Chemopreventive effect of Annona squamosa extract

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Abstract- Annaona squamosa is commonly known as custard apple which is eatable. In the folk medicine, the different parts of plant are used for medicinal purposes, .**Objectives:** The aim of this study was to assess the anticarcinogenic, antioxidant and antibacterial effects of Annaona squamoasa *leaves* and seeds extract in mouse skin Papilloma model, DPPH method and disk diffusion methods. **Materials and Methods:** Chemopreventive activity 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**.** The antioxidant and antibacterial effects were assessed by using DPPH and Disk diffusion methods respectively.

Results: A significant reduction in tumor incidence, tumor burden and cumulative number of papillomas was observed in mice treated tropically with *Annona sqamosa extracts* as compared to the control group treated with DMBA and croton oil .. The antioxidant and antibacterial effect of *Annona sqamosa* extract was also observed

Conclusion: The above studies reveal information about the prevention of cancer. Therefore, the present study is immensely important in future drug development programs for the cancer treatment.

Index Terms- papilloma, Annona sqamosa ,Antibacterial , antioxidant.

I. Introduction

Annaona squamoasa is commonly known as custard apple which is eatable. In the folk medicine, the different parts of plant are used .The leaves are used as a vermicide and are applied to abscesses, insect bites and other skin complaints. Scrapings of root- bark are used for toothache. Powdered seeds are used to kill head-lice and fleas. Annaona squamoas (Custard apple) seeds are generally thrown away as waste materials. They are known to possess insecticidal, anti-ovuatory, abortyfacient and anti-implantation properties (Vohora et al. 1975; Rao et al.1979; Damasceno et al.2002). In one study the anti- cancer properties of custard apple has been reported due to a class of compounds called acetogenins which are very long chain fatty acids (McLaughlin, 2008) and specific to Annonaceaous species (McLaughlin, 2008). Anti-diabetic properties of Annona spp. appear to be related to stimulation of insulin production and enhanced uptake of glucose by muscles leading to stabilization of blood sugar concentrations (Gupta et al., 2005a). Leaf extracts are effective in lowering blood glucose levels and several reports indicates that Annona squamosa leaf extract can substitute effectively for externally administered insulin (Gupta et al., 2005b) The fruit of Annona spp. have been shown to have anti-microbial activities (Wiart et al., 2005) which showed good anti-bacterial activity of the crude methanol extract of sugar apple fruit, and an isolated diterpene, against Staphylococcus aureus and Streptococcus pneumonia .Custard apple was listed as one of the foods with strong anti-obese activity (Niwano et al., 2009). Beppu et al. (2009) showed that oral administration of ethanol extracts of fresh custard apple fruit potently lowered plasma triglyceride concentrations. Studies report widely differing levels of anti-oxidants in Annona spp. Studies conducted in India (Kaur and Kapoor, 2005), Taiwan (Chen, et al. 2006; Kaleem et al,2006 & Damsceno et al). Hole et al. (2006) reported the protective effect of aqueous extract of the fruits on isoproterenol induced myocardial infarction (death of heart tissue) in rats.

II. Materials and Methods:

1. Chemicals

7, 12 - Dimethylbenz (a) anthracene (DMBA), croton oil from Sigma Chemicals Co. (St. Louis, MO. USA). The other chemicals were obtained from local firms and were of the highest purity. DMBA was dissolved in acetone at a concentration of 100 ug/100ul and croton oil was diluted in acetone to give a 1% dilution.

Animals

Random bred male Swiss albino mice (7- 8 weeks old), weighing 24 ± 2 gm were used for the experiments. These animals were housed in polypropylene cages in the animal house at temperatures of 22 ± 3ºC. The animals were provided with standard mice feed and tap water ad libitum.

Preparation of *Annona sqamosa* extract

Plant material (*Annona sqamosa*) was collected locally and identified and the specimen was authentified at DRI, Chitrakoot (MP), and India. The leaves and seeds were washed, air dried, powdered and extracted separately, with 50 % methanol using separating funnel.

Extract thus obtained were vacuum evaporated to make it in powder form. These extract was again dissolved in DDW just before topically application.

Experimental design for Skin Carcinogenesis The dorsal skin on the back area of the animals was shaven 1 day before the commencement of the experiment and only those animals in the resting phase of the hair cycle were chosen for the study. For induction of tumors a 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 as per our previous modified method of Berenblum (1975) reported elsewhere **.**The animals were randomly allocated into 7 groups comprising six mice each. The treatment was provided topically on shaved area

Treatment Groups
Group 1 (Untreated control): No treatment
Group 2 (Vehicle control): 100 µl acetone 2 times /week up to 16 weeks
Group 3 (DMBA Alone): - 100 µg DMBA was dissolved in 100 µl acetone and single application was given.
Group 4 (Croton Oil Alone): - 1 % Croton oil was applied on skin 2 times a week up to 16 week.
Group 5 (*Annona sqamosa leaves & seeds extract*s Alone): - *Annona sqamosa leaves and seeds* extract were applied on skin in different mice at the dose of 1000 mg/kg, 2 times a week up to 16 week.
Group 6 (DMBA + Croton Oil): - 100 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards 1 % Croton oil was applied on skin 2 times a week up to 16 week.
Group 7 (DMBA + *Annona sqamosa leaves and seeds extract* + Croton Oil): -

100 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards the 100 µl dose of *Annona sqamosa leavesand* seeds extract were applied at different mice *at* the dose of 1000 mg/kg b. wt. dose was given one hour before the each application of 1% croton oil 2 times a week up to 16 weeks.

The animals of all groups were kept under observation for gross and microscopic changes in skin were observed . During the period of 16 weeks of experimentation, mice of all groups were weighed carefully examined once a week for skin papillomas and these were recorded. The no. of papillomas every weeks were recorded.

Anti-bacterial activities

Antibacterial activities of hydro-methanolic extract from *Annona sqamosa leaves was*

Investigated using the Disk diffusion method given by Kerby-Bauer Disk Diffusion Susceptibility test.

Bacterial strain :

Following gram negative and gram positive bacterial strain i.e. *Pseudomonas aeroginosa, Escherichia coli, Shigella flexneri, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis* used for the Antibacterial activities which were received from stock culture of our laboratory.

Media

Nutrient agar broth media were used for the antibacterial activities. Nutrient broth is prepared i.e. 1.3g in 100ml of double distilled water, poured in 6 different test-tubes and added 6 bacterial strain in each test-tube. Nutrient Agar media prepared poured in Petri plates after solidifying swab the bacterial cultures on the plates and allowed for incubation at 37^0C for 24 hrs.

Concentration

4 different concentrations of crude extract were prepared (100%, 75%, 50%, 25%).

100%= 1g crude extract in 1ml of double distilled water freshly prepared afterward serial dilution prepared 75%= 75mg in 1ml, 50%= 50mg in 1ml, 25%= 25mg in 1ml.

Study parameter
Measurement of Zone of Inhibition (In mm).

Anti-oxidant activities
Anti-oxidant activities of *Annona sqamosa leaves* extract (10-100 µg/ml) were determined according DPPH method.

The **Formula:**
% Inhibition = Abs. 532nm control- Abs. 532nm test Abs. 532nm /control x 100
Study parameter
Results are concluded on the basis of plotting graph = % inhibition of TBARS vs Concentration.

III. RESULTS

Papilloma Test

It was observed that the topical application of *Annona Squamosa* on Hydromethnolic seed & leaves extract on skin at the dose of 1000 mg/kg body weight have prevented the appearance of papillomas. A significant reduction was observed in tumor incidence, tumor burden, tumor weight, tumor size, cumulative number of papillomas, in *Annona Squamosa* - treated groups relative to the carcinogen treated control. The papillomas appearance was delayed by about 2 weeks in the animals which received prior application of *Annona Squamosa* extract as compared to the DMBA

+ Croton Oil group. The mean no. of papillomas in DMBA+ *Annona Squamosa* leaves and seed extract + DMBA + Croton oil group was 5 and 2.4 respectively as compared to 6.6 in DMBA + Croton Oil group. The tumour incidence was 83 % and 66 % in leaves and seed extracts as compared to 100 % in control group. (Table 1 -2 & graphs 1-4) .

The antibacterial effects of Annona sqamosa leaves and seed extract was also observed in gram negative and positive bacteria's (Table3-4 & graphs 5-6).

The dose dependent antioxidant effect of leaves and seed extracts were also observed in DPPH method (Table 5 & graph 6).

IV. DISCUSSION

The anticarcinogenic nature of Annona sqamosa leaves and seeds extract may be due to presence of Acetogenins and flavonoids as Active compounds (Alali et al,1999) . The plant was reported for anticancer activity on MCF 7 (epithelial cells of human breast cancer) and A 549 (epithelial cells of human lung cancer) under in vitro conditions by MTT assay (McLaughlin, J. (2008) . *Annona Squamosa* showed predominantly significant anti-inflammatory activity (Mukhlesur 2005). Several studies suggested that compounds possessing anti-inflammatory property inhibit 12-O-tetradecanoyl phorbol-13- acetate induced tumor

promotion in mouse skin. Aurore *et al* (1997) reported that anti-inflammatory steroids drastically inhibit the epidermal DNA synthesis and cellular proliferation induced by phorbol ester tumor promoters, a pre-requisite for tumorigenesis. Though the exact mechanism underlying the anti-inflammatory activity of *Annona Squamosa* has not been ascertained but it may be inferred that due to anti-inflammatory property of *Annona Squamosa* might have played a synergistic role in the inhibition of tumorigenesis as observed in the present investigation. In our previous report the anticancer activity of Annona sqamosa extract has been reported in mouse skin carcinogenesis model (Agrawal et al, (2018) .The antioxidant and antibacterial activity of Annona extracts have been also observed. The overall results indicates the promising baseline information for the potential uses of the methanol extracts of Annona sqamosa leaves as an anti cancer agent.

REFERENCES

[1] Agrawal, R.C., Singh, Snehal and Maheshwari, S.K. Chemopreventive potential of Annona sqamosa leaves extract in Swiss albino mice. International journal of Modern Biological Research 2018, Vol.6 page 1-8.

[2] Agrawal RC, Jain R, Raja W and Ovais M (2009): Anticarcinogenic effect of Solanum lycopersicum fruit extract on Swisis albino and C57BL mice.Asia Pacific Journal of Cancer prevention 10(1-4),379-382.

[3] Alali, F. Q., Liu, X. X. and McLaughlin, J. L.(1999). Annonaceous acetogenins: recent progress. Journal of Natural. Products. 62: 504-540

[4] Aurore, V, Thoma JS, Michael, W., Weinsteen LB (1997): Effects of anti-inflammatory agents on mouse skin tumor promotion, epidermal DNA synthesis, phorbol ester induced cellular proliferation and production of plasminogen activator. Cancer Reserch, 37, 1530-1536.

[5] Berenblum ,I (1975: Sequential aspects of chemical carcinogenesis : skin in cancer '' A comprehensive treatise'' . Plenum press, New York, 1, 323-344.

[6] Beppu, F., Niwano, Y., Kyan, R., Yasura, K., Tamaki, M., Nishino, M., Midorikawa, Y. and Hamada, H(2009): Hypolipidemic effects of ethanol extracts of Citrus depressa and Annona atemoya, typical plant foodstuffs in Okinawa, Japan on KKAy mice fed with moderately high fatdiet. Food Science and Technology Research. 15(5): 553-556.

[7] Chen, T., Wen-Ju Yang, Ning-Sing Shaw and Tzong- Shyan Lin. (2006): Antioxidant activity of fruits produced in Taiwan. Abstract, 27th International Horticultural Congress, Korea, 2006.

[8] Damasceno,D.C.,Volpto,G.T.,Sartori,T.C.,Rodrigues, P.F.,Perin,E.A.,Calderon I.M.,Rudge,M.V.,2002.

[9] Effects of Annona squamosa seed extract on early pregnancy in rates. Phytomedicine 9,667-672.

[10] Gupta, R. K., Kesari, A. N., Watal, G., Murthy, P. S., Chandra, R. and Tandon, V. (2005a): Nutritional and hypoglycaemic effect of fruit pulp of Annona squamosa in normal healthy and alloxan-induced diabetic rabbits. Annals of Nutritional Metabolism: 49, 407-413.

[11] Gupta, Rajesh Kumar, Achyut Narayan Kesari, Geeta Watal, P.S. Murthy, Ramesh Chandra, Kapil Maithal and Vibha Tandon (2005b): Hypoglycaemic andantidiabetic effect of aqueous extract of leaves of Annona squamosa (L) in experimental animal. Current Science: 88, 8, 1244-1254.

[12] Hole, R., Wakade, A., Juvekar, A., Nachankar, R. and Kulkarni, M. (2006): Protective effect of Annona reticulata L. fruits on isoproterenol induced myocardial infarction in rats. International Congress, Phytopharm, St. Petersburg, Russia.

[13] Kaleem, M., Asif, M., Ahmed, Q. U. and Bano, B. (2006): Anti-diabetic and antioxidant activity of Annona squamosa extract in streptozotocin-induced diabetic rats. Singapore Medical Journal, 47(8): 670- 675.

[14] Kaur, C. and Kapoor, H. (2005): Antioxidant activity of some fruits in Indian diet. Acta Horticulturae, 696.

[15] McLaughlin, J. (2008): Paw paw and cancer: Annonaceous acetogenins from discovery to commercial products. Journal of Natural Products: 71:1311-1321.

[16] Mukhlesur Rahman M, Shahnaj Parvin, Ekramul

[17] Islam M, Antimicrobial and cytotoxic constituents from the seed of Annona Squamosa Fitoterapia 76:2005, 484-489.

[18] Niwano, Y., Beppu, F., Shimada, T., Kyan, R., Yasura, K., Tamaki, M., Nishino, M., Midorikawa, Y. and Hamada, H. (2009): Extensive screening for plant foodstuffs in Okinawa, Japan with anti-obese activity on adipocytes in vitro. Plant Foods Human Nutrition, 64:6-10.

[19] Rabintossaporn, P., Saenthaweesuk, S., Thuppia, A., Ingkaninan, K. and Sireeratawong, S. (2009): Antihyperglycemic and histological effects on the pancreas of the aqueous leaves extract of Annona squamosa L. and diabetic rats. Songklanakarin Journal of Science and Technology, 31(10):73-78.

[20] Rao,V.S.N., Dasradhan , P., Krishnaiah,K.S.,1979. Antifertility effect of some indigenous plants. Ind.J.Med.Res.70,517-520.

[21] Vohora, S.B.,Kumar, I., Naqvi,S.A.,1975. Phytochemical, pharmacological,antibacterial and anti-ovulatory studies on Annona squamosa. Planta Med.28,97-100.

[22] Wiart, C., Au, T.S., Mohd, Y., Hamimah, H. and Sulaiman, M. (2005): 16 ? hydroxy-(-) kauran-19-oic Acid: an antibacterial diterpene from sweet apple (Annona squamosa L. Annonaceous). International Journal of Pharmacology: 1 (3):296-298.

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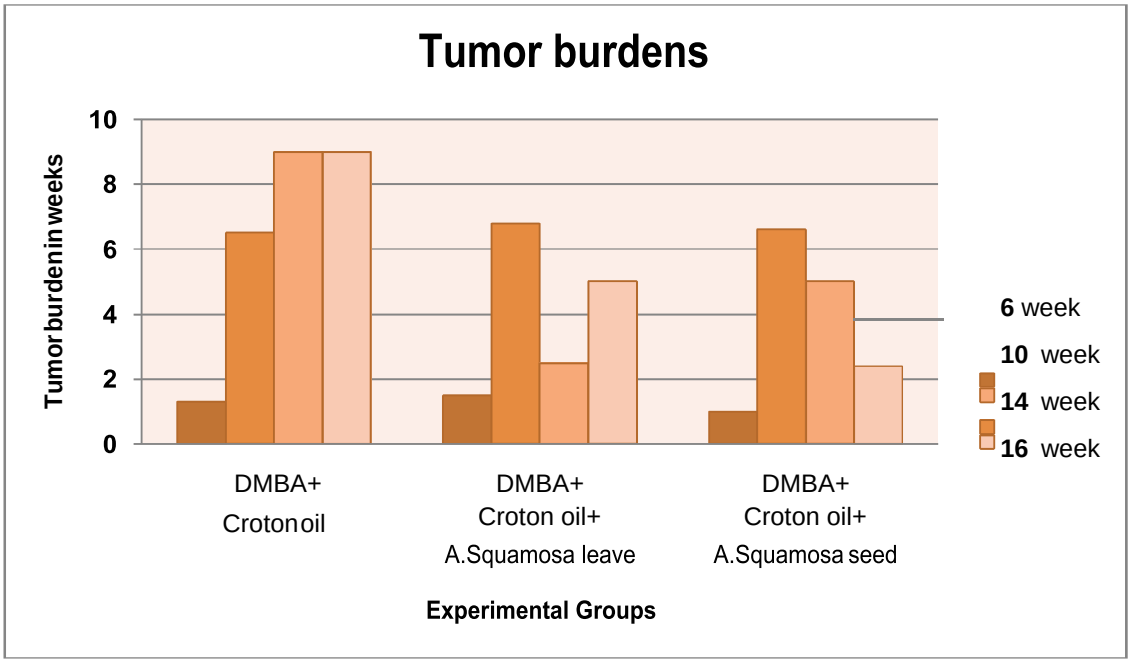
Table No.1 Effect of *Annona Squamosa* on Hydromethnolic seed & leaf extract on DMBA induced carcinogenesis Swiss albino mice.

S.No.	Group	6 Week	10 Week	14 Week	16 Week	Total
1	Untreted	0	0	0	0	0
2	DMBAAl one	0	0	0	0	0
3	Croton Oi l Al one	0	0	0	0	0
4	DMBA+Control	8/ 6 (1.3)	39/ 6 (6.5)	37/ 4 (9.02)	18/ 2 (9)	100%
5	DMBA+A.Sl eavel +Croton Oi l	9/ 6 (1.6)	41/ 6 (6.8)	10/ 4 (2.5)	30/ 6 (5.0)	83%
6	DMBA+A.SSeed+Croton Oi l	6/ 6 (1)	40/ 6(6.6)	30/ 6(5)	12/ 5 (2.4)	66%



Graph No.1 Effect of *Annona Squamosa* on Hydromethnolic seed & leaf extract on DMBA induced carcinogenesis Swiss albino mice.

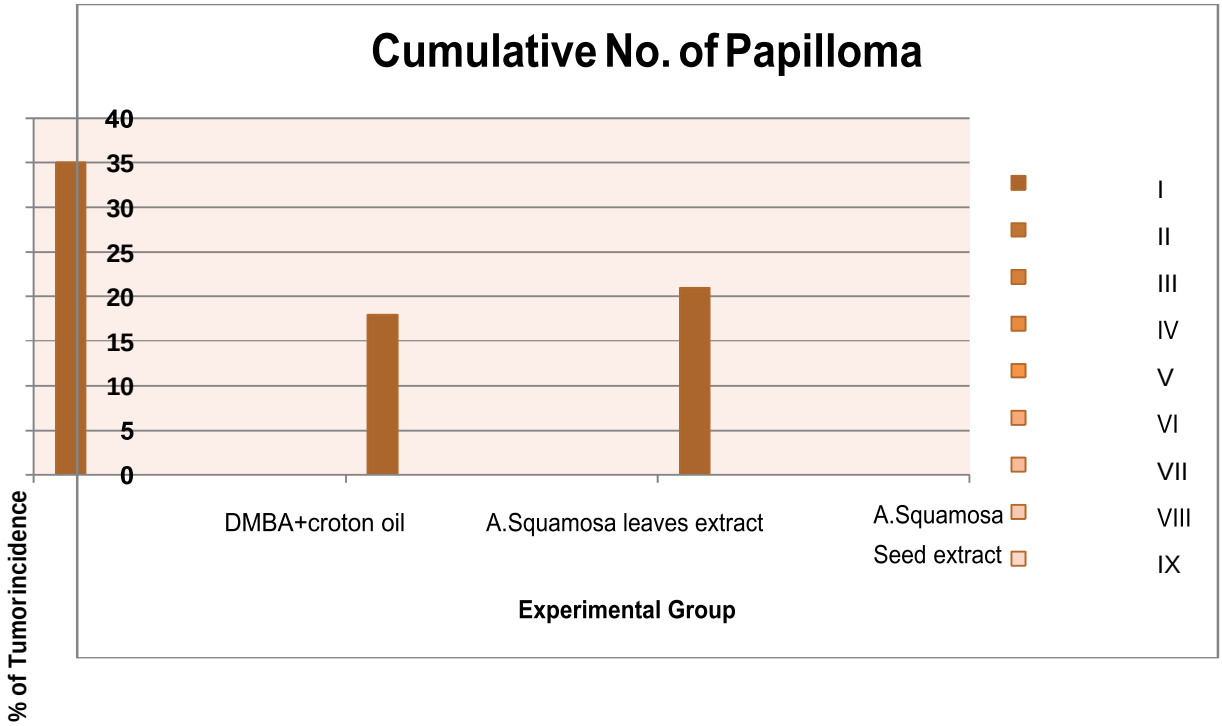
Table 2

Effect of *Annona Squamosa* on Hydromethnolic leaf & seed extract on DMBA induced carcinogenesis Swiss albino mice.

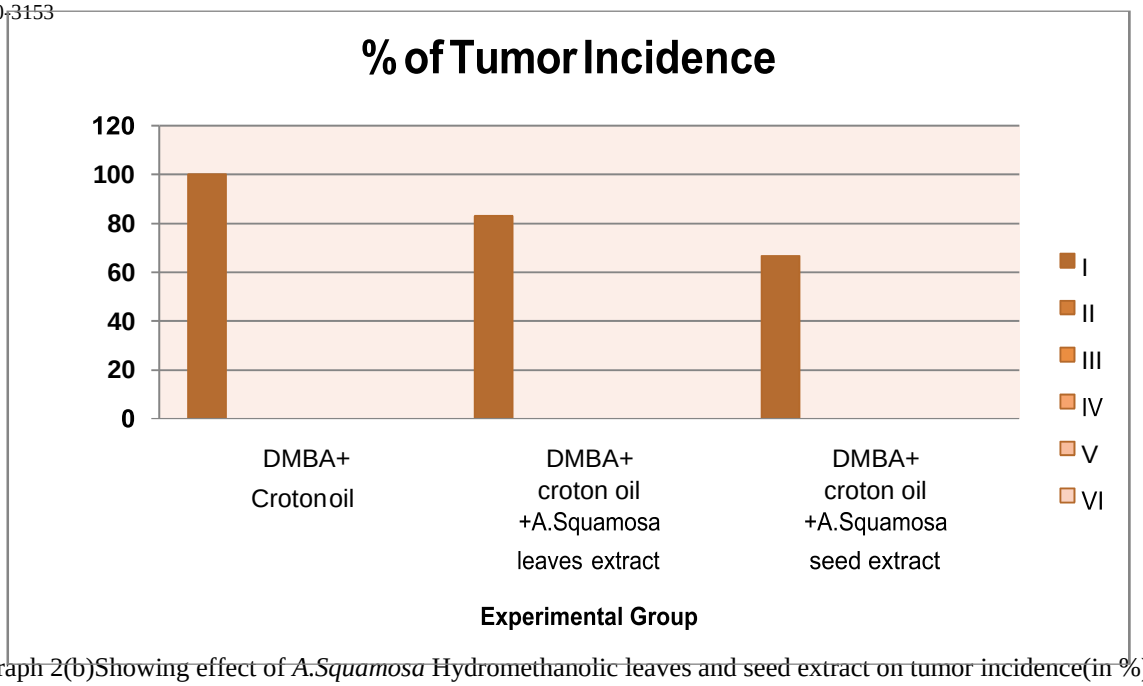
S.No.	Group	Treatment Doses	Ist Appearance of Papilloma (In days)	Cumulative No. of Papilloma	Tumor incidence (%)	Tumor Burden	Number of Papilloma with Tumor Tumor yield	Size (in mm)	
								<2	< -4
1	I (N=6)	Vehicle alone (100 µl acetone)	0	0	0	0	0	0	0
2	II (N=6)	DMBAalone (100 µg/ 100 µl actone)	0	0	0	0	0	0	0
3	III (N=6)	Croton Oil (100µl of 1% concentration)	0	0	0	0	0	0	0

5	V(N=6)	*A.Squamosa* l eaves extract al one (1000 mg/ kg bwt)	0	0	0	0	0	0	0
6	VI (N=6)	*A.Squamosa* Seed extract (1000 mg/ kg bwt)	0	0	0	0	0	0	0
7	VII(N= 6)	DMBA(100µg)+ Croton Oi l (1%)	40	35	6/ 6 (100%)	6.6	6.6	25	8
8	VIII(N=6)	DMBA(100 µg/ 100 µl *A.Squamosa* l eaves + Croton Oi l (1%)	58	18	5/ 6(83 %)	5	5	18	4
9	IX(N=6)	DMBA(100 µg/ 100 µl *A.Squamosa* Seed + Croton Oi l (1%)	56	21	4/ 6(66. 6%)	2.4	2.4	12	3

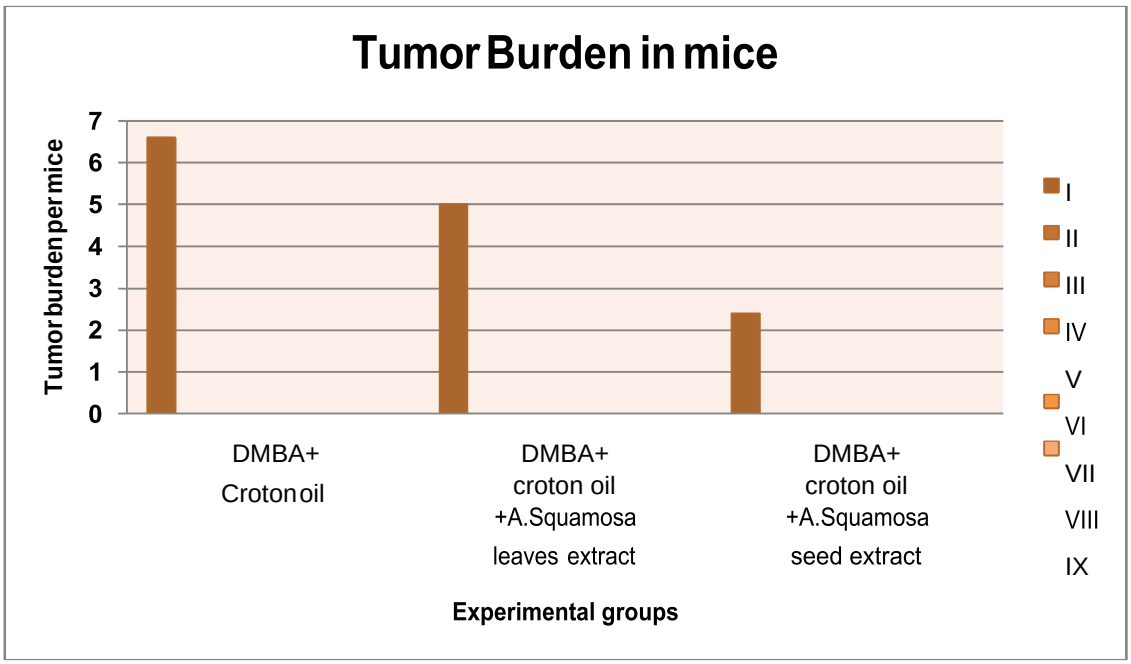
* DMBA = 7,12 dimethylbenz (a)nthracene ,CO= Croton oil , *A.Squamosa= Annona Squamosa*



Graph 1(a) Showing effect of *A.Squamosa* Hydromethanolic leaves and seed extract on cumulative no. of papilloma induced by DMBA+Croton oil.



Graph 2(b)Showing effect of *A.Squamosa* Hydromethanolic leaves and seed extract on tumor incidence(in %) induced by DMBA+Croton oil.



Graph 3(c) Showing effect of *A.Squamosa* Hydromethanolic leaves and seed extract on tumor burden induced by DMBA+Croton oil.

Antibacterial Activity

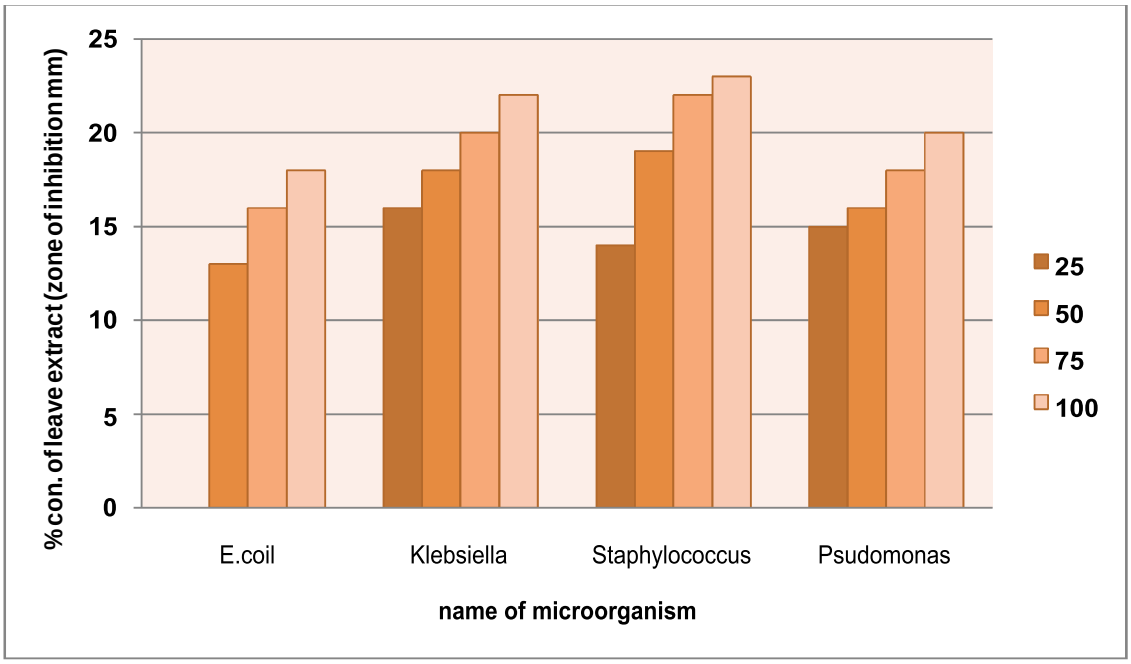
50% methanolic extract of leaves of Annona Squamosa at the different concentration

25%, 50%, 75%,100% exhibited antibacterial against *E.coli, Klebsiella, Staphylococcus, and Psuedomonous.*

Table no. 3 :- Antibacterial activity of *Annona Squamosa* leaves against bacterial strai

S.no	Name of Microorganisms	% Concentration of Seed Extract (Zone of inhibition (mm)			
S.no	Name of Microorganisms	(Zone of inhibition (mm)			
		25	50	75	100
1	*E.coli*		13	16	18
2	*Klebsiella*	16	18	20	22
3	*Staphylococcus*	14	19	22	23
4	*Pseudomonas*	15	16	18	20

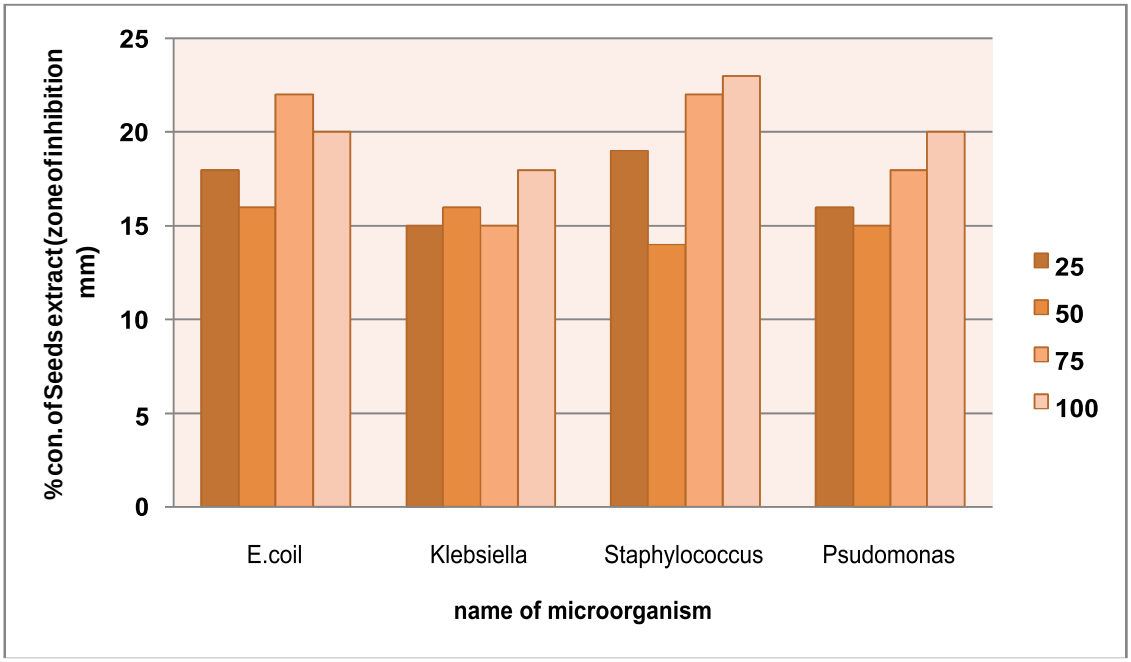
ns.



Graph4 :- showing of zone of inhibition of *A.S* leaves extract against different strains of bacteria.

Table:- Antibacterial activity of *Annona Squamosa* Seeds against bacterial strains.

		25	50	75	100
1	*E.col i*	18	16	22	20
2	*Kl ebsi el la*	15	16	15	18
3	*Staphyl ococcus*	19	14	22	23
4	*Pseudomonas*	16	15	18	20



Graph:- showing of zone of inhibition of *A.S* seeds extract against different strains of bacteria.

3. Antioxidant Activity

:- The In vitro Anti-oxidant potential of *A.Squamosa* hydromethonolic

leaves and seeds extract have been evaluated using DPPH methods. It was observed that Annona sqamosa leaves and seed extract caused dose dependent TBARS inhibitionand caused antioxidant activities.

Antioxidant activity:- Table No.4

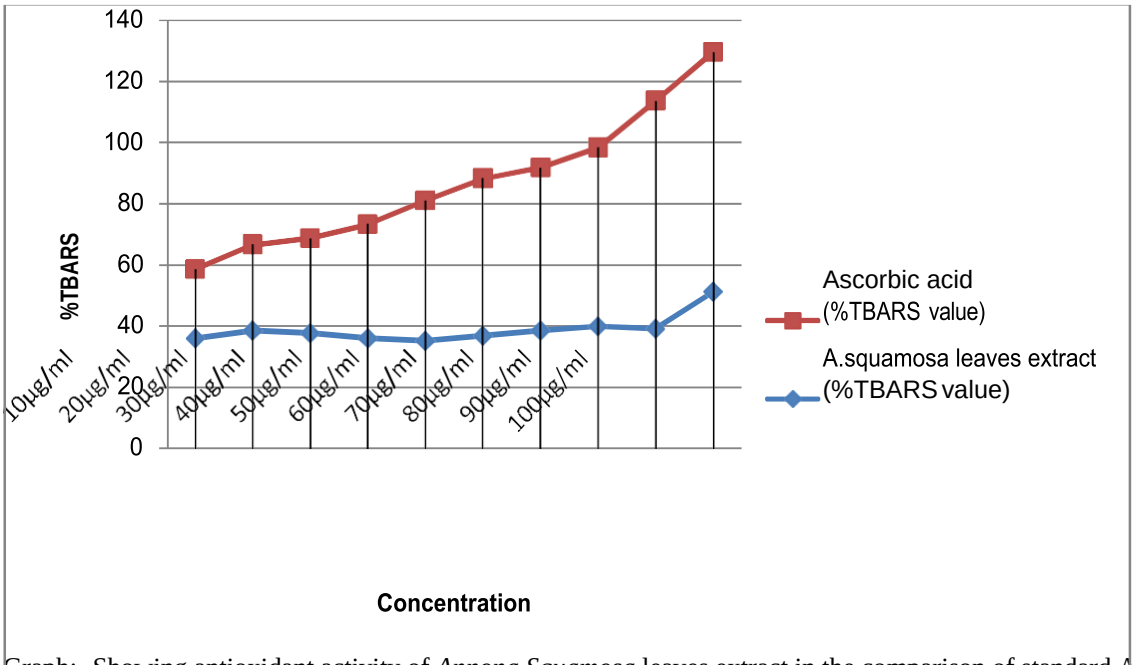
In vitro Anti-oxidant potential of *A.Squamosa* hydromethonolic leaf extract in the

comparison of standard Anti-oxidant.

S.No.	Con. Of Ascorbi c Aci d	Ascorbi c Aci d		*Annona Squamosa*	
				Leaves	
		0.D517nm	(% TBARSVal ue)	0.D517nm	(% TBARSVal ue)
1	100	0.098	22.55	0.130	36.01
2	200	0.11	28.1	0.139	38.50
3	300	0.132	31.02	0.136	37.67
4	400	0.159	37.27	0.141	36.01
5	500	0.157	45.72	0.127	35.18
6	600	0.175	51.35	0.130	36.85
7	700	0.187	53.27	0.144	38.51
8	800	0.192	58.49	0.138	39.88
9	900	0.199	74.45	0.134	39.11
10	1000	0.213	78.35	0.181	51.12

IC_{50}= Concentration at which % inhibition of TBARS is 50%.

S.No.	Group	Concentrati on	IC50 val ue
1	*A.Squmosa* l eaves extract	600µg/ ml	36.85
2	Ascorbi c aci d	700µg/ ml	38.51



Graph:- Showing antioxidant activity of *Annona Squamosa* leaves extract in the comparison of standard Ascorbic acid.