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ANTIOXIDANT, ANALGESIC AND CYTOTOXIC ACTIVITIES OF *MIMUSOPS ELENGI* LINN. LEAVES

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ABSTRACT

Mimusops elengi Linn. (Family: Sapotaceae) is a tree which is traditionally used against a number of diseases including ulcers, headache, dental diseases, wound and fever. In the present study crude methanolic extract of *Mimusops elengi* Linn. leaf was investigated for possible antioxidant, analgesic and cytotoxic activity. The extract exhibited statistically significant antioxidant activity in DPPH free radical scavenging and Nitric oxide scavenging test. The analgesic activity of the sample was studied using acetic acid induced writhing of white albino mice and hot plate test. The extract produced 45.61% and 63.85% ($P<0.001$) writhing inhibition at the doses of 250mg/kg and 500 mg/kg body weight respectively which is comparable to the standard drug diclofenac sodium was found to be 76.69% at a dose of 25 mg/kg body weight. In hot plate test the extract exerted significant ($P<0.001$) prolongation in the response of latency time to the heat stimulus. The cytotoxic activity of the extract was assessed by brine shrimp lethality bioassay as an indicator of toxicity in which LC_{50}= 80µg/ml and LC_{90} = 320µg/ml for sample. All the results tend to justify the traditional uses of the plant and require further investigation to identify the chemicals.

INTRODUCTION: In the past few years, there has been growing interest in the involvement of reactive oxygen species (ROS) in several pathological situations like cancer, chronic pain, cardiovascular diseases and arthritis. ROS produced in vivo include superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl).H_2O_2 and O_2^- can interact in the presence of certain transition metal ions to yield a highly-reactive oxidizing species, the hydroxyl radical (OH^-) [1].

The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease [2]. Although the body possesses such defense mechanisms, as enzymes and antioxidant nutrients, which arrest the damaging properties of ROS, continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them, and cause irreversible oxidative damage [3].

Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated [4]. In this respect, polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity [5]. Restriction is being imposed on the use of synthetic antioxidants because of their carcinogenicity, the need for natural antioxidants therefore become

imperative and desirable [6, 7]. Therefore, as sources of natural antioxidants, much attention is being paid to plants and other organisms. Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years [8].

Mimusops elengi Linn. (Family: Sapotaceae), locally known as Bokul is a medium sized deciduous long tree of 15-20 m high with smelt flower and 3-5 cm long lateral leaves, grows well in subtropical climates and all over Bangladesh. *Mimusops elengi* Linn. possesses antibacterial, antifungal, anticariogenic, free radical scavenging, antihyperglycemic, antineoplastic, gastro-protective, antinociceptive and diuretic effects, thus lending pharmacological support to the tree's ethnomedicinal uses in Ayurveda.

Previous investigations on this plant in different areas of Bangladesh have revealed that leaves are traditionally used in fever, postural eruptions of skin, ulcer, headache, dental diseases, wound, etc [9]. Recent studies showed that color compounds isolated from *Mimusops elengi* Linn. contains flavonoid moieties in their molecule. The leaves of this plant are antiulcerant and hypotensive [10]. Different derivatives of Triterpenes and Saponnins have been isolated from *Mimusops elengi* Linn. [11]. As a part of our ongoing investigations of medicinal plant of Bangladesh, in this paper we are investigating the antioxidant, analgesic and cytotoxic activity of the leaves of *Mimusops elengi* Linn.

MATERIALS AND METHODS:

Plant Material and Extraction: The leaves of *Mimusops elengi* Linn. were collected from Khulna University campus. Khulna, Bangladesh in August, 2009. The plant was identified by the expert of Bangladesh National Herbarium, Mirpur, Dhaka (Accession no. DACB - 34469). A voucher specimen has been deposited in Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh. The identified leaves were dried under shade. After complete drying, the sample was cut into small pieces and then slashed to coarse powder with the help of mechanical grinder and the powder was stored in a suitable container.

About 500 mg of powder was extracted by maceration over 20 days with 1200 ml of 80% methanol. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through filter paper. The filtrate thus obtained was evaporated by using a rotary evaporator to get a viscous mass. The viscous mass was then vacuum dried to get a dried methanolic extract (approx. yield value 16%). The extract thus obtained was used for experimental purposes.

Test Animals: Young Swiss-albino mice aged 4-5 weeks, average weight 20-25 gm were used for the experiment. The mice were collected from the Animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). They were kept under standard environmental condition for one week for adaptation after their collection and fed ICDDR, B formulated rodent food and water *ad libitum*. Experiments on animals were performed based on animal ethics guidelines [12].

Chemicals: DPPH (1, 1-diphenyl-2-picryl hydrazyl) was obtained from Sigma Chemical Co. USA. Ascorbic acid was obtained from SD Fine Chem. Ltd. Biosar, India. Sodium molibdate and Sodium nitropruside were purchased from Merck, Germany. Ethylene diamine tetraacetic acid (EDTA) and sodium phosphate, sulphanilamide and N (1- napthyl) ethylenediamine dihydrochloride were purchased from BDH, England. The standard drugs Diclofenac sodium and Chloramphenicol were collected from Beximco Pharmaceuticals Ltd. Dhaka, Bangladesh.

Phytochemical tests: The crude extract was subjected to preliminary phytochemical screening for the detection of major functional groups [13]. Then, the extract was used for pharmacological screening.

Determination of Antioxidant Activity:

DPPH Radical Scavenging Activity: A suitably diluted stock sample solutions were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% 1-1-diphenyl-2-picryl hydrazyl (DPPH) in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow on purple background) were noted. DPPH forms deep pink color when it is dissolved in ethanol. When it is sprayed on the chromatogram of the extract, it forms pale yellow

or yellow color which indicates the presence of antioxidants [14].

Assay of Nitric Oxide Scavenging Activity: The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitropruside (10mM) in phosphate buffered saline (PBS) was mixed with different concentration of methanolic extract of *Mimusops elengi* Linn. dissolved in methanol and incubated at room temperature for 150 minutes. The same reaction mixture without the methanol extract but the equivalent amount of methanol served as control. After the incubation period, 0.5 ml of Greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed was read at 546nm [15].

Reducing power: The reducing power of *Mimusops elengi* Linn. was determined according to the method previously described by Oyaizu, 1986. Different concentrations of *Mimusops elengi* Linn. extract (25-500μg) in 1 ml of distilled water were mixed with phosphate buffer (2.5ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5ml. 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the reference [16].

Determination of Analgesic Activity:

Acetic acid induced Writhing Test: Experimental animals were randomly selected and divided into four groups denoted as Group I, Group II, Group III and Group IV consisting of five mice in each group. Each group received a particular treatment, i.e., control, standard (Positive control) and two doses of extract. Group I served as the control group and received 1% Tween 80 in distilled water. Group II received diclofenac sodium 25mg/Kg body weight per oral (po) the standard drug used to compare the analgesic activity of the extracts. The last two group i.e. group III and Group IV were treated with the crude extract suspension 250mg/kg and 500mg/kg body weight per oral respectively. Thirty minutes after drug treatment for proper absorptions each group was treated intraperitoneally (ip) with 0.2 ml 0.7% acetic acid. Five minute after acetic acid administer, the number of writhes (Abnormal contraction or stretches) were counted for the next ten minutes and recorded. The recorded number of acetic acid induced writhes that occurred in the standard and test group compared with those in the control group [17].

Hot Plate Test: The hot plate test was used to measure the response latencies according to Wolfe and MacDonald, 1944 method. In this experiment the hot plate was maintained at 55±2°C. Animals were placed into the Perspex cylinder on the heated surface, and the time between placement and licking of the hind paws or jumping movements was recorded as response latency. The extract (250 and 500mg/kg, p.o.) and morphine (5mg/kg, i.p.) were administered 30 minutes before beginning of the experiment. Mice were observed before and at 30, 60, 120, 180, 240 and 300 minute after substance administration. The cut-off time was 20 seconds [18].

Determination of Cytotoxic Activity:

Brine shrimp: The investigation was done on *Artemia salina* (Brine shrimp). One spoon of cyst were hatched for 48 h in saline water, prepared by dissolving 30mg pure NaCl and 53mg table salt into 1.5 litre water. The cysts become nauplii.

Lethality Bioassay: Solution of different concentrations was prepared with the extract by using dimethyl sulfoxide (DMSO) as solvent. Eight test tubes were used, in each test tube 10 shrimps were taken and solution of different concentration applied on it. Finally volume of liquid was adjusted by saline water. The test tubes were kept for 24 hours. For blank control, a test tube with saline water was kept for observation with 10 shrimps under the same condition with the test sample. For positive control, in another test tube 10 shrimps were taken with saline water. A known drug

chloramphenicol as standard was introduced in the test tube with a concentration of 200µg/ml. The percent of mortality of the brine shrimp nauplii was calculated for every concentration to determine LC_{50} (lethal concentration) [19, 20, 21].

Statistical analysis: Student's t-test was used to determine significant differences between the control group and test group.

RESULTS: In the preliminary phytochemical screening the extract showed the presence of alkaloids, flavonoids, saponins, reducing sugars, tannins and steroids (**Table 1**).

TABLE 1: RESULTS OF PRELIMINARY PHYTOCHEMICAL ANALYSIS

Plant Extract	Alkaloids	Glycosides	Steroids	Gums	Tannins	Saponins	Flavonoids	Reducing sugar
Extract of *Mimusops elengi* Linn.	+	-	+	-	+	+	+	+

+ = Presence; - = Absence

DPPH Radical Scavenging Activity: During qualitative analysis it was found that DPPH forms deep pink color when it is dissolved in ethanol. When it was sprayed on the chromatogram of the extract, it forms pale yellow or yellow color which indicates the presence of antioxidants.

Assay of Nitric oxide Scavenging Activity: The result of NO scavenging activity of the plant extract and ascorbic acid is shown the **Table 2 and Table 3**.

TABLE 2: NITRIC OXIDE SCAVENGING ACTIVITY OF THE METHANOLIC EXTRACT OF *MIMUSOPS ELENGI* LINN.

Extract Conc. (µg/ml)	Abs. S1	Abs. S2	Average Abs.	St. dev	% inhibition
10	0.3990	0.4070	0.4030	0.0057	57.04
25	0.4020	0.4250	0.4135	0.0163	55.92
50	0.4400	0.4330	0.4365	0.0050	53.47
125	0.4500	0.4470	0.4485	0.0021	52.19
250	0.5300	0.4800	0.5050	0.0354	46.16
500	0.3880	0.4730	0.4305	0.0601	54.10

TABLE 3: NITRIC OXIDE SCAVENGING ACTIVITY OF THE ASCORBIC ACID

Ascorbic acid Conc.(µg/ml)	Abs. S1	Abs. S2	Average Abs.	St. dev	% inhibition
10	0.211	0.297	0.254	0.0608	72.92
25	0.182	0.185	0.184	0.0021	80.38
50	0.215	0.209	0.212	0.0042	77.40
125	0.220	0.275	0.248	0.0389	73.56
250	0.247	0.255	0.251	0.0057	73.24
500	0.323	0.257	0.290	0.0467	69.08

Analgesic test (Acetic acid induced Writhing Method): Crude extract at both dose level produced significant ($p<0.001$) and dose dependent analgesic effect (**Table 4**). Maximum inhibition of writhing responses as exhibited by the extract (63.85) at the dose of 500mg/kg body weight which was comparable with that of diclofenac sodium (76.69%).

TABLE 4: EFFECT OF METHANOLIC EXTRACT OF *MIMUSOPS ELENGI* LINN. ON ACETIC ACID INDUCED WRITHING IN MICE

Animal Group	Treatment	Writhing Count (% Writhing)	%Writhing Inhibition
Group I: Control (n=5)	1% tween-80 solution in water	29.6 ±1.99 (100)	--
Group II: Positive Control (n=5)	Diclofenac sodium (25mg/kg)	6.9±1.25* (23.31)	76.69
Group III: Test group (n=5)	Methanolic Extract (250mg/kg)	16.1±1.24* (54.39)	45.61
Group IV: Test group (n=5)	Methanolic Extract (500mg/kg)	10.7±1.45* (36.15)	63.85

Values are expressed as mean ± SEM, SEM=Standard error of Mean, n=No. of mice, **P* < 0.001 vs. control

Analgesic test (Hot Plate Method): It was demonstrated that oral administration of the extract (250 and 500mg/kg) exerted significant prolongation in the response latency time to the heat stimulus (**Table 5**).

The effect began early at 30 min after administration of MeOH extract and persists until the following fifth hour. Morphine (5mg/kg i.p.) significantly increased the latency time to the nociceptive response compared to control group.

TABLE 5: EFFECT OF METHANOLIC EXTRACT OF *MIMUSOPS ELENGI* LINN. ON HOT PLATE TEST IN MICE

Animal Group/Treatment	Latency time						
Dose	0 min	30 min	60 min	120 min	180 min	240 min	300 min
Control (Vehicle) 10ml/kg	6.3±1.2	7.5±1.1	7.7±1.0	7.5±0.7	6.7±1.0	6.9±0.8	6.4±0.5
Morphine 5mg/kg	7.2±1.2	14.1±0.*	15.3±0	16.1±0.7**	15.8±0.7**	15.4±0.6**	14.9±0.3**
MeOH Extract 250mg/kg	6.2±1.2	9.1±0.6 (ns)	9.7 ±1.0	10.1 ±0.9#	10.0 ±0.6#	9.8 ±0.6#	9.6±0.6*
MeOH Extract 500mg/kg	6.1±1.2	11.9±0.8*	12.1 ±0.9	12.4±0.8**	12.3 ±0.8#	11.5 ±0.9#	11.5±0.9**

Values are mean ± S.E.M; (n=6); #p<0.05; *p<0.01; **p<0.001 (student's t test); ns= not significant

Brine Shrimp Lethality Bioassay: The result of the brine shrimp lethality bioassay is given in **Table 6** and **Table 7**. *Mimusops elengi* leaf extract showed cytotoxic activity. LC_{50} = 80µg/ml and LC_{90} = 320µg/ml which is comparable to chloramphenicol LC_{50} = 40µg/ml and LC_{90} = 160µg/ml.

TABLE 6: RESULT OF BRINE SHRIMP LETHALITY BIOASSAY OF METHANOLIC EXTRACT OF *MIMUSOPS ELENGI* LINN.

Sample	Conc. of extract (µg/ml)	Number of Shrimp taken	Number of Shrimp alive Test-I	Number of Shrimp alive Test-II	Average number of Shrimp alive	Number of Shrimp died	% Mortality
Methanolic extract of ***Mimusops elengi* Linn.**	5	10	10	10	10	00	0
	10	10	10	10	10	00	0
	20	10	09	07	08	02	20
	40	10	08	08	08	02	20
	80	10	06	04	05	05	50
	160	10	04	02	03	07	70
	320	10	01	01	01	09	90

LC_{50} = 80 µg/ml and LC_{90} = 320 µg/ml

TABLE 7: RESULT OF BRINE SHRIMP LETHALITY BIOASSAY OF CHLORAMPHENICOL

Sample	Conc. of extract (µg/ml)	Number of Shrimp taken	Number of Shrimp alive Test-I	Number of Shrimp alive Test-II	Average number of Shrimp alive	Number of Shrimp died	% Mortality
Chloramphenicol	5	10	10	10	10	00	0
	10	10	09	09	09	01	10
	20	10	08	06	07	03	30
	40	10	04	06	05	05	50
	80	10	02	04	03	07	70
	160	10	01	01	01	09	90
	320	10	00	00	00	10	100

LC_{50} = 40µg/ml and LC_{90} = 160µg/ml

DISCUSSION: Most of the free radical production within the body involves oxygen, and thus the free radicals are often referred to as reactive or reduced oxygen species. Presence of free radicals in the body may cause cell and tissue damage. This sort of damage is known as oxidative damage [22, 23].

Several mechanisms for the production of free radicals within the body have been proposed. The mitochondria and ischemia - injury have been areas of focus. Free radicals cause cellular damage by reacting with phospholipids bilayer of cellular membrane. This reaction results in the production of measurable end products, primarily malondialdehyde. The most effective way to eliminate free radicals is with the help of antioxidant nutrients such as ascorbic acid (vitamin c), alpha tocopherol (Vitamin E) and beta carotene (Vitamin E) which can be found in vast amounts in fruits and vegetables. Literature review of this plant confirms the presence of polyhydric phenolic compounds, flavonoids, sesquiterpene etc. Any of these phytoconstituents may be responsible for antioxidant activity of the crude extract.

Phenolic compounds are mainly found both in edible and inedible vegetable and they have been reported to have multiple biological effects including antioxidant effects. Phenolic compound and flavonoids have also

been reported to be associated with antioxidant effects in biological systems acting as scavengers of singlet oxygen and free radicals [24, 25]. The antioxidant property of phenolic compounds are mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing reactive oxygen [26]. Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with super-oxides, such as NO_2, N_2O_4, N_3O_4, NO_3^-, NO_2^- are very reactive. This compounds are responsible for altering the structural and functional behavior of many cellular components. Incubation of solutions of sodium nitroprusside in PBS (phosphate buffered saline at 25^oC for 2 hrs resulted in linear time-dependent nitrite production, which is reduced by the tested methanolic extract.

The writhing test is generally used for screening analgesic effects (peripheral mechanisms) whereas the hot plate test is believed to show central mechanisms [17, 27]. With respect to analgesic test, the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intra-peritoneally absorption of acetic acid [28]. They found high level of prostaglandin PGE_2 and $PGE_{2\alpha}$ during the first 30 minutes after acetic acid injection.

Nevertheless it was found that intraperitoneally administration of acetic acid induces the liberation not only the prostaglandins, but also the symptomatic nervous system mediators [29]. The methanol extract of *Mimusops elengi* Linn. showed significant inhibition of acetic acid induced writhing response compared to reference drug diclofenac sodium (25mg/kg body weight) in mice.

Diclofenac sodium reduces inflammation, swelling and arthritic pain by inhibiting prostaglandin synthesis [30]. Results of this test showed that the extract may possess ingredients that have the capability to inhibit prostaglandin synthesis. The central analgesic effect of the MeOH extract may be supported by the results recorded in the hot plate test which is a selective method able to screen centrally acting opiate analgesic drugs [31].

Brine shrimp lethality bioassay was employed to explore a number of noble antitumor, antibacterial and pesticide of natural origin [20]. In this cytotoxic activity study, mortality of the nauplii was observed in all experimental groups. Control group nauplii remained unchanged (no lethality/mortality), is indicative of the cytotoxicity of the extract. The rate of mortality of the nauplii found to be increased with increased concentration of the sample. However, further investigations using carcinoma cell line are necessary to isolate the active compound(s) responsible for the activity. The positive response obtained in this assay suggests that the extracts may have bioactive compounds.

CONCLUSION: The study clearly indicates that the extract possess antioxidant, analgesic and cytotoxic substances. At the same time its ability to suppress abdominal writhes confirms the analgesic property of the extract. These studies justify the traditional use of this plant in the treatment of headache, dental diseases such as pyorrhea, ulcers and wound. Further study is necessary for elucidating the active principles.

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REFERENCES:

1. Aruoma OL, Halliwell B, Hoey BM, Butler J. (1989) The antioxidant action of N-acetylcystiene: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide and hypochlorous acid. Free Rad. Biol Med.6:593-597.
2. Liao, K.L. and M.C. Yin, (2000) Individual and combined antioxidant effects of seven phenolic agents in uman erythrocyte membrane ghosts and isolation of active flavonoid constituent, Planta Med., 59: 333-336.
3. Halliwell B, Aeschbach R, Loliger J, Aruoma Ol. (1995) The characterization of antioxidants. Food Chem. Toxicol. 33: 601-617.
4. Soares JR, Dinis TCP, Cunha AP and Almeida LM. (1997) Antioxidant Activities of some Extracts of *Thymus zygis*. Free Radical Research. 26: 469-478.
5. Brown, J. E.; Rice-Evans, C. A. (1998) Luteolin-rich artichoke extract protects low-density lipoprotein from oxidation in vitro. Free Radical Research. 29 (3): 247-255.
6. Behera BC, Verma N, Sonone A, Makhija U. (2006) Determination of antioxidative potential of lichen Usnea ghattensis in vitro. LWT. 39:80-85.

7. Grice H. C. (1986) Safety evaluation of Butylated Hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract. Food Chem. Toxicol. 24: 1127-1130.
8. Jayaprakasha GK, Jaganmohan Rao L. (2000) Phenolic constituents from Lichen Parmotrema stuppeum (Nyl.) Hale and their antioxidant activity. Food Control.55:1018-1022
9. Ghani A. (2003). Medicinal Plants of Bangladesh with Chemical constituents & uses. Asiatic Society of Bangladesh. 2nd edition. 1-16, 381.
10. Dar A, Behbahanian S, Malik A, Jahan N. (1999) Hypotensive effect of the methanolic extract of *Mimusops elengi* in normotensive rats. H. E. J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan. Phytomedicine. 6 (5):373-8
11. Lavaud C, Massoit G, Becch M, Misra G, Nigam SK (1996). Saponins from three species of *Mimusops*. Phytochem. 41: 887-893.
12. Zimmermann M. (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain.16:109-110
13. Evans WC. (1989). Trease and Evan's Textbook of Pharmacognosy. Cambridge University Press, London. 13th edition. p. 546.
14. Sadhu SK, Okuyama E, Fujimoto H, Ishibashi M. (2003) Separation of *Leucas aspera*, a Medicinal Plant of Bangladesh, Guided by Prostaglandin Inhibitory and Antioxidant Activities. *Chem. Pharm. Bull.* 51(5), 595-598.
15. Sreejayan, N., Rao, M.N.A. 1997. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol. 49:105–107.
16. Oyaizu, M. 1986. Studies on product of browning reaction prepared from glucosamine. *Japn. J. Nutri.* 44: 307-315.
17. Koster R, Anderson M, De Beer EJ. (1959) Acetic acid for analgesics screening Fed. Proc. 18: 412-417.
18. Wolfe, G. & Macdonald, A. D. (1944). The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J. Pharmacol. Exp. Ther. 80, 300-307.
19. McLaughlin JL, Anderson JE, Chang CJ (1988). Bioactive components of *Allamanda schottii.* J. Nat. Prod. 51(2): 307-8.
20. Meyer BN, Ferringm NR, Puam JE, Lacobsen LB, Nichols DE, MeLaughlin JL. (1982) Brine Shrimp: a convenient general bioassay for active constituents. Planta Medica. 45: 31-32
21. Persoone G. (1980) Proceeding of the International Symposium on Brine Shrimp, *Artemia salina,* Vols. 1-3. Universa Press, Witteren, Belgium.
22. Halliwell B, Gutteridge JMC. (1989) Free Radicals in Biology & Medicine. 2nd edition, Clarendon Press, Oxford, London.
23. Halliwell B. (1996) Oxidative stress, Nutrition & Health: Experimental strategies for optimization of nutritional antioxidant intake in humans. Free Radical Research. 25: 1-32.
24. Jorgensen, K., Kiebler, T., Hylander, I., Vermehren, C., 1999b. Interaction of a lipid-membrane destabilizing enzyme with PEG-liposomes. Int. J. Pharm. 183, 21–24.
25. Rice-Evans CA, Sampson J, Bramley PM, Holloway DE. (1997) Why do we expect carotenoids to be antioxidants in vivo? Free Rad. Res. 26: 381-398.
26. Osawa T. (1994) Novel natural antioxidants for utilization in food and biological systems. In: Uritani 1, Garcia VV, Mendoza EM (Eds) Post harvest biochemistry of plant food-materials in the tropics. Japan Scientific Societies Press, Tokyo, Japan. pp. 241-251.
27. Ghule BV, Ghante MH, Saoji AN, Yeole PG. Hypolipidemic and antihyperlidemic effects of *Lagenaria siceraria* Stand. fruit extracts. Indian J Exp Biol 2006; 44:9059.
28. Deraedt R, Jouquey S, Delevallee F, Flahaut M. (1980) Release of prostaglandin E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol. 61: 17-24.
29. Hokanson GC. (1978) Acetic acid for analgesic screening. J. Nat. Prod. 41: 497-498.
30. Small RE. (1989) Drug Reviews: Diclofenac Sodium. Clinical Pharm. 8:545-558.
31. Frances V. Abbott[a], Ronald Melzack[a] and Brian F. Leber[a] (1982) Morphine analgesia and tolerance in the tail-flick and formalin tests: Dose-response relationships. Pharmacology Biochemistry and Behavior, Volume 17, Issue 06, 1213-1219.
32. Rang H. P., Dale M. M. (1993). Pharmacology, 2nd edition, Churchill Livingstone Publisher, UK, p. 706-71
33. Tripathi K. D (2004). Essentials of Medical Pharmacology. 6th Edition, Jaypee New Delhi, India

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