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Screenings of antimicrobial, cytotoxic and anti-inflammatory potential of crude methanolic extract of *Crinum latifolium leaves*

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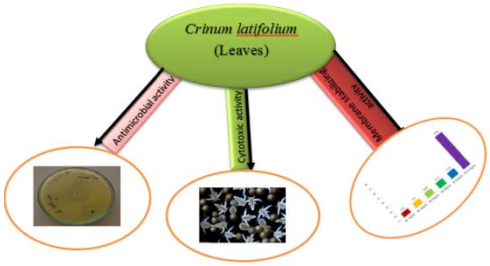
ABSTRACT



Crude methanolic extract of *Crinum latifolium* plant leaves was assayed for various pharmacological properties. Antimicrobial potential of crude methanolic extracts of *Crinum latifolium* was accomplished by most commonly used disc diffusion method against a wide range of Gram positive and Gram negative bacteria. Extracts showed slight antimicrobial activity against Gram positive bacteria while significant antimicrobial activity against Gram negative bacteria. In contrast to Vincristine Sulphate, the crude metanolic, n-hexane soluble, petroleum ether soluble and chloroform soluble extracts showed slight to moderate cytotoxic properties with LC_{50} value of 7.06µg/ml, 48.978µg/ml, 242.83µg/ml and 153.93µg/ml respectively. Plant extract showed significant anti-inflammatory properties i.e 16.21% & 20.55%10mg/ml for hypotonic solution and heat induced condition respectively. So, this plant extract demands further research for revealing all its potency to have new safe drug for the entire respective field of medical science.

Keywords: Crinum latifolium, Zone of inhibition, Brine shrimp lethality bioassay, anti-inflammatory.

INTRODUCTION

Cancer or tumor is the most common cause of death in both developed and developing countries. Cancer preliminary affects a specific part of our body and then invade to the other parts of our body very quickly and ultimately causes death of the patient.[1,2] There are several approaches of cancer treatment including surgery, radiation therapy and chemotherapy. Each approach possesses several side effects. The later approaches aimed to destroy cancerous cell from the body.[3] Antibiotic resistance has become a great concern of treating infectious disease globally which offer great challenges for clinicians and pharmaceutical industry.[4] Many of our currently used antibiotics have become less active against a wide range of pathogen due to emergences of drug resistance. On the other hand, newly discovered drug possess many unwanted side effect. So the

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analysis of medicinal plants to explore antimicrobial agents will be a fruitful task in generating new way of treatment.[5,6]

It is established that stabilization of lysosomal membrane limiting the inflammatory response through inhibiting the release of lysosomal constituents such as bactericidal enzymes and proteases which cause further tissue inflammation and damage upon extracellular release.[7] It is evidence that RBC membrane represents the lysosomal membrane. So, if the drugs effect on the stabilization of erythrocyte membrane could be resembled to the stabilization of lysosomal membranes [8].Anti-inflammatory agent causes the red blood cells membrane stabilization, subjected to hypotonic stress, through the release of hemoglobin (Hb) from RBCs.[9] Therefore, the stabilization of red blood cells hypotonic solution induced condition represent useful technique for the assessing the anti-inflammatory activity of various plant extractives.[10] *Crinum latifolium* is an herb belonging to the family Amaryllidaceae that arises from an underground bulb. It is locally known as sukhdarsan. Phytochemical screening of leaves reveals the presence of a wide variety of compounds such as alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, amino acids, steroid saponins, and antioxidants. Traditionally Bulbs are used as a rubefacient for rheumatism. Juices of the leaves are used for earaches. Crushed and toasted bulbs are used for piles and abscesses to hasten suppuration.[11] The purpose of our current study is to analyze antimicrobial, cytotoxic and membrane stabilizing potentials of the plant methanolic extract.

MATERIALS AND METHOD

Collection and identification of plant material

The fresh leaves of *Crinum latifolium* were collected from Noakhali, a coastal region of Bangladesh on 26th July 2012 and taxonomically identified by taxonomist and botanist of Bangladesh National Herbarium, Mirpur, Dhaka. Their given Accession number was -37751.

Plant extract preparation and isolation

The leaves were allowed to sun dry for ten days and pounded into coarse powder means of a suitable grinder. From which 400 gm of pounded material was taken into a suitable clean, flat-bottomed glass container and extracted with 1600 ml of 80% Methanol. Then the container with its contents was sealed with a suitable aluminum foil and kept at room temperature for 14 days.[15] During this time the sample mixture were shacked and stirred at regular interval of time. The mixture was then passed through markin cloth in order to obtain maximum quantity of extract. It was then filtered through whatman filter paper and allowed to evaporate at a convenient rotary evaporator. The filtrate (Methanol extract) was then placed in a water bath. After a certain period of time the extract converted into a brownish black color residue, properly preserved at 4º C temperature, which was then used as a sample for further study.

ANTIMICROBIAL ACTIVITY

To determine the antimicrobial potential of the plant antimicrobial screening were usually performed.[12] There are a wide range of accepted method for antimicrobial screening like-Disc diffusion method, Serial dilution method and Bioautographic method.[13,14] But for the convenience of our project we used slightly modified Disc diffusion method.[15-16]

Test organisms:

Strain of Gram positive (*Staphylococcus aureus*)[17] and three strains of Gram negative (*Escharichia coli, Salmonella typhi, Pseudomonas aeruginosa*) bacteria were used as a test organism for antimicrobial activity. The strains of these organism were collected from the Department of Microbiology,Noakhali Science and Technology University, Sonapur-3814, Noakhali, Bangladesh and are sub-cultered in nutrient broth and nutrient agar culture media.

Media preparation

Nutrient agar medium (DIFCO) is the popular media for determining the response of the organism against test materials and to prepare fresh cultures. In the preparation of media, all the constituents of the media were taken into a previously sterilized conical flask, according to the media preparation chart. Distilled water then added to the flask for required volume of 1000 ml. The pH of the media was maintained at 7.2-7.6 by using NaOH or HCL and the mixture was boiled at a suitable magnetic stirrer for perfect dissolution. After that, the tip of the flask was capped with a flag of cotton and aluminum foil and sterilized by autoclaving machine at a pressure of 15 lbs/sq inch, for 25 minutes at 125ºC temperature. About 20ml and 5ml of media was placed in a number of screw cap test tube for the purpose of plate and slant preparation and kept at water bath to lower the temperature to 45-50ºC. These slants were used for preparing fresh culture of microorganisms, which further used for sensitivity test.

Preparation of sub-culture

The test organisms were then transferred into agar slant from pure culture in an aseptic condition by using an inoculating loop under the defined condition of laminar air cabinet. The slants were incubated at a temperature of 37ºC for a period of 18-24 hours to allow proper growth of the microorganism. These newly prepared cultures were used for further analysis i.e. for sensitivity test.

Test plate preparation

The organisms under investigation were then transferred into the test tube containing about 20ml of previously melted and sterilized agar media by the help of inoculating transferring loop in an aseptic condition. The tube was then shaken to get uniform mixture of organism by gentle rotation. Immediately after that, the bacterial suspensions were transferred into sterile petri dishes in an aseptic laminar air cabinet and allowed to rotate in clockwise-anticlockwise direction for several times to ensure homogenous distribution of test organism.

Test sample preparation

In order to prepare test sample, 10 mg of dried crude extract was dissolved in 10 ml of methanol, so that the final concentration could be 1mg/ml and loaded into dried & sterilized filter paper discs with different volume (25 µl/disc, 50 µl/disc, 75 µl/disc, and 100 µl/disc respectively) using micropipette.

Application of discs, diffusion and incubation

These freshly prepared sample discs and commercially available standard antibiotic disc were transferred to each petri

dish carefully using a sterile forceps. The discs were placed in such a way that, the disks were no closer than 15 mm to the edge of the plate and far enough from each other to prevent overlapping of zones of inhibition. The plates were then inverted and kept in a refrigerator for about 24 hours at 4°C to allow sufficient diffusion of the materials from the discs to the surrounding area of the medium. The dishes were then incubated at 37˚C for 24 hours to allow optimal growth of microorganism.

Measurement of zone of inhibition:

Antibacterial activity of test sample was measured by calculating zone of inhibition,[22] which can be expressed in millimeter or centimeter unit by using suitable antibiotic zone scale. Different antibiotics discs (Ampicillin, Imipenem, Penicillin and Cefixitime) and sterile filter paper disc with respective solvent (methanol) of 25 µl were used as positive and negative control respectively. If the test sample possesses any antimicrobial activity, it will reduce the growth of the microorganisms and a clear, distinct zone of inhibition will be appeared surrounding the medium.

Brine shrimp lethality bioassay

The measurement of toxicity plays a vital role in drug discovery and is a useful tool in biological, especially ecological investigations.[18] It's also serves as a tool for screening plant extracts of possible medicinal value. In this study, we used simple brine shrimp bioassay test of Meyer with slight modification by using *Artimia salina* as test organism, which was collected from a pet shop.[19]

Brine shrimp hatching

Sea water was prepared by dissolving 38 gm sea salt (pure NaCl) in one liter of distilled water, which is then filtered to get clearsolution of 3.8% concentration.[20] In a suitable plastic or glass vessel sea water was taken and shrimp eggs were added to one side of the vessel and allowed to hatch for 24 hours till the mature nauplii were found. Continuous oxygen and light supply were provided to support the hatching process.

Sample preparation

Test solution was prepared by mixing 4mg of methanolic, extract with 200µL of DMSO in a suitable vial by using vortex mixer. 100µL of sample was taken from the vial and mixed with 5ml of sea water in a test tube. Thus the concentration we obtained was 400µg/ml. Then a series of solution at a concentration of (200 µg/ml ,100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.5625 µg/ml, 0.78125µg/ml) were prepared by serial dilution method.

Negative control group test

100 µl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii to use as negative control groups.

Positive control group test

Here we used vincristine sulphate (VINCRIRST ®, Techno Drugs Ltd., Bangladesh) as a positive control. Measured amount of vincristine sulphate was dissolved in DMSO to get an initial concentration of 40 µg/ml from which serial dilutions were made using DMSO to get 20 µg/ml, 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml and 0.078125 µg/ml respectively. Then the positive control solutions were added to the pre-marked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups.[21]

Counting of nouplii

After 24 hours, the number of survived nauplii in each vial was counted by using magniflying glass. From this data the percent (%) of mortality of brine shrimp nauplii was calculated for each concentration.

Membrane stabilizing activity

The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution hemolysis of human erythrocytes following the method developed by Omale et al. (2008).[8]

Table 1:Antimicrobial effect of crude methanolic extract of *Crinum latiflium.*

Test microorganism(Bacteria)		Zone of inhibition of extract in various conc.				Response of the standard against microorganism
Gram Positive		25µl	50µl	75µl	100µl	10/30 µl/Disc
	Staphylococcus Aureus	_	_	_	1.0 ±0.039* cm (+)	Ampicillin (10µl) +++
Gram Negative	*Salmonella Typhi*	_	_	_	_	Penicillin (10µl +++
	E. coli	1.0±0.025*cm (+)	1.2±0.18cm (+)	1.4±0.37cm (++)	1.6±0.004** cm (++)	Cefoxitin (30µl) +++
	Pseudomonas Aeruginosa	_	_	_	_	Imipenem(10µl) +++

Here, (+++) = highly active; (++) = moderately active; (+) = slightly active; (-) = No activity against microorganism.***= P < 0.001, **= P < 0.01, *= P < 0.05

Statistical analysis

All the above assays were conducted in triplicate and repeated threes for consistency of results and statistical purpose. The data were expressed as Mean±SD and analyzed by one way analysis of variance (ANOVA) followed by Dunnett 't' test using SPSS software of 10 version. P<0.05 was considered statistically significant.

RESULTS

Antimicrobial Activity

From the experiment we could see that, crude methanolic extracts of *Crinum latifolium* showed slight activity against Gram positive *Staphylococcus aureus* bacteria. On the other hand, it showed good antibacterial properties against Gram negative *E. coli* bacteria. The overview of the results is shown in the table 1.

Findings of brine shrimp lethality bioassay:

By using brine shrimp bioassay, developed by Meyer we could understand the cytotoxic potential and anti-tumor properties. In our current study we used various solvent soluble extracts of *Crinum latifolium.* Different solvent soluble extracts (ME, HSE, PESE, and CSE) showed various rate of mortality at different concentration. By plotting the log of concentration against percent of mortality for all test sample, we found a linear correlation. On the basis of this correlation the LC50 (the concentration at which 50% of mortality of brine shrimp nauplii occurred) was determined for each solvent soluble extracts. We also found that, there was no rate of mortality obtained, in case of control study. The overview of the results is shown in table 2

Table 2: results of brine shrimp lethality bioassay of crude methanolic extract of *Crinum latiflium*

Sample	LC_{50} (µg/ml)	Regression Equation	R^2
Vincristine Sulphate (positive Control)	0.79	y = 2.65x+2.60	1.71
ME (Leaves)	7.06	y = 2.74x + 2.57	3.40
HSE	48.978	y = 42.88x – 22.502	0.671
PESE	242.83	y = 66.137x - 102.82	0.9125
CSE	153.93	y = 29.79x – 15.16	0.93

Here, ME=methanoloic extract, HSE= n-hexane soluble extract, PSESE= petroleum ether soluble extract, CSE= chloroform soluble extract

Anti-inflammatory activity:

The anti-inflammatory activities of the Crude methanolic extracts of *C. latifolium* are showed in Table 3 & 4. The crude methanolic extractsdose dependently increased in anti-inflammatory study, whereas 10 mg/ml concentration most significantly showed 16.21% & 20.23% inhibition of hemolysis respectively by hypotonic solution and heat induced hemolysis. Acetyl salicylic acid was used as standard in membrane stabilization. ASA (0.10 mg/mL) revealed 70.01% & 56.32% inhibition of hemolysis respectively induced by hypotonic solution and heat induced hemolysis correspondingly.

Table 3: Effect of different conc. of methanolic extract of *C. latifolium* on hypotonic solution-induced hemolysis of erythrocyte membrane.

Treatment	Concentration	Optical density of samples in hypotonic solution (Mean ± SD)	% inhibition of haemolysis
Control	_ _ _	3.701±0.058	
ME	2 mg/ml	3.423±0.075	7.51±0.0077
ME	4 mg/ml	3.265±0.108	11.78±0.0080
ME	6 mg/ml	3.234±0.082	12.61±0.0088
ME	8 mg/ml	3.123±0.1012*	15.62±0.0092
ME	10 mg/ml	3.101±0.098**	16.21±0.0101
Acetyl salicylic acid	0.10 mg/ml	1.712±0.043***	53.74±0.0265

Here, ME stands for methanolic extract and values are represented as mean ± SEM (n=6); *p<0.05, **p<0.01, ***p<0.001

Table 4: Effects of different conc. of methanolic extract of *C. latifolium*on heat induced hemolysis of erythrocyte membrane.

Treatment	Concentration	OD of sample ±SD		% inhibition of Hemolysis
		Heated Solution	Unheated Solution	
Control	- - -	1.093±0.035		
ME	2 mg/ml	0.879±0.107	0.856±0.011	9.7±.069%
ME	4 mg/ml	0.780±0.004	0.743±0.017	10.57±.043 %
ME	6 mg/ml	0.715±0.014	0.650±0.022	14.67±.062 %
ME	8 mg/ml	0.587±0.082	0.461±0.342*	19.93±.036 %
ME	10 mg/ml	0.405±0.008	0.227±0.021**	20.55±.087 %
Acetyl Salicylic Acid	0.10mg/ml	0.672±0.025	0.129±0.029***	56.32±0.228 %

Here, ME stands for methanolic extract and values are represented as mean ± SEM (n=6); *p<0.05, **p<0.01, ***p<0.001

DISCUSSION

The medicinal properties of the plants lie in a several chemical group such as tannins, flavonoids, alkaloids and phenolic compound. Many parts of the plant specially leave possess antimicrobial properties due to presence of tannins and flavonoids.[22,23] Plants also synthesize huge amount of aromatic compound among which phenols or their oxygen-substituted derivatives are predominant.[24]

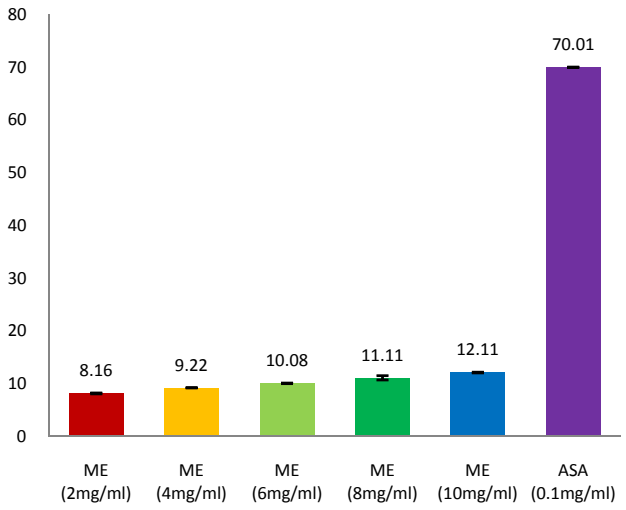

Figure 1: Effect of different conc. of *C. latifolium* on heat induced hemolysis of erythrocyte membrane.

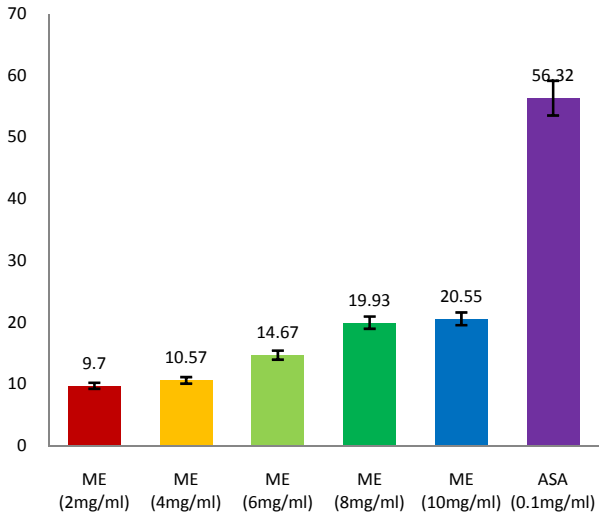

Figure 2: Effect of different conc. of *C. latifolium* on hypotonic solution induced hemolysis of erythrocyte membrane.

These compounds provide protection against microbes for the plant.[25] This is great to see our plant extract showed to have phytochemicals responsible for anti-microbial effect.[11] May be that is why Extracts showed slight antimicrobial activity against Gram positive bacteria while Surprisingly showed significant antimicrobial activity against Gram negative bacteria *E. coli.* Cancer-related research is conducted all over the world for discovering new hopes for patient suffering with cancer. These studies frequently able to originate biologically active agents from plants used and will be used for treating different carcinoma[26].In addition, it is important to understand the mechanisms of anticancer agents for future application in cancer therapy.[27] Our present study investigated the cytotoxic activity of the methanolic extract of *Crinum latifolium.* It was found that many of the phytochemicals provide protection against cancer due to poly-phenyl antioxidant and anti-inflammatory effect. Several studies also suggest that these phytochemical provide protection against colorectal plus other types of cancer.[28-30] Our plant part also contain polyphenol so this plant was one will be one of the most trusted source for discovering anticancer drug, that was so far established through our present study as our plant methanolic extract showed remarkable cytotoxic activity. *Crinum latifolium* methanolic extract inhibited hypotonic solution and heat induced hemolysis of erythrocyte at varying percentage that was comparable with membrane stabilizing activity shown by standard Acetyl salicylic Acid. As through the standard anti-inflammatory drug showed higher stabilization activity than the experimental plant methanolic extract, but our plant extract will be the existing source of anti-inflammatory activity with fewer or no side effects. The moderate membrane stabilizing activity shown by our plant methanolic extract may be due to the presence of flavonoid contents.[31] It has been established by many experimental study that plants with flavonoids shown profound stabilizing effects on lysosomes both *in vitro* and *in vivo* laboratory condition.[32]

CONCLUSION

From the above experiments we can conclude that the crude methanolic and various solvent soluble extracts of *Crinum latifolium* (leaves) showed slight to moderate cytotoxic activities. It also revealed excellent antibacterial and membrane stabilizing activities.

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REFERENCES

1. W.C. Evans. *Treaseand Evanspharmacognosy*. London: WB Saunders **2002**.
2. J. Ueda, Y. Tezuka, A.H. Banskota, Q. Tran, Harimayay, SaikiI, etal. Antiproliferative activity of Vietnamese medicinal plants. *Biol Pharm Bull.* **2002**, 25, 753-60.
3. S.E. Kintzios, M.G. Barberaki. Plants that fight cancer. Boca Raton: CRCPress. **2004**.
4. J. Bauer, R. Rojas, B. Bustamante. Antimicrobial activity of selected Peruvian medicinal plants. *J Ethnopharmacol*. **2003**, 88, 199- 204.
5. A. Tamrakar, A. Singh, M. Chodhrary, P. Kodgire. Fighting with Gram-negative enemy: Can outer membrane proteins aid in the rescue?. *Chemical Biology Letters*, **2017**, 4(1), 9-19.
6. D. Runyoro, M. Matee, N. Olipa, C. Joseph, H. Mbwambo. Screening of Tanzanian medicinal plants for anti-Candida activity. *BMC Complement Altern Med.* **2006**, 6, 11.
7. V.Rajendran, K.S.Lakshmi. In vitro and In vivo anti-inflammatory activity of leaves of *Symplocos cochinchnensis* (Lour) Mooressp laurina.*Bangladesh. J. Pharmacol*.**2008**, 3, 121.
8. J.Omale, P.N.Okafor. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. *Afr. J. Biotechnol.* **2008**, 7, 3129-3133.
9. R.A.Naibi, V.S. Sethuraman, N.Suluchana, J.Sadique. Satellite symposium on traditional medicine as Asian congress of Pharmacology.*Tamil University of Thanjavur*.**1985**, 140.
10. O.O.Oyedapo, F.C.Sab, J.A.Olagunju. Bioactivity of fresh leaves of *Lantana camara*. *Biomed. Lett.* **1999**, 59, 175- 183.
11. S.M.R. Dewan, D. Abhijit. Investigation of in vitro thrombolytic potential and phytochemical nature of *crinum latifolium* leaves growing

in coastal region of Bangladesh. *International Journal of Biological & Pharmaceutical Research*. **2013**, 4(1), 1-7.
12. N. Gupta, C. Gupta, S. Sharma, R.K. Sharma, H.B. Bohidar. Comparative study of antibacterial activity of standard antibiotic with silver nanoparticles synthesized using ocimum tenuiflorum and garcinia mangostana leaves. *Chemical Biology Letters*, **2015**, 2(2), 41-44.
13. A. Mittal, K. Thanki, S. Jain, U. Banerjee. Comparative studies of anticancer and antimicrobial potential of bioinspired silver and silver-selenium nanoparticles. *Appl. Nanomed.*, **2016**, 1(1), 1-6.
14. W.C. Evans. Trease and Evans Pharmacognosy (Microbial Assay). WB Saunders Company Ltd, London.**1996**,14, 473.
15. A. Bauer, W. Kirby, J.C. Sherris, M. Turck. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*. **1966**, 45, 493.
16. S. Prabhu, S.J. Britto, P. Thangavel, L. Raj. Antibacterial and Antioxidant Activity of Leaves of *Canavalia Mollis* Wight and Arn (Horse Bean). *Int. J. Pharm. Sci. Drug Res* **2011**, 2.
17. H. Jafri, F. Husain, I. Ahmad. Antibacterial and antibiofilm activity of some essential oils and compounds against clinical strains of Staphylococcus aureus. *J. Biomedical Therapeutic Sciences*, **2014**, 1(1), 65-71.
18. A.Opler, R.Mizell, A.Robert, M.Cervantes-Cervantes, D.Kincaid, E.J.Kennelly. Use of a brine shrimp assay to study herbal teas in the classroom. *The American Biology Teacher*. **2002**, 64, 8596-8604.
19. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, J.L. McLaughlin .Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med*. **1982**, 45, 31–34.
20. A.V. Krishnaraju, T.V.N. Rao, D. Sundararaju, M. Vanisree, H.S. Tsay, G.V. Subbaraju. Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test). *International Journal of Applied Science and Engineering*. **2006**, 4(2), 115-125.
21. M.K.Islam, Z.EtiI,J.A.Chowdury.Cytotoxic Studies on Two Meliaceae plants: *Chukrasia Tabularis* and *Aglaia Roxburghiana*. *J. Sci. Res*. **2009**, 1(2), 399-403
22. A.Scalbert. Antimicrobial properties of tannin. *Phytochemistry* **1991**, 30, 3875-3883.
23. K.T. Chung, T.Y. Wong, Y.Wei, Y.W. Huang, Y. Lin. Tannins and human health. A review. *Crit Rev Food Sci Nutr*. **1998**, 8, 421-464.
24. T.A. Geissman. Flavonoid compounds, tannins, lignins and related compounds. In: Florkin M and Stotz EH (eds). Pyrrole Pigments, Isoprenoid Compounds and Phenolic Plant Constituents, New York, USA: Elsevier Press. **1963**, 265.
25. M.M. Cowan. Plant products as antimicrobial agents. *Clin Microbiol Rev*. **1999**, 12, 564-582.
26. P. Kumari, R. Gautam, A. Milhotra. Application of Porphyrin nanomaterials in Photodynamic therapy. *Chem Biol Lett*, **2016**, 3(2), 32-37.
27. Half E, Arber N. Colon cancer: Preventive agents and the present status of chemoprevention. *Exp. Opin. Pharmacother*. **2009**, 10, 211–219.
28. D.S. Michaud, D. Feskanich, E.B. Rimm. Intake of specific carotenoids and risk of lung cancer in two prospective U.S. cohorts. *Am J Clin Nutr*. **2000**, 72, 990-997.
29. E.R. Greenberg, J.A. Baron, T.D. Tosteson. A clinical trial of antioxidant vitamins to prevent colorectal cancer. *N Engl J Med*. **1994**, 331, 141-147.
30. D.F. Birt, S. Hendrich, W.Q. Wang. Dietary agents in cancer prevention: flavanoids and isoflavonoids. *Pharmacol Ther*. **2001**, 90, 157–177.
31. J. Singh, S. Kumar, B. Rathi, K. Bhrara, B.S. Chhikara. Therapeutic analysis of Terminalia arjuna plant extracts in combinations with different metal nanoparticles. *J. Mat. NanoSci*., **2015**, 2(1), 1-7.
32. J.E. Middleton. Biological properties of plant flavonoids: An overview. *Int. J. Pharmacognosy*. **1996**, 34, 344-348.