Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 09, pp.38899-38904, September, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

IDENTIFICATION AND ESTIMATION OF PHYTOCHEMICALS AND EVALUATION OF ANTICANCER ACTIVITY OF *LAGENARIA SICERARIA* LEAVES AND FRUIT EXTRACT

***Agrawal, R. C. and Shiwa Mishra**

Department of Research, Priyamvada Birla Cancer Research Institute, J.R.Birla Road, Satna-485005, Madhya Pradesh, India

ARTICLE INFO

Article History:
Received 22nd June, 2016
Received in revised form
25th July, 2016
Accepted 17th August, 2016
Published online 30th September, 2016

Key words:

Papilloma,
DMBA,
Croton oil,
Phytochemical,
HPTLC.

ABSTRACT

Cancer is one of the leading causes of mortality worldwide. The present study was carried out to evaluate the anti-cancer activity of methanolic extract of *Lagenaria siceraria* on skin Papilloma model in mice. *Lagnaria sciceria* leaves and fruit extract against 7, 12 - dimethylbenz (a) anthracene (DMBA) induced papillomagenesis in Swiss albino mice was studied. The methanolic extract of *Lagnaria sciceraria* was analyzed for chemopreventive activity. It was evaluated by two stage protocol consisting of initiation with a single topical application of a carcinogen (7, 12 - dimethylbenz (a) anthracene (DMBA) followed by a promoter (croton oil) two times in a week were employed. A significant reduction in tumor incidence, tumor burden and cumulative number of papillomas was observed, along with a significant increase in average latent period in mice treated topically with *Lagnaria sciceraria* extract as compared to the control group treated with DMBA and croton oil. The Phytochemical analysis of methanolic extract of leaves and fruits of Lagnaria sciceraria showed presence of Alkaloids, triterponoids, flavonoids steroid, glycoside, tannin resin and saponin. However in methanolic extract of fruits of Lagnaria shows presence of all above compounds except alkaloids. The carbohydrate and protein were present in fruit extract which were absent in leaves sample. The above studies revealed information about the anticancer activity of *Lagnaria sciceraria* extracts.

Citation: Agrawal, R. C. and Shiwa Mishra, 2016. "Identification and estimation of phytochemicals and evaluation of anticancer activity of *Lagenaria siceraria* leaves and fruit extract", *International Journal of Current Research*, 8, (09), 38899-38904.

INTRODUCTION

Cancer is one of the most fatal diseases in human population and one of the most frequent causes of death worldwide. An extremely promising strategy for cancer prevention today is chemoprevention which is defined as the use of synthetic or natural agents (alone or combination) to block the development of cancer in humans. Plants, vegetables and herbs used in the folk and traditional medicine have been accepted currently as one of the main sources of cancer chemoprevention in drug discovery and development. Plant derived natural products such as flavonoids, terpenoids alkaloids and steroids have received considerable attention due to their diverse pharmacological properties which include cytotoxic and chemopreventive effects (Abdullaev, 2001; Uddin *et al*., 2003; Koduru *et al*., 2006; Zahan *et al*., 2011; Sodde *et al*., 2011; Kundu Sen *et al*., 2011). The plant, *Lagenaria siceraria* (Mol.) Stanley from Cucurbitaceae family, popularly known as bottle gourd (English), has wide occurrence throughout India as an edible vegetable. It is a pubescent or trailing herb, with bottle or dumb-bell shaped fruits. Both of its aerial parts and fruits are commonly consumed as vegetable. Traditionally it is used as medicine in India, China, European countries, Brazil, Hawaiian island etc. for its cardiotonic, general tonic, diuretic, antiproliferative properties (Kirtikar and Basu, 2003). Further, antihepatotoxic, analgesic, anti-inflammatory, hypolipidemic, antihyperglycemic and antioxidant activities of its fruit extract have been reported (Deshpande *et al*., 2008; Deshpande *et al*., 2007; Ghule *et al*., 2006a, b; Shirwaikar and Sreenivasan, 1996). *Lagenaria siceraria* fruits are good source of Vitamin B complex, ascorbic acid, fibers, proteins, cucurbitacins, saponins, fucosterols and compesterols, polyphenolics, flavones-C-glycoside (Ghule *et al*., 2006b; Shirwaikar and Sreenivasan, 1996; Krauze-Baranowska and Cisowski, 1994; Duke, 1999; Sturm and Stuppner, 2000). Methanol extract of its leaves was reported the presence of sterols, polyphenolics, flavonoids, saponin, protein and carbohydrates (Shah and Seth, 2010). A novel protein, Lagenin from its seeds was reported antitumor, immunoprotective and antiproliferative properties (Wang and Ng, 2000). The present investigation was therefore,

****Corresponding author: Agrawal, R. C.***
Department of Research, Priyamvada Birla Cancer Research Institute, J.R.Birla Road, Satna-485005, Madhya Pradesh, India.

carried out to evaluate anticancer activity of methanol extract of *L. siceraria* leaves and fruit against Skin Papilloma tumor model in mice and identification of phytoconstituents present in fruit and leaves of Lagnaria sciceraria.

MATERIALS AND METHODS

Phytochemical analysis:

Preliminary phytochemical screening of the extract was carried out using standard methods (Kokate, 1994).

Collection and identification of plant material

The plant of *L. Siceraria* was collected from garden of agriculture college Sehore (Madhya Pradesh India). The identification of the plant *Lagenaria siceraria* (family*: cucutbitacea*) was done by Dr. Manoj Tripathi (Voucher Specimen TFRA/AS/S/115), Deendayal Research Institute, Chitrakoot, Satna Madhya Pradesh (India) The project was approved by Institutional Animal Ethical Committee (IAEC). Project no 5 Ref no 670/2251. The experiment was carried out according to the committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines.

Extraction Procedure

The leaves and fruits of *L.Siceraria* was dries in shade and powdered with mechanical grinder. The powder was passed through sieve no 40 and stored in airtight container for further studies. About 50 gm of *L.Siceraria* leaves and fruits powder was kept in petroleum ether to de fat for 1hr and to remove the lipid present in plant material. The powder (L. Siceraria leaves and fruits) was dried in filter paper. After dry it was poured in separating funnel for extraction using 50% methanol solvent at room temperature for 24 hr and then filtered. Again the 50% methanol solvent was added and allowed to stand for overnight and then filtered to concentrated it. The filtrate was kept at 55-60'C in water bath. The collected residue was finally transferred into the hot air oven to dry it. About 14 gm crude extract was obtained which was used for the studies. The yield of methanolic extract was 30%. The determination of Alkaloid by Harborne (1973) method, Flavonoid by Bohm and Kocipai – Abyazan (1994) method and saponin by Obadani and Ochako (2001) method was done.

HPTLC fingerprint profile

HPTLC fingerprinting of methanolic extract of the leaves and fruit of Lagnaria sciceria was carried out by using Ethyl acetate: Methanol: Water (10:13.5:10)solvent system. A total number of 8 spots (peaks) at different Rf values and peak area at 366 nm were observed in the HPTLC chromatograms while 4 peaks were observed in HPTLC chromatogram at 254 nm. The total number of phytoconstituents (no. of peaks) in the extract and their retention factors (Rf) are given in the Table and chromatographic profile had been shown in Plate 1 & Table 4.

Animals

The random breed, 6-7 weeks old male *Swiss albino* mice of weight 25±2 gm body were used in the study. These mice were maintained under controlled conditions of temperature (25±2 °C) and light (12 light: 12 dark). They were fed on standard mice feed procured from Golden feeds, New Delhi and water was given ad. libitum. One day before the commencement of the experiment, hairs on the interscapular region of the mice were removed using hair removing cream.

Chemicals

7, 12-dimethylbenz (a) anthracene (DMBA), and Croton oil were procured from Sigma chemicals Co., St. Louis, U.S.A. and other chemicals were procured locally and were reagent grade.

Procedure

Experiment was performed as per the method reported by Berenblum (1975) and standardized by us (Agrawal *et al*., 2009). The animals were randomly divided into different groups and each group comprised of six animals. Hairs were removed with the help of hair removing cream from the dorsal region with proper care in the area of 2cm2 in all the groups.100 µg DMBA was dissolved in 100µl acetone and was given initially and 1% Croton oil was given 2 times a week up to 16 weeks. Skin tumor formation was recorded weekly and the papillomas greater than 1mm in diameter and if they persisted two weeks or more were included in to counting of total number of papillomas / mouse, Tumor incidence and tumor yield was also calculated. The animals were divided into 8 different groups for each extract as follows: Total No. of animals for each group were 6

Treatment Groups

Group I (Vehicle Control) - 100µl acetone 2 times/ week up to 16 weeks.

Group II (DMBA alone)-100 µg DMBA was dissolved in 100µl acetone and single application was given.

Group III (Croton oil alone)-1% Croton oil was applied on the skin 2 times a week up to 16 weeks.

Group IV (DMBA+Croton oil)- 100 µg DMBA was dissolved in 100µl acetone and single application was given afterwards, 1% Croton oil was applied on skin 2 times a week up to 16 weeks.

Group V *L.siceraria* leaves extract alone)- *L.siceraria* leaves Methanolic extract alone was applied (3000mg/kg body wt.) on skin 2 times a week up to 16 weeks.

Group VI (*L.siceraria* fruit extract alone)- *L.siceraria* fruit Methanolic extract (3000mg/kg body wt.) was alone applied on skin 2 times a week upto 16 weeks.

Group VII (DMBA+*L.siceraria* leaves extract + Croton oil)- 100 µg DMBA was dissolved in 100µl acetone and single application was given afterwards the 100µl dose of *L.siceraria* leaves Methanolic extract at the dose of 3000mg/kg b.wt. was given one hour before each application of 1% Croton oil 2 times a week up to 16 weeks.

Group VIII (DMBA+ *L.siceraria* fruit extract + Croton oil)- 100 µg DMBA was dissolved in 100µl acetone and single application was given afterwards the 100µl dose of *L.siceraria* fruit Methanolic extract at the dose of

3000mg/kg b.wt. dose was given one hour before each application of 1% Croton oil 2 times a week up to 16 weeks. Cumulative No. of Papillomas, Tumor Incidence, Tumor Yield, Tumor burden. Average latent period were calculated and the differences of the tumors among different groups were considered to be significant at 5% significance level ($p<0.05$) which were evaluated by Student's 't' test.

RESULTS

The Phytochemical analysis of methanolic extract of leaves of Lagnaria sciceria shows presence of Alkaloids, triterponids, flavonids steroid, Glycoside, tannin Resin and Saponin However in methanolic extract of fruits of Lagnaria shows presence of all above compounds except alkaloids. It also shows presence of carbohydrate and protein which are absent in leaves sample. (Table 1) Table 2 shows the quantitative

Table 1. Preliminary phyto-chemical screening of Lagnaria sciceria methanolic extract

S. No.	Name of Experiments	Observation	Result (Leaf)	Result (fruit)
1.	Alkaloids			
	a) Mayer' test	Yellow colour appear	Present	Absent
	b) Wagner's test	Brown colour appear	Present	Absent
	c) Dragendorff's test	Orange colour appear	Present	Absent
2.	Carbohydrate			
	a) Anthrone's test	Dark colour appear	Absent	Present
	b) Fehling's test	Green colour appear	Absent	Present
	c) Molisch's test	No red – violet ring disapper	Absent	Present
3.	Proteins			
	a) Bieuret's test	Green colour appear	Absent	Present
	b) Millon's test	White ppt are not appeared	Absent	Present
4.	Triterpinoids test			
	a) Libermann's Buchard test	Violet colour ring is formed	Present	Present
5.	Resins	Turbidity are seen	Present	Present
6.	Saponins	Honey comb – like structure are form	Present	Present
7.	Starch	Red colour is formed	Absent	Absent
8.	Flavonoid		Present	
	a) Ferric chloride test	Reddis pink colour is appear	Present	Present
	b) Alkaline reagent test	On addition of dilute acid yellow colour disappear	Present	Present
9.	Steroid			
	a) Salkowski's reaction	A red colour is disappear in the chloroform layer	Present	Present
10.	Glycoside			
	a) Borntrager's Test	Colour is change	Present	Present
11.	Tannin	Greenish colour appear	Present	Present
	a) Lead acetate Test	Reddish brown bulky ppt. are formed	Present	Present

Table 2. Quantitative Phyto-chemical Analysis *Lagineria siceraria* (Leaves & Fruit)

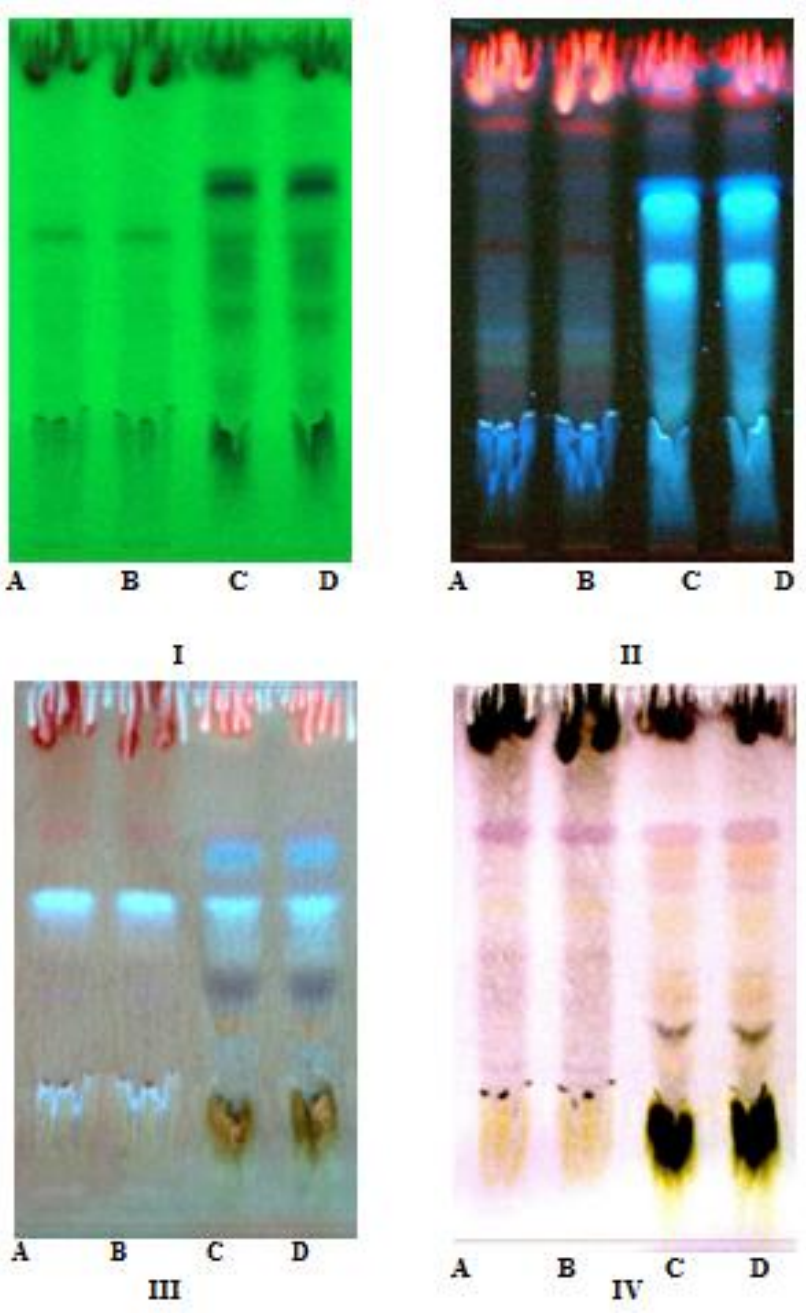
S. No.	Name of tests	Sample-1 *(Lagineria siceraria* (Leaves)	Sample-2 *(Lagineria siceraria* (Fruit)
1	Alkaloids	1.7642%	2.4878%
2	Flavonoids	10.5794%	34.0275%
3	Saponins	23.4551%	23.5079%

Table 3. Cumulative No. of Papilloma in the animals treated with *Lagnaria leaves and fruit* extract

Groups	Treatment	*Bodyweight (Mean±SEM)*		*Cumulative no. of Papilloma*	Tumor Incidence (%)	Tumor Burden	Tumor Yield	Average Latent Period	Number of Papilloma with Tumor size, mm	
		Final	Final							
I (n=6)	Vehicle alone (100µl acetone)	27.6±2.2	31.60 ± 0.36	-	-	-	-	-		
II (n=6)	DMBA alone (104µg/100µl acetone)	26.7±1.6	35.6 ± 0.63	-	-	-	-	-		
III (n=6)	Croton oil(100µl of 1% concentration)	27.0±1.5	30.9±1.1	-	-	-	-	-		
IV (n=6)	L.siceraria leaves extract alone (3000mg/kg b.wt.)	27.0±1.5	29.9±1.1	-	-	-	-	-		
V (n=6)	L.siceraria fruit extract alone (3000mg/kg b.wt.)	27.0±1.5	29.9±1.	-	-	-	-	-		
VI (n=6)	DMBA (104µg/100µl acetone)+ Croton oil(100µl of 1% concentration)	20.74±1.2	25.99±0.6	25	6/6 100%	3.83± 0.91	3.83± 0.91	8.60±1.78	17	8
VII (n=6)	DMBA + Croton oil + *L.siceraria* leaves extract (3000mg/kg)	27.35±0.39	28.9±0.5	7	3/6 50%	1.16± 0.59*	2.1± 0.55*	13.43± 2.6	5	2
VIII (n=6)	DMBA + Croton oil +L.siceraria fruits extract (3000mg/kg)	26.6 ± 0.56	29.66 ± 0.48	11	4/6 66.66%	1.83±0.80	1.83±0.80	2.75±0.25*	10	1

Rf Values	Before derivatization			
	At 254nm		At 366nm	
	Track-A&B (Sample -1, Leaf)	Track-C&D (Sample-2, Fruit)	Track-A&B (Sample -1, Leaf)	Track-C&D (Sample-2, Fruit)
R_f-1	0.61(black)	0.45 (black)	0.37(greenish yellow)	0.53 (sky blue)
R_{f-2}	-	0.59(black)	0.43 (blue)	o.67 (sky blue)
R_{f-3}	-	0.70(black)	0.60 (brownish red)	0.72 (blue)
R_{f-4}	-	-	0.83 (red)	0.83 (red)
	After derivatization			
	At 366nm		At visible light	
	Track-1&2 (Sample -1, Leaf)	Track-3&4 (Sample-2, Fruit)	Track-1&2 (Sample -1, Leaf)	Track-3&4 (Sample-2, Fruit)
R_f-1	0.60(sky blue)	0.37 orange	0.60 (light yellow)	0.37 (green)
R_{f-2}	0.73 (pink)	0.60 (sky blue)	0.73 (pink)	0.69 (orange)
R_{f-3}	-	0.68 (blue)	-	0.73 (pink)

Plate-1: HPTLC Fingerprint Profile *Lagineria siceraria* (Leaf & Fruit)
Solvent System : *Ethyl acetate: Methanol: Water* (10:13.5:10)
Derivatizing Reagent: *Vanillin Sulphuric Aci*



I=254nm; II=366nm; and after derivatization III=366nm; IV=Day Light; Track-A&B= Sample-1(Leaf) and Track-C&D= Sample-2 (Fruit)

determination of methanolic extract of leaves Lagnaria sciceraria Alkalods (1.76%), Flavonoids (10.5 7 %) and Saponins (23.45 %) where as in methanolic extract of fruits of Lagnaria sciceria Alkalods (2.48 %), Flavonoids (34.02 %) and Saponins (23.50 %) were estimated. The above compounds have been detected also by HPTLC fingerprinting which shows the rf Values of the different compounds. The findings of the present antitumour study are depicted in Tables 3. Animals of Group- VI (control) in which a single topical application of DMBA, followed by croton oil produced skin papillomas, which started appearing from the 5th week onwards. The incidence in DMBA/croton oil treated mice (carcinogen control) reached 100% by the termination of the experiment (i.e. 16 weeks). In the skin papilloma model, significant prevention of tumor incidences was observed in the *Lagnaria sciceria* extract treated experimental groups (50 %and 66 %) in group VII and VIII respectively as compared to carcinogen control (100 %) group. The cumulative number of papillomas was also reduced in the *Lagnararia sciceria leaves and fruit extract* treated experimental groups (7 in group VII and 11 in group VIII) as compared to carcinogen control (25) group. The tumor burden and tumor yield were significantly decreased (1.16 and 1.8 3) as compared to DMBA croton oil treated control (3.83) group.

DISCUSSION

Chemoprevention is currently an important strategy for controlling the process of cancer induction. Therefore, there is a need to explore medicinal plants or other natural agents that can work as chemopreventive agents. The current study demonstrates the chemopreventive potential of Lagnaria sciceraria extracts of leaves and fruit on DMBA-induced skin tumorigensis in male Swiss albino mice. The skin carcinogenesis model in experimental animals has been found to be a very useful system for investigating the influence of dietary chemopreventors both mechanistically and operationally (Kausar *et al*., 2003). The present study demonstrated that topical application of the Lagnaria sciceria methanolic extracts of leaves and fruit (3000 mg/kg body weight) at the pre promotion phase showed a significant reduction in tumor incidence, tumor burden, tumor weight, tumor size, and cumulative number of papillomas in Lagnaria sciceria treated groups relative to the carcinogen treated control. The mechanism of anticarcinogenic activity of Lagnaria sciceria extract has not been well documented. However, evidence has been accumulated to suggest that this is perhaps due to reactive oxygen species. Which play an important role in tumor initiation/promotion by enhancing or facilitating the metabolic activation and/or initiating effects of carcinogens (Ather 2002). The plant extract may have inhibited the metabolism of DMBA to its active form, delayed the promotion phase of carcinogenesis, or down regulated reactive oxygen species formation (Kausar, 2003; Sancheti, 2005 and Kumar *et al*., 2006). There are few reports about the cytotoxic and antiproliferative effects of Lagnaria sciceria in In vitro cell lines (Navneet *et al*., 2013). Plant derived natural products such as flavonoids, terpenoids alkaloids and steroids have shown cytotoxic and Chemopreventive effects (Abdullaev, 2001; Uddin *et al*., 2003; Koduru *et al*., 2006; Zahan *et al*., 2011; Sodde *et al*., 2011; Kundu Sen *et al*., 2011) The phenols or phenolics and flavonoids, (polyphenolic compounds) are important secondary metabolites of plants and these compounds are natural antioxidants which have wide spectrum pharmacological potentials e.g., anti-allergic, antibacterial, anticancer, anti-inflammatory, neuroprotective activities. The HPLTC technique is an important analytical tool for identification, detection, separation, and some other assessments of plants and their products A total number of 8 peaks at different Rf values and peak area at 366 nm were observed in the HPTLC chromatograms while 4 peaks were observed in HPTLC chromatogram at 254 nm. The anticarcinogenic activity of Lagnaria sciceria in skin papilloma model in Swiss albino mice was assured. The present study is immensely important because Lagnaria sciceria is an important vegetable and medicinal plant.

Acknowledgements

The authors are thankful to Dr.Manoj Tripathi of Deendyal Research Institute, Chitrakoot for HPTLC fingerprinting of the compound.

REFERENCES

Abdullaev, F.I. 2001. Plant-Derived Agents Against Cancer. In: Pharmacology and Therapeutics in the New Millennium, Gupta, S.K. (Ed.). Narosa Publishing House, New Delhi, pp: 345-354.

Agrawal, RC, Jain, R, Wasim Raja, Ovais, M. 2009. Anticarcinogenic Effects of Solanum lycopersicum Fruit Extract on Swiss Albino and C57 BL Mice. *Asian Pacific Journal of Cancer Prevention*, 10:379-381.

Ather M. 2002. Oxidative stress and experimental carcinogenesis. *Indian J Exp Biol*., 40:656-667.

Berenblum, J., Shubik, P. A 1947.new quantitative approach to the study of stages of chemical carcinogenesis in the mouse skin. *Br. J. Cancer*, 1:373-391.

Boham BA and Kocipai-Abyazan R. 1974. Flavonoids and condensed tannins from leaves of Hawaiian Vaccinium vaticulatum and V. calycinium" *Pacific Science*, 48: 458-463.

Deshpande, J.R., A.A. Choudhari, M.R. Mishra, V.S. Meghre, S.G. Wadodkar and A.K. Dorle, 2008. Beneficial effects of Lagenaria siceraria (Mol.) Standley fruit epicarp in animal models. *Indian J. Exp. Biol*., 46: 234-242.

Deshpande, J.R., M.R. Mishra, V.S. Meghre, S.G. Wadodkar and A.K. Dorle, 2007. Antioxidant activity of Lagenaria siceraria (Mol) Stand. *Fruit. Nat. Prod. Rad*., 6: 127-127.

Duke, J.A. 1999. Handbook of Phytochemical and Constituents of GRAS Herbs and other Economic Plants. CRC Press, Boca Raton, FL., USA., pp: 98-119.

Ghule, B.V., M.H. Ghante, A.B. Upaganlawar and P.G. Yeole, 2006. Analgesic and anti-inflammatory activities of Lagenaria siceraria Stand. fruit juice extract in rats and mice. Pharmacogn. Mag., 2: 232-238.

Ghule, B.V., M.H. Ghante, A.N. Saoji and P.G. Yeole, 2006. Hypolipidemic and antihyperlipidemic effects of Lagenaria siceraria (Mol.) fruit extracts. *Indian J. Exp. Biol*., 44: 905-909.

Harborne JB. Phytochemical methods, London. Chapman and Hall, Ltd. 1973; 49- 188.

Kausar H, Bhasin G, Zargar MA, Ather M. 2003; Palm oil alleviates 12-O tetradecanoyl–phorbol-13 acetate-induced tumor promotion response in murine skin. *Cancer Lett*. 192:151-160.

Kirtikar, K.R. and B.D. Basu, 2003. Indian Medicinal Plants. Vol. 5, 2nd Edn., Oriental Enterprises, Uttaranchal, India, pp: 1551-1554.

Koduru, S., D.S. Grierson, M. van de Venter and A.J. Afolayan, 2006. In vitro antitumour activity of Solanum aculeastrum berries on three carcinoma cells. *Int. J. Cancer Res*., 2: 397-402.

Kokate, C.K., 1994. Preliminary Phytochemical Screening, Practical Pharmacognosy. Vallabh Prakashan, New Delhi, pp: 107-113.

Krauze-Baranowska, M. and W. Cisowski, 1994. High-performance liquid chromatographic determination of flavone C-glycosides in some species of the Cucurbitaceae family. *J. Chromatogr.* A, 675: 240-243.

Kumar, N, Kale, R.K. and Tiku, A.B. 2013. Chemopreventive effects of Lagnaria siceraria in two stage DMBA plus croton oil induced skin papillomagenesis, *Nutrition and Cancer*, 65 (7) 999-1001.

Kumar M, Soni KA, Shukla S, Kumar A. 2006 Chemopreventive potential of Tribulus terrestis against 7,12 dimethylbenz (a)anthracene induced skin papillomagenesis in mice. *Asian Pac J Cancer Prev*., 7:289-294.

KunduSen, S., M. Gupta, U.K. Mazumder, P.K. Haldar, P. Saha and A. Bala, 2011. Antitumor activity of Citrus maxima (Burm.) Merr. leaves in Ehrlich's Ascites Carcinoma cell-treated mice. *ISRN Pharmacol*., 1: 1-4.

Obdoni BO and Ochuko PO. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria *Global J. Pure Appl. Sci*., 8: 203-208.

Sancheti G, Jandal A, Kumari R, Goyal PK. 2005. Chemopreventive action of Emblica officinalis on skin carcinogenesis in mice. *Asian Pac J Cancer Prev*., 6:197-201.

Shirwaikar, A. and K.K. Sreenivasan, 1996. Chemical investigation and antihepatotoxic activity of the fruits of Lagenaria siceraria. *Indian J. Pharm. Sci*., 58: 197-202.

Sodde, V., N. Dashora, K.S. Prabhu and R. Lobo, 2011. Evaluation of anticancer activity of Macrosolen parasiticus (L.) danser on ehrlich's ascites carcinoma treated mice. *Int. J. Cancer Res.*, 7: 135-143.

Sturm, S. and H. Stuppner, 2000. Analysis of cucurbitacins in medicinal plants by high-pressure liquid chromatography-mass spectrometry. *Phytochem. Anal*., 11: 121-127.

Uddin, K., A. Sayeed, A. Islam, A.A. Rahman, A. Ali, G.R.M.A.M. Khan and M.G. Sadik, 2003. Purification, characterization and cytotoxic activity of two flavonoids from Oroxylum indicum vent. (Bignoniaceae). *Asian J. Plant Sci*., 2: 515-518.

Wang, H.X. and T.B. Ng, 2000. Lagenin: A novel ribosome inactivating protein with ribonucleolytic activity from bottle gourd (Lagenaria siceraria) seed. *Life Sci*., 67: 2631-2638.

Zahan, R., M.B. Alam, M.S. Islam, G.C. Sarker and N.S. Chowdhury *et al*. 2011. Anticancer activity of Alangium salvifolium flower in Ehrlich Ascites Carcinoma bearing mice. *Int. J. Cancer Res*., 7: 1-9.

****