*. Idea Group Inc. Manila, Philippines
10. Kredy, L. (2010) Phacoemulsification-induced injury in corneal endothelial cells mediated by apoptosis: In vitro model. Elsevier Inc., Volume 34, Issue 12, Pages 2146–2152.
11. Lacaille-Dubois M.A. (2007) Bioactive saponins from plants: recent development in Yaniv Z. and U. Bacherach (eds.) *Handbook of Medicinal plants: India*, Harworth Press
12. Lawinsohn E. and Y. Tadmor (2007) *Biotechnology in medicinal crop improvement* in Yaniv Z. and U. Bacherach (eds.) *Handbook of medicinal plants: India*, Harworth press.
13. Maiscott, M. (2000) *Curing everyday ailments the natural way*. New York: The Reader's Digest n Association, Inc.
14. Maneemegalai, S. et al., (2010) Antioxidant, antiglycation and insulinotrophic properties of *Coccinia grandis* (L.) in vitro: Possible role in prevention of diabetic complications. U.S. National Library of Medicine. 8600 Rockville Pike, Bethesda MD, 20894 USA
15. Meyer, R. & McLaghin et. Al., (2004) Fe<sub>3</sub>O<sub>4</sub>@NiFe<sub>2</sub>O<sub>4</sub> Nanoparticles with Enhanced Electrocatalytic Properties for Oxygen Evolution in Carbonate Electrolyte. ACS Publications, 1155 Sixteenth Street N.W. USA
16. Nair, P. et al., (2008) *Advancing Medicine through Nanotechnology and Nanomechanics Applications*. IGI Global. Copyright.
17. Okwu, D. & Igara, F. (2009) Comparative Phytochemical, Nutrient and Anti-Nutrient of Stems of *Ipomoea Involucrata* Beauv, *Ipomoea. Triloba* L. and *Ipomoea Batatas* Lam. U. A. Essiet<sup>1</sup>, U. J. Ukpong. Department of Botany and Ecological Studies University of Uyo, P. M. B. 1017, Uyo. Akwa Ibom State-Nigeria
18. Prajapati N.D. et al (2007) *A Handbook of Medicinal Plants: A complete source Book-India*, Agrobios publishers.
19. Pugulayan, R. et al., (2004) Molecular phylogeny of Philippine freshwater sardines based on mitochondrial DNA analysis. IE Samonte RC Pagulayan WE Mayer. *Journal of Heredity*, Volume 91, Issue 3, 1 May 2000, Pages 247–253,
20. Soetan, K. et al., (2006) Assessment of the Anthelmintic Efficacy of an Aqueous Crude Extract of *Vernonia amygdalina*. *Journal Pharmaceutical Biology*. Volume 45, 2007 - Issue 7
21. Wadher, B. et al., (2012) Treatment of superficial pseudomonal infections with citric acid: an effective and economical approach. Elsevier Inc.