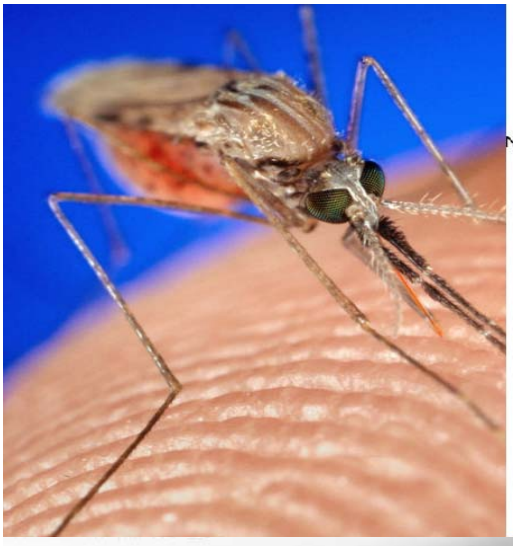
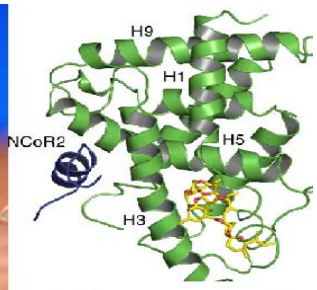
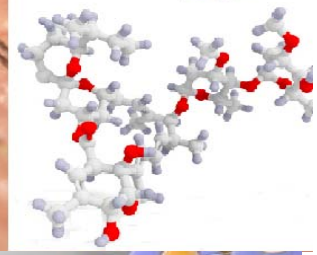
PhytoChem & BioSub Journal

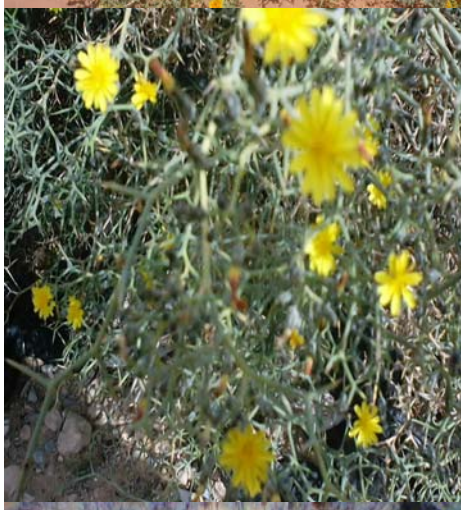
Peer-reviewed research journal on Phytochemistry & Bioactives Substances

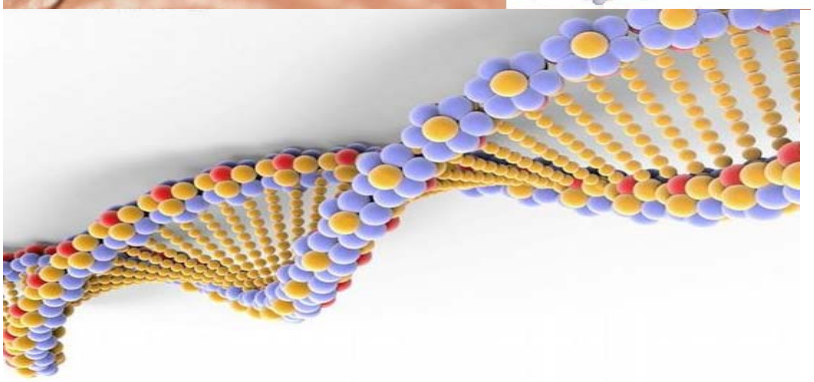
ISSN 2170 - 1768











PCBS Journal

Volume 10 N° 3 **2016**

ISSN 2170-1768

PhytoChem & BioSub Journal

ISSN 2170 – 1768

Peer-reviewed research journal on Phytochemistry & Bioactives Substances

CAS Source Index (CODEN: PBJHB3)

Editor in Chief

Pr *Abdelkrim* CHERITI

Phytochemistry & Organic Synthesis Laboratory

08000, Bechar, Algeria

PhytoChem & BioSub Journal (***PCBS Journal***) is a peer-reviewed research journal published by Phytochemistry & Organic Synthesis Laboratory. The PCBS Journal publishes innovative research papers, reviews, mini-reviews, short communications and technical notes that contribute significantly to further the scientific knowledge related to the field of Phytochemistry & Bioactives Substances (Medicinal Plants, Ethnopharmacology, Pharmacognosy, Phytochemistry, Natural products, Analytical Chemistry, Organic Synthesis, Medicinal Chemistry, Pharmaceutical Chemistry, Biochemistry, Computational Chemistry, Molecular Drug Design, Pharmaceutical Analysis, Pharmacy Practice, Quality Assurance, Microbiology, Bioactivity and Biotechnology of Pharmaceutical Interest). Contributions in all areas at the interface of Chemistry, Pharmacy, Medicine and Biology are welcomed.

Submission of an article to the ***PCBS Journal*** implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors.

The ***PCBS Journal*** reserves the right to submit all received manuscripts to *ad hoc* referees, whose names will be kept confidential, and will have the authority to decide on the pertinence for acceptance. Referees may send back manuscripts to Editor-in-Chief, for transmission to the author(s) with suggestions for necessary alterations, which are to be made in order to conform to the standards and editorial rules of the Journal. All manuscripts should be prepared in MS-Word format, and submitted online to **Phytochem07@yahoo.fr.** Upon receipt of paper submission, the Editor sends an E-mail of confirmation to the corresponding author within 1-4 working days. The Editors reserve the right to edit or otherwise alter all contributions, but authors will receive proofs for approval before publication.

Editorial Board

PhytoChem & BioSub Journal Vol. 10(3) 2016
ISSN 2170-1768
CAS-CODEN:PBJHB3

PCBS Journal
ISSN 2170-1768

PhytoChem & BioSub Journal

2016
Vol.10 No. 3

*In vivo*Acute Toxicity Studies Using Swiss Albino Mice and *In vitro* Cytotoxicity (BST) Assay of the Methanol Extracts of Stem bark of *Echinaceae angustifolia DC*

Abdu ZAKARI [1], Dimas KUBMARAWA [2], Sunday A. OSEAMEAHON [2] & Christiana A. KENDESON [1]

[1] *Department of Chemical Sciences. Federal University Kashere PMB 0182 Gombe – Nigeria*
[2] *Department of Chemistry, Modibbo Adama University of Technology Yola PMB 2076 Yola – Nigeria*

Received: July 12, 2016; Accepted: September 16, 2016
Corresponding author Email *abdu.zakari@fukashere.edu.ng*

DOI:10.163.pcbsj/2016.10.3.89

Abstract. *The methanol extract of Echinaceae angustifolia DC was tested for acute toxicity (LD_{50}) using Swiss Albino Mice and was screened for cytotoxicity assay using Artemia salina larvae. The results showed that the plant was toxic to the mice at even a low concentration and caused death at a concentration of 500mg/Kg body weight of the live mice. The LD_{50} was found to be 1,224.74mg/Kg. The result of the cytotoxicity showed that the extract was toxic to the naupli with an $LC_{50}$and LC_{90} values of 125.89µg/mL and 794.33µg/mL.*

***Key Words*:** *In vivo,* Acute Toxicity, Albino mice, *In vitro,* Cytotoxicity

Introduction

Plants are potential sources of modern drugs. It was reported in a survey of United Nations Commission for trade and development (UNCTAD) that about 13% of drugs produced within developed countries are derived from plants. Today less than 15% of the plant, were known to have been investigated pharmacologically (Kubmarawa*et al*, 2013).

Drugs from the plants are easily available, less expensive, safe and efficient, largely and/or rarely have side effects. According to WHO medicinal plants would be the best source to obtain variety of drugs. Large percentage of peoples from developed countries uses traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety and efficiency (Abdul Rani*et al.*, 2010).

A soxhelet extractor was invented in 1879 by Franz von Soxhelet and was originally designed for the extraction of a lipid from a solid material. It is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material (Lilybeth and Olga, 2013).

Studies have shown that medicinal plants have been prescribed and used with almost no change in the form or the way they have been used, and with a strong belief in their usefulness in disease prevention and control. The toxicity of plants is very important and measured by the LD_{50} and LC_{50}/LC_{90}as the case may be. The terms refer to lethal dose/concentration that killed 50% of the organism population and 50%/90% of the living cells respectively (Abdu et al, 2016).
Toxicity testing was given much attention following early 1960s thalidomide catastrophe; with thousands of children born worldwide with severe birth defects. After this incidence many countries of the world have resolved to go for toxicity testing and teratogenicity in both sexes so as to prevent further tragedies. Toxicity testing is considered paramount in the screening of drugs and/or chemical substances before it can be used on humans. According to the Organization of Economic Cooperation and Development (OECD), toxicity is the determination of potential hazards a test substance may likely produced and the characterization of its action (OECD, 2013).
The advantages of using animal models in toxicity testing include the possibility of clearly defined genetic constitution and their amenity to controlled exposure, controlled duration of exposure, and the possibility of detailed examination of all tissues following necropsy. Results obtained from toxicity testing can serve as the basis for hazard classification and labeling of chemicals and will help to check how safe a test substance is and to characterize the possible toxic effects it can produce. The present study is an effort to highlight the toxicity of the plant extract intended to be used in antimalarial testing.The importance of toxicity studies include the establishment of a dose response curve, to ensure safety of new chemicals for use as pesticides, drugs, or food additives before they are registered for general use in industry or doctors clinics, establish the mode of action or mechanism for a toxic effect that may have been seen in other studies, produce epidemiological studies to explain observations in the population, for instance, the long investigation into the association of smoking with lung, to validate new methods of testing or investigation, particularly those conducted in vitro rather than in animals(David and Enegide, 2013).

Materials and Method

Plant Collection and Identification

The fresh samples of the plants were collected in Girei Local Government Area of Adamawa State, Nigeria in November, 2015. The plant was identified by Clifford and authenticated by Botanists in the Department of Biological sciences Federal University Kashere.

Preparation of Plant Parts for Extraction

The plant samples were air dried and ground into powder with pestle and mortal and weighed. Around 60g of the powder was packed in a thimble of filter paper prepared manually. The thimble was then inserted into the Soxhlet apparatus, 500ml methanol was transferred down the thimble into the pot. A temperature of 75 ^{0}C was maintained and extraction continued for 6 hours. Then the methanol extract was collected and the cake recovered from the thimble was kept. The methanolic extract was evaporated on a rotary evaporator (R110) at 40^{0}C, altogether, 200g of each sample were extracted and labeled F01 (Abdu and Dimas, 2016).

*In Vivo*Acute Toxicity Test (LD_{50})

Laboratory Animals Used in the Research

The animal models involved in this study were the Swiss albino mice acquired from the National Veterinary Research Institute (NVRI) Vom in Jos – Nigeria.The experimental procedures relating to the animals were authorized by the Ethical committee of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja-Nigeria before starting the study and were conducted under the internationally accepted principles for laboratory animal use and care

(EEC Directive of 1986; 86/609/EEC). The parameters evaluated during this assay include body weight of the mice, survival time, paw licking, salivation, stretching/ writhing, erect fur, calmness, reduced movement, weakness, coma, convulsion, sleep and death. Albino mice were coded, weighed and randomized into three groups/dose with 3 mice per group/dose in two phases. In phase one, nine mice were randomized into three groups of three mice each and were given (5ml) each of 30, 300 and 500 mg/ kg body weight (b. wt) of the extract orally. The mice were observed for paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first four (4) hours and subsequently daily for seven (7) days. In phase two, another fresh set of nine mice were randomized into three groups of three mice each and were given 1600, 2900 and 5000mg kg^{-1} b. wt of the extract orally on the result of the first phase. They were observed for signs of toxicity and mortality for the first four critical hrs and thereafter, daily for 7 days. The LD_{50} was then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose i.e the geometric mean of the consecutive doses for which 0 % and 100% survival rates were recorded in the second phase, the oral median lethal dose were calculated using the formula:

LD_{50}=√ Minimum toxic dose X maximum toxic dose or
Oral median lethal (LD_{50}) dose =√ Minimum toxic dose X maximum toxic dose

Screening of Plants Extract in the Brime Shrimp Lethality Test

Brime shrimp eggs (*Artemia salina* premium grade) was generously provided by Professor O. A. Adoum of the Department of Pure and Industrial Chemistry Bayero University Kano – Nigeria.
Artemia salina eggs were added into a hatching chamber ¾ filled with ocean sea water. The chamber was kept in an open space for 24hours, after which the eggs hatched into shrimp larvae. 4ml ocean water was then added and ten (10) larvae of *Artemia salina* were introduced into each vial. After 24 hours of introducing larvae, the number of survivals were counted in triplicate and recorded. To each sample vial, a drop of DMSO solvent was added, ten shrimps were transferred using a Pasteur pipette, and artificial seawater was added to make a total volume of 5 ml. The nauplii were counted against a lighted background. Counting for the chronic LC50 began 24 hours after initiation of tests. Nauplii were considered dead if they were lying immobile at the bottom of the vials, and the percentage of deaths at each dose and at the control were determined (Abdu *et al*, 2016).

$$\% \text{ Deaths} = \frac{\text{No of dead shrimps}}{\text{No of survival shrimps in control}} \times 100$$

Statistical Analysis

Microsoft Excel spreadsheet application was used to formulate the regression equations from the data of mean results of percentage mortality of the brine shrimp versus the log of concentrations. These equations were later used to deduce the $LC_{50}$and LC_{90} values for the extract (Subrata *et al*., 2011).

Results

Volume of extract per live weight of mice

Volume of extract solution = (Weight of mice X Dose)/Stock concentration
Weight of mice = 35g
Stock concentration = 250mg/ml
Dose = 5000mg/Kg
1000g ⟶ 1Kg
35g ⟶ x

x = 0.035Kg
Vol. = (0.035Kg X 5000mg/Kg)/250mg/ml
Vol. = 0.70ml

The results for the acute toxicity test were presented in tables 1-3.

Table 1: Phase I: *In vivo*Acute Toxicity Test of Methanol Extract of Stem bark of *Echinaceae angustifolia DC*

30mg/Kg (3mg/mL)	**Vol. (mL)**	**Signs of toxicity**	**Survival**
28g	0.28	None	1
22g	0.22	None	1
19g	0.19	None	1
300mg/Kg(30mg/mL)			
29g	0.29	raised tail	1
28g	0.28	weakness	1
30	0.30	salivation	1
500mg/kg(50mg/mL)			
31g	0.31	convulsion	death
31g	0.31	paralysis	death
35g	0.35	convulsion	death

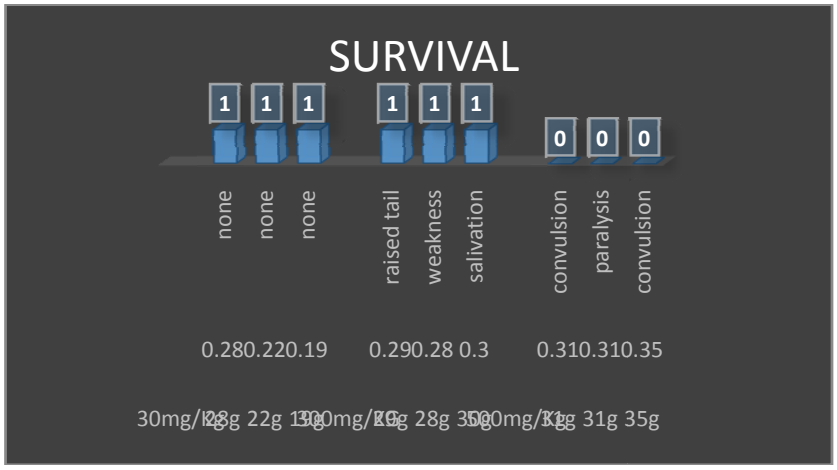


Fig 1: Phase I Acute Toxicity Test of Methanol Extract of Stem bark of *Echinaceae angustifolia*

Table 2: Phase II : Acute Toxicity Test of Methanol Extract of Stem bark of *Echinaceae angustifolia*

16000mg/Kg (160mg/mL)	**Vol. (mL)**	**Signs of toxicity**	**Survival**
28g	0.28	paralysis	Death
28g	0.28	convulsion	death
18g	0.18	coma	death
2900gmg/Kg(290mg/mL)			
32g	0.32	erect fur	death
26g	0.26	convulsion	death
21	0.21	death	death
5000mg/kg(500mg/mL)			
33g	0.33	death	
26g	0.26	death	
22g	0.22	death	

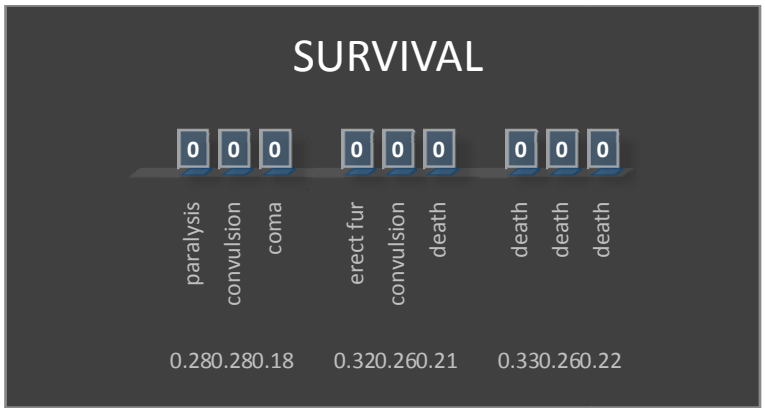


Fig 2: Phase II Acute Toxicity Test of Methanol Extract of Stem bark of *Echinaceae angustifolia*

By definition Oral Median Lethal Dose (LD_{50}) =
ɬ minimum toxic dose X maximum toxic dose
= ɬ 300 X 5000
= 1,224.74mg/Kg

Table 3: BST Assay Results of MeOH extract of Stem bark of *Echinaceae angustifolia DC*

Conc. µg/mL	Survival V_1	V_2	V_3	Death V_1	V_2	V_3	% Mortality	Log_{10}Conc.	LC_{50} (µg/mL)	LC_{90}(µg/mL)
1000	1	2	1	9	8	9	86.67	3		
500	3	1	2	7	9	8	80.00	2.7		
250	2	3	3	8	7	7	73.33	2.4		
125	5	3	5	5	7	5	56.67	2.1	125.89	794.33
65.5	6	7	8	4	3	2	30.00	1.8		
Ctrl(+)	0	0	0	10	10	10	100.00			
Ctrl(-)	10	10	10	0	0	0	0.00			

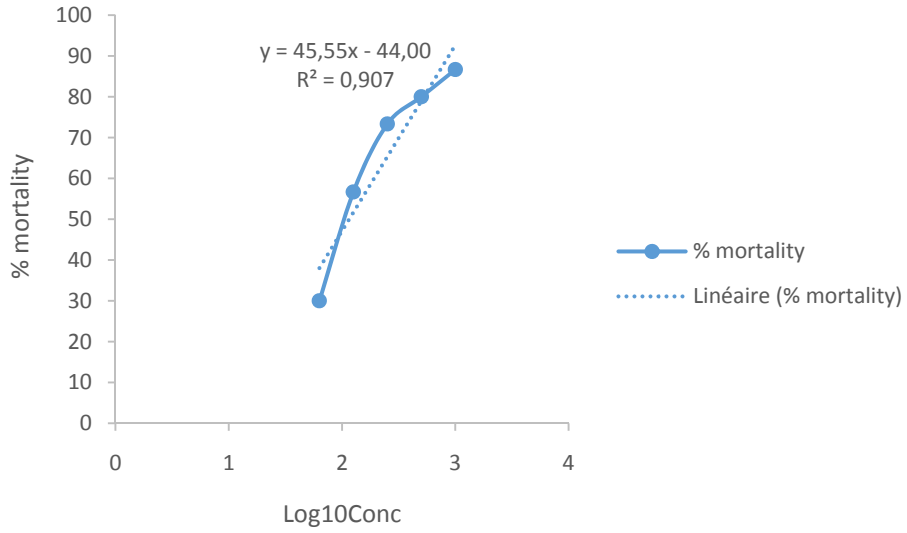


Fig 3: Graph of % mortality versus log10 Conc. For Stem bark extract of *Echinaceae angustifolia*

Discussion

The acute oral toxicity of methanol extracts of *E. angustifolia* was determined in the present study. The evaluations of the in vivo acute toxicity as well as determining the $LC_{50}$and LC_{90} values using the brine shrimp lethality test were done. The screening of the toxicity of the plant was crucial to assure the safety and effectiveness of the plant extracts. While the *in vivo* method provided hint of toxicity of the extract in the animal body, the*in vitro* cytotoxicity test gave result with limited information. Signs of toxicity such as pain, distress, allergic reactions, physical changes in the tested animals can be detected in the *in vivo* assays but not effects on vital functions like the cardiovascular, central nervous, and respiratory systems.In this study of acute oral toxicity, 36 Swiss albino mice were employed to observe the toxicity effects of methanol crude extract of *E. angustifolia* leaves. From the result, mortalities were reported as well as adverse toxicity signs were observed on the tested mice right from the lower dose. The physical appearance such as, fur, raised tails, salivation, paw licking were observed which indicated that the crude extract did affect the animals. Hence, *E. angustifolia* has caused acute toxicity effects with an LD_{50} value of 894.42mg/Kg which suggest the possibility of the use of the extract as a potential source for the development of pharmacological agent to treat various types of ailment.
The Brine shrimp bioassay was used to determine the potential toxicity of *E.angustifolia* methanol extract to become lethal to *A. salina* nauplii due to its toxic expression. According to Abdul Rani *et al* 2010, extracts derived from natural products which have $LC_{50} \leq 1000\mu g/mL$ are known to be toxic. In this study, the plotted graphs show that the LC_{50} and LC_{90}values of the crude extract were 125.89µg/mL and 794.33µg/mL respectively.

Conclusion

Thus, the results proved that the methanol extract of *E. angustifolia* is toxic both *in vivo* and *in vitro* and can be further explored in research such as antimalarial properties for which the plant is traditionally known for in Adamawa State – Nigeria and in the research for the development of natural product-based pharmaceutical products.

References

1. Abdu Z., Dimas K., Sunday A.O., Isyaka M.S., Sa'id J.(2016):Qualitative investigation of phytochemicals and brine shrimp lethality test of the root, stem bark and leaves extract of *Isoberlinia doka (Fabaceae*);*International Journal of Chemical Studies 4(1) 112-116.*
2. Abdu Zakari and Dimas Kubmarawa (2016):*In vitro* Cytotoxicity Studies and Qualitative Investigation of Phytochemicals of Stem Bark Extracts of *Detarium microcarpum (Caesalpinioideae), Echinaceae angustifolia (Compositae) andIsoberlinia doka (Fabaceae)National Journal of Multidisciplinary Research and Development 1(1): 22-26*
3. Abdul Rani Muhamad Syahmi , Soundararajan Vijayarathna , Sreenivasan Sasidharan ,Lachimanan Yoga Latha , Yuet Ping Kwan , Yee Ling Lau , Lai Ngit Shin and Yeng Chen (2010); Acute Oral Toxicity and Brine Shrimp Lethality of *Elaeis guineensis* Jacq., (Oil Palm Leaf) Methanol Extract; *Molecules 15*,8111-8121;doi:10.3390/molecules15118111
4. David Arome and Enegide Chinedu (2013):Importance of toxicity testing;*J. Pharm. BioSci. 4, 146-148*
5. D. Kubmarawa, J.A. Akiniyi, D.A. Okorie (2013): Ethnomedicinal survey of the traditional medicine of Lala people of Nigeria; International Journal of Medicinal Plant and Alternative Medicine, Vol. 1(3), pp. 039-057.
6. Lilybeth F. Olowa and Olga M. Nuñeza (2013): Brine Shrimp Lethality Assay of the Ethanolic Extracts of Three Selected Species of Medicinal Plants from Iligan City, Philippines; *International Research Journal of Biological Sciences*Vol. 2(11),74-77

7. OECD (2002): OECD guidelines for the testing of chemicals: Proposal for a new guideline 223 – Avian acute oral toxicity test, available at: http://www.oecd.org/dataoecd/16/41/1836204.pdf. ACCESSED 30/6/16.
8. Subrata Kumar Biswas, Anusua Chowdhury, Joysree Das, Utpal KumarKarmakar, Manik Chandra Shill and Sheikh Zahir Raihan(2011):Assessment of cytotoxicity, antibacterial activity and phytochemical screening of ethanol extract of *Phyllanthus acidus* L. (family: Euphorbiacceae) bark;*Journal of Applied Pharmaceutical Science* 01 (06): 112-114.

PhytoChem & BioSub Journal

Peer-reviewed research journal on Phytochemistry & Bioactives Substances

ISSN 2170 - 1768

PCBS Journal

ISSN 2170-1768

Edition LPSO - Phytochemistry & Organic Synthesis Laboratory-
http://www.pcbsj.webs.com
https://sites.google.com/site/phytochembsj/
Email: phytochem07@yahoo.fr