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Studies on Antioxidant and Anticancer Potentials of Fruit Pulp Extract of *Semecarpus anacardium*

A.D. Naveen Kumar[1*], Berihu Haftu[1] and M.V.S. Mahesh Kumar[2]

[1]Department of Biomedical Sciences, College of Medicine and Health Sciences, Adigrat University, Adigrat, PO Box: 50, Ethiopia.
[2]Department of Biotechnology, Institute of Science, GITAM University, Visakhapatnam-530 045, Andhra Pradesh, India.

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Corresponding Author Email: adurganaveen@gmail.com

Abstract

Cellular damage mediated by reactive oxygen species (ROS) has been implicated in the pathogenesis of several diseases. Natural antioxidants have significant importance in human health. Focus towards the natural sources of antioxidants has been increasing these days to fight against the lethal effects of free radicals. *Semecarpus anacardium*, a medicinal plant of Anacardiaceae family used as remedy in the alleviation of disorders. The present study was focused to evaluate the antioxidant and anticancer potentials aqueous extract of *S.anacardium* fruit pulp by most accepted methods. The aqueous extract exhibited significant DPPH, hydroxyl radical and nitric oxide scavenging activities. In addition, the aqueous extract also exhibited significant anti-proliferative activity and cathepsin B and MMP-9 inhibitory activities. Therefore, *S.anacardium* fruits may serve as a novel therapeutic agent for the treatment of radical mediated diseases.

Keywords
Semecarpus anacardium, radical scavenging, natural antioxidant, antioxidant activity, anti-proliferative activity.

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INTRODUCTION

Cancer is the second leading cause of death worldwide and hence combating cancer is of paramount importance today [1]. In recent times, focus on medicinal plant research has increased all over the world even though large body of evidences has been collected to show immense potential of medicinal plants [2]. The use of medicinal plant products as antioxidants is wide spread and still medicinal plants are huge source of antioxidants that might serve as novel drugs for the treatment of oxidative damage related diseases such as cancer [3]. Antioxidants are one of the major plant products that play a role as anticancer agents through acting as reducing agents, hydrogen donators, and singlet oxygen and nitrogen quenchers that suppress the naturally produced free radicals and delaying oxidative stress-related reactions such as lipid oxidation [4]. Clinical studies have reported that many medicinal plant extracts have tumoricidal

activity against various cancers. In addition, low toxicity and high efficiency of natural products might be the reason for the growing movement towards the use of medicinal plants. Therefore, the usage of natural products has a great importance for the treatment of cancer [5].

Semecarpus anacardium (*Anacardiaceae*) commonly known as 'Bhilwa' or 'Ballataka' is well-known for medicinal value in ayurvedic and siddha system of medicine [6]. Different parts of this plant such as stem bark, nuts and leafs have been traditionally used to treat rheumatism, asthma, neuralgia, helminthic infection, psoriasis and cancer [7]. The aqueous stem bark extract was reported to have antimicrobial, CNS stimulant, hypoglycemic, anti-atherogenic and anti-carcinogenic activities [8]. Previously, we reported antioxidant and cytoprotective activites of stem bark extract against Fenton reaction induced lipid peroxidation and heat induced hemolysis [9]. Two flavonoids 3-O-methyl quercetin and kaempferol have been isolated from the stem bark of *S.anacardium* and their protective role against H_2O_2 induced oxidative stress have been studied at molecular level [10]. However, antioxidant and anticancer activities of *S.anacardium* fruit were not completely explored and the present study was aimed to evaluate the antioxidant and anticancer potentials of methanolic extract of *S.anacardium* fruit pulp by widely accepted in vitro methods.

MATERIALS AND METHODS

Chemicals and reagents:

1,2-dipheny1-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-striazine (TPTZ), nitrobluetetrazolium (NBT), thiobarbituric acid (TBA), Bacterial Collagenase-I, were purchased from Sigma, USA., Sodiumnitropruside, Sodium azide (NaN_3), deoxyribose, queretin were purchased from Merck, Germany. All the chemicals and solvents used in the present study were of analytical grade obtained from local supplier in pure quality.

Collection of plant material

The fruits of *S.anacardium* was collected from the Eastern Ghats of Vizianagaram region and authenticated by the faculty in the department of Botany, Andhra University, and Visakhapatnam.

Preparation of plant extract

The fruits were thoroughly cleaned with distilled water, peeled off and the pulp was extracted with mechanical grinder. The pulp was extracted with soxhlet extractor using n- hexane, ethyl acetate, methanol and water for 48 hrs. The extracts were concentrated to dryness in desiccator. The solvent extracts were used to determine the DPPH radical scavenging ability and total antioxidant activity. Further, different concentrations of aqueous extract (25, 50,100,250,500,750 and 1000µg) were prepared in aforesaid solvents and were used to assay antioxidant and anticancer activities.

Antioxidant assays

DPPH radical scavenging activity

DPPH scavenging activity was measured by the method of Cuendet *et al* [11]. To 3.0ml of methanolic solution of DPPH (0.1mM), 1.0 ml of different concentrations of fruit pulp extract (25, 50, 100, 250, 500, 750 and 1000µg/ml) were added. In control, the extract was replaced by methanol. The reaction mixture was incubated for 30 min at 37^0C and absorbance was measured at 517nm using UV-visible spectrophotometer. The percentage of inhibition was calculated from the following equation: A_0-Ax100/A_0, where A_0 and A are the absorbance of control and test sample, respectively. Quercetin is used as standard.

Determination of total antioxidant activity

The total antioxidant potential of fruit pulp extract of *S.anacardium* was assayed by using FRAP method as described Wong *et al* with some modifications [12]. Briefly, 0.2ml of different concentrations extracts were added to 3.0ml of FRAP reagent (mixture of 300mM sodium acetate buffer (pH 3.6), 10mM, TPTZ solution and 20mM $FeCl_3$ in a ratio of 10:1:1. The reaction mixture was incubated in a water bath at 37°C for 30min. The increase in the absorbance was measured using spectrophotometer at 593nm. The total antioxidant capacity was assessed based on the ability to reduce ferric ions by the extracts. The percent of total antioxidant activity was calculated using a formula

Percent of antioxidant activity = [(A593 of sample – A593 of control)/ A593 of sample] x 100

Determination of hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radical's generated by fenton reaction, a method originally described by Gulhan *et al* [13]. 0.1ml of fruit extract was added to the reaction mixture containing 0.1ml of 3.0mM deoxyribose, 0.5ml of 0.l mM $FeCl_3$, 0.5 ml of 1mM H_2O_2 and 0.8 ml of 20mM phosphate buffer, pH 7.4 in a final volume of 3.0ml and incubated at 37°C for 1hr. The thiobarbituric acid reactive substances (TBARS) formed were measured by treating with 1.0ml of TBA (1.0%) and 1.0 ml of TCA (2.8%) at 100°C for 20min. After the mixtures were cooled, absorbance was measured at 532 nm against control,

which is devoid of plant extract. Percentage of inhibition was calculated as

(I) = [(Absorbance of control- Absorbance of test / Absorbance of control)] x 100.

Nitric oxide scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH was measured by Griess reaction [14]. The reaction mixture (3ml) containing sodium nitropruside (10mM) in phosphate buffer saline and the test extracts were incubated at 25°C for 150 min, after incubation 1.5ml of the reaction mixture was removed and 1.5ml of the Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% Napthylethylinediamine hydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm. Percent inhibition of nitric oxide scavenging was calculated using the formula.

Percentage Inhibition = (A of Control – A of Sample)/A of Control× 100. A- Absorbance.

In vitro cell cytotoxicity of Fruit pulp extracts of *S.anacardium*:

ER positive breast cancer cells (MCF7) were grown as monolayer cultures in Dulbecco's Modified Eagle's Medium (DMEM) and ER negative breast cancer cells (BT-549) were grown as monolayer cultures in RPMI-1640. Both media are supplemented with 5% foetal bovine serum, 100U/ml each of penicillin and streptomycin and maintained at 37OC in a 5% CO_2 incubator. The cell cytotoxicity of methanolic fruit pulp extracts of *S.anacardium* against MCF-7 and BT-549 was determined by MTT assay [15]. Cells were seeded into 96-well plate at 1×10^4 cells/well density and treated with extracts (25, 50 and 100µg/ml) for 48 h. After 48 h MTT was added to each well and solubilized the formazan crystals by DMSO. Then absorbance was measured at 570 nm in a microplate ELISA reader. Effect of extract was quantified as the percentage of control absorbance of reduced dye at 570nm.

Percent of inhibition [100-(absorbance of test wells/absorbance of control wells) x 100] were calculated. All experiments were performed in triplicate.

In vitro inhibition of MMP-9 (Collagenase -1) activity:

Matrix metalloproteinase-9 activity assay was performed in 50mM Tris–HCl buffer, pH 7.5 containing 0.15M NaCl, 10mM $CaCl_2$, 0.02% NaN_3, 0.05% Brij 35 and 50µM ZnSO4 as previously described [16]. The inhibitory activity of plant extracts on MMP-9 (collagenase -1) was determined using synthetic fluorogenic substrate. 0.1ml of extract was incubated with 0.3ml of 1M substrate and 0.2ml of bacterial collagenase-1 (Sigma, USA) at 37 °C for 20h and the reaction was terminated by the addition of 1.0ml of 3% acetic acid. The residual activity of MMP-9 was determined by measuring the fluorescence intensity at 495nm (excitation) and 520nm (emission) using fluorescence microplate reader. The inhibitory activity of plant extracts was expressed as percent control.

In vitro inhibition of cathepsin B:

Effect of fruit pulp extracts on human liver cathepsin B (Sigma, USA) activity was determined using Na-CBZ-L-Lysine p-Nitrophenyl Ester (Sigma, USA) as substrate according to method of Bajkowski and Frankfurter with some modification [17]. 2.84ml of 20mM sodium acetate buffer, pH 5.0 containing 1mM EDTA and 5mM L-cysteine was mixed with 0.05ml of 5.2mM Na- CBZ-L-Lysine -p-Nitrophenyl Ester substrate, 0.1 ml of plant extract and mixed well by inversion. Then increase in absorbance at 326nm was monitored for two min and 0.01ml of cathepsin B enzyme (Sigma, USA) solution in sodium acetate buffer was added. For control, 0.1ml of sodium acetate buffer was added instead of plant extract. Immediately mixed by inversion and increase in A326nm was recorded for 3min. The A326nm/min was obtained using the maximum linear rate for both the test and the control.

Percent of inhibition was calculated as [A326 of control – A326 of test/ A326 of control] x 100.

Statistical analysis:

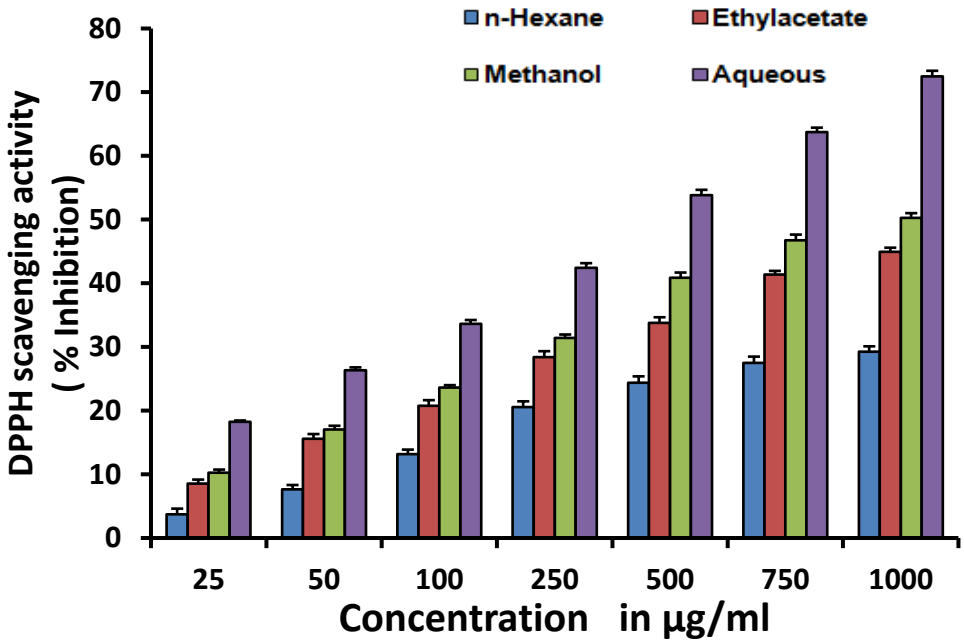
Each experiment was carried out three times separately. Data was expressed as mean ± SE of minimum of three independent experiments. Statistical differences between control and target groups for all experiments were determined using Student's t-test. The statistical significance was determined at 5% ($p<0.05$) level.

RESULTS AND DISCUSSION

Several inflammatory diseases are thought to be related to oxidative injury and oxygen free radicals have been projected as an important causative agent of various disorders, aging and various types of cancers [18]. Reactive oxygen species are responsible for initiating and progression of the multistage carcinogenesis process [19]. Antioxidants form medicinal plants shield from the free radical induced cellular damage by terminating free radical intermediates. Epidemiological and *in vitro* studies have strongly supported that medicinal plant constituents with antioxidant properties exerting protection against oxidative stress in inflammatory, age related diseases and lung disorders [20]. In the present study, fruit pulp extracts of *S.anacardium*

were used to study their reducing ability, antioxidant activity, radical scavenging ability and inhibitory activity of the enzymes MMP-9 and cathepsin B.

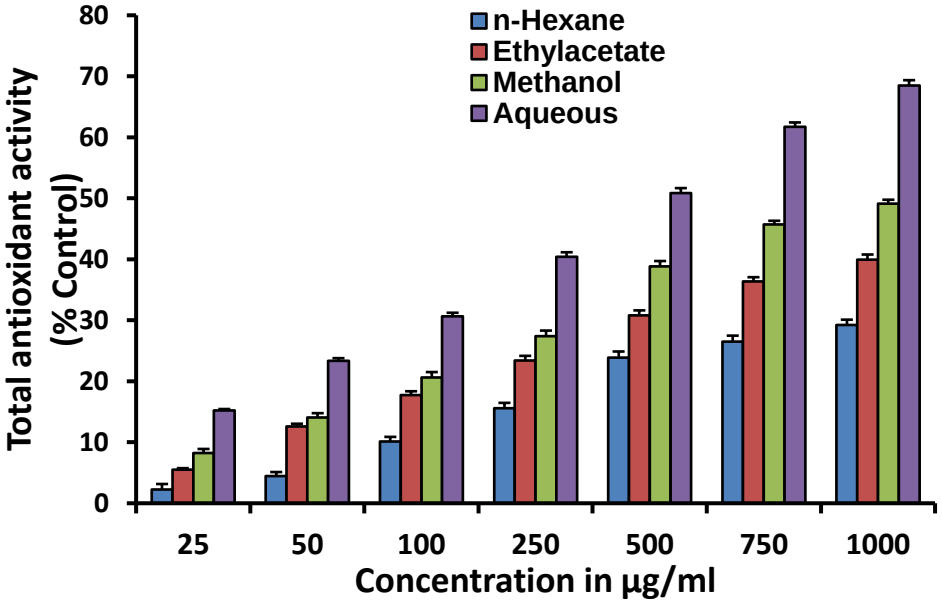
Oxygen derived radicals represent the most important class of radical species generated in living systems [21]. The harmful effect of free radicals causing potential biological damage is oxidative stress [22]. DPPH is stable and non-physiological radical and most widely used method for screening antioxidant activity of plant extracts [23]. DPPH is reduced to diphenylpicryl hydrazine with plant extracts in a concentration-dependent manner. The radical scavenging ability of different solvent extracts on DPPH radical is in the following order: aqueous > methanol > ethyl acetate > hexane with 72.5, 50.28, 44.92 and 29.25% respectively, at 1000 µg/ml concentration (Fig. 1a). Among the four extracts, aqueous extract had shown the highest DPPH scavenging activity similar to natural antioxidant quercetin (78.25%).



DPPH Scavenging of n-Hexane, ethylacetate, methanol and aqueous extracts of fruit pulp of *S.anacardium*. Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis. Each value represents mean ± SE of three independent experiments. The values are significant at *p*<0.05.

Fig 1a: DPPH Scavenging Activity

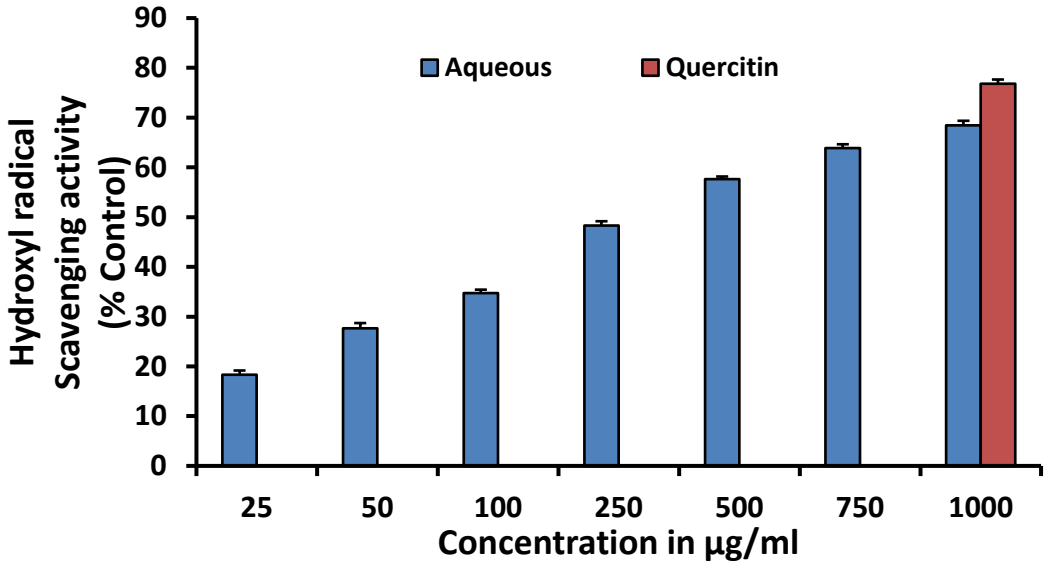
FRAP assay is a simple, expedient method extensively used to determine the total antioxidant activity of biological samples [24]. As shown in the figure-1b, aqueous, methanol, ethyl acetate and n-hexane extracts of *S.anacardium* stem bark exhibited 68.5, 49.10, 39.92 and 29.25%, respectively, at 1mg/ml. Ascorbic acid represented the standard showed 76.75% total antioxidant activity at 1000 µg/ml. The results from this antioxidant assay recommended that the aqueous extract showed higher FRAP reducing activity than other solvent extracts. The significant FRAP activity of aqueous extract may be due to extraction of more hydrogen donation compounds by methanol than other solvents used in the present study. FRAP is reduced by the compounds that can donate hydrogen atoms to free radicals and convert them into stable nonreactive molecules. As aqueous extract exhibited significant as it is exhibited significant antioxidant activity and DPPH radical scavenging activity, further studies were carried out with aqueous extract.



Total antioxidant activity of n-Hexane, ethylacetate, methanol and aqueous extracts of fruit pulp of *S.anacardium*. Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis. Each value represents mean ± SE of three independent experiments. The values are significant at $p<0.05$.

Fig 1b: Total Antioxidant Activity

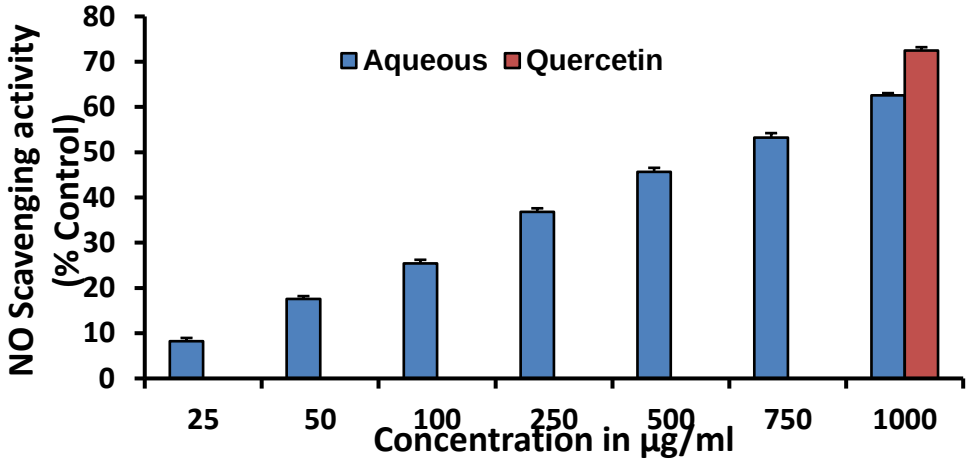
Hydroxyl radical is the most lethal and reactive radical among the ROS with shortest half-life compared to other free radicals. The oxygen derived hydroxyl radicals in presence of transition metal ion (Fe^{2+}) causes the degradation of deoxyribose into malondialdehyde which produces a pink chromogen with thiobarbituric acid [25]. The aqueous extract of fruit pulp of *S.anacardium* exhibited hydroxyl radical scavenging activity in a dose dependent manner and significant inhibition was observed at 1000 µg/ml concentration with 68.45% when compare to the percent of inhibition natural antioxidant quercetin with 76.8%.



Hydroxyl radical scavenging activity of aqueous extract of fruit pulp extract of *S.anacardium* compared to that of standard Quercetin (1mg/ml). Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on Xaxis and % inhibition taken on Y-axis. Each value represents mean ± SE of three independent experiments. The values are significant at $p<0.05$.

Fig 2a: Hydroxyl Radical Scavenging Activity

Nitric oxide (NO) is a powerful inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, and inhibition of platelet aggregation and regulation of cell mediated toxicity [26]. It is a diffusible signaling molecule considered as proinflammatory mediator that plays a key role in the pathogenesis of inflammatory disorders [27]. Generally, scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions [28]. Aqueous extract of *S.anacardium* fruit pulp showed noteworthy nitric oxide scavenging activity in dose dependent manner with percent of inhibition 62.58 at 1000µg/ml concentration compared with Ascorbic acid 72.45% (Fig. 2a).

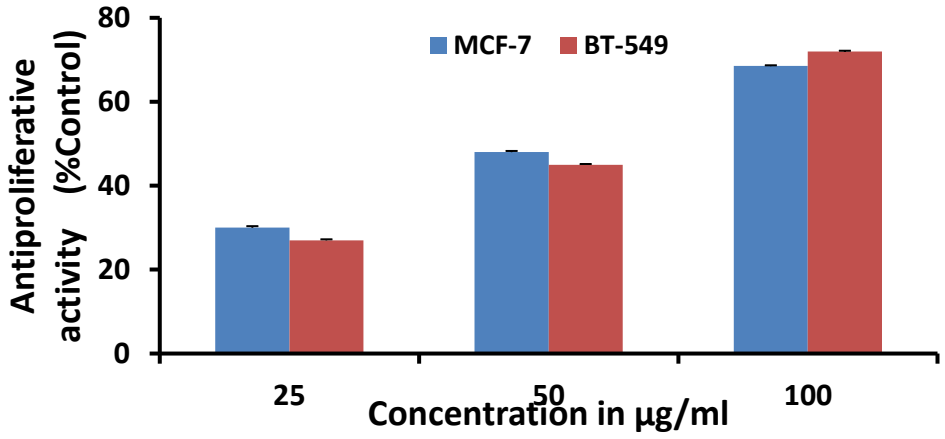


Nitric oxide scavenging activity of aqueous extract of fruit pulp of *S.anacardium* compared to that of standard Ascorbic acid (1mg/ml). Each value is expressed as mean ± standard deviation (n=3). Concentration (µg/ml) taken on X-axis and % inhibition taken on Y-axis. Each value represents mean ± SE of three independent experiments. The values are significant at $p<0.05$.

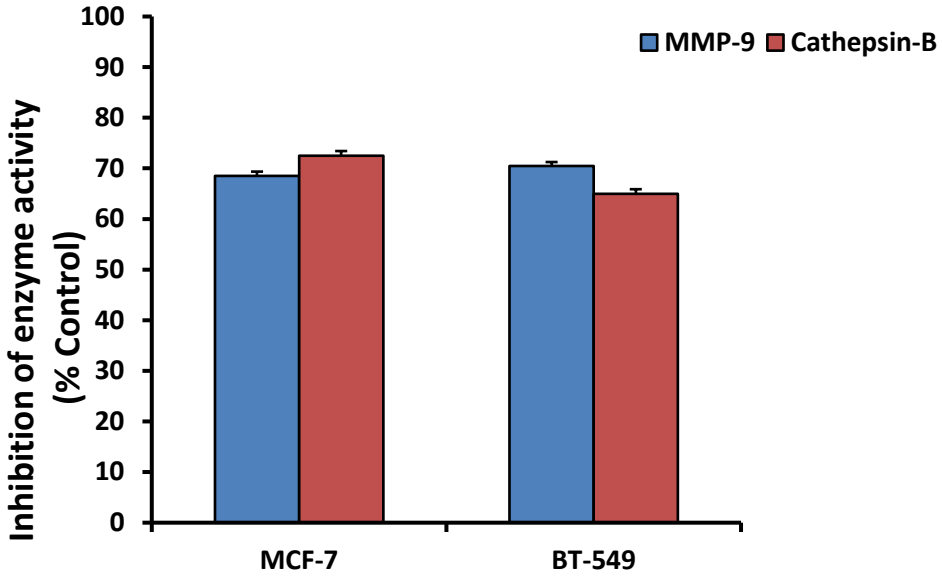
Fig 2b: Nitric Oxide Scavenging Activity

The cytotoxic effect of fruit pulp extract of *S.anacardium* was evaluated by using anti-proliferative assay with ER positive breast cancer cell line (MCF-7) and ER negative breast cancer cell line (BT549). The aqueous fruit pulp extract of *S.anacardium* exhibited 68.5% of anti-proliferative activity against MCF-7 compared to control and 72% of anti-proliferative activity against BT-549 cells compared to control (Figure 3a). MTT assay was used to evaluate the cytotoxic activity of *S.anacardium* fruit pulp extract on MCF-7 and BT549 cell lines. MTT assay is a well-known in vitro method to determine the cytotoxic activity of the extracts against cancer cell lines. Cytotoxicity screen models provide significant primary data to help selecting plant extracts with potential anti-neoplastic properties for future work and provide scientific support to the use of *S.anacardium* for the treatment of cancer patients by traditional healers.

A)



B)



a) Antiproliferative activity of aqueous extract of pulp extract of *S.anacardium* against MCF-7 and BT-549 breast cancer cell lines. Monolayer breast cancer cells were treated with aqueous extracts for 48h and cytotoxicity was determined by MTT assay. Each value represents mean ± SE of three independent experiments. The values are significant at $p<0.05$.
b) Antiprotease activity of aqueous extract of fruit pulp of *S.anacardium* against MMP-9 and cathepsin B. Activity of enzymes was determined in presence or absence of extracts as described in "Material and Methods".

Cathepsin B and MMP-9 are important tissue remodeling enzymes essential for metastasis of solid tumors. Both Ayurveda preparation as well as the extracts of various parts of *S.anacardium* has been reported to exhibit anti-tumour property and it is a rich source of phytoconstituents such as flavonoids, bioflavonoids, diterpenes, tannins & high amount of antioxidants; shows good cytotoxic, cytostatic & anticancer property [29]. Recent studies have confirmed that ROS activates MMP-9 and Cathepsin B to intervene the breakdown of extra cellular matrix and responsible for metastasis (Liu *et al*., 2005) [30]. The present study demonstrated that aqueous extract of aqueous fruit pulp extract of *S.anacardium* have significant inhibition on proliferation of breast cancer cell lines as well as both cathepsin B and MMP-9 activities. This study indicates that the anticancer potential of *S.anacardium* fruit pulp is relatively due to its inhibitory activity on cancer cell proliferation and also inhibition of tissue remodeling enzymes such as MMP-9 and Cathepsin B probably by scavenging oxygen free radicals.

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CONCLUSION
In summary, our study demonstrated that aqueous fruit pulp extract of *S.anacardium* is a potential source of natural antioxidants as confirmed DPPH, superoxide and hydroxyl radical and hydrogen peroxide scavenging activities. This study also demonstrated the possible anti-cancer activity of *S.anacardium* as aqueous extracts showed significant inhibitory effect on tissue remodeling proteases, such as MMP-9 and Cathepsin B. Further studies need to be conducted to identify the antioxidant compounds and to understand its anti-cancer activity mechanism.

CONFLICT OF INTEREST
None

REFERENCES
[1] Cragg, G.M.; Newman, D.J. Natural product drug discovery in the next millennium. *Pharmaceut. Biol*., 2001, *39 Suppl 1*, 8-17.

[2] Dahanukar SA, Kulakarni RA, Rege NN: Pharamacology of medicinal plants and natural products. *Indian Journal of Pharmacology* 2000, 32: 81-118.
[3] Linn CC, Huang PC: Antioxidant and hepatoprotective effects of *Acothopanaz senticosus*. *Phytother* 2000, 14(7): 489-494.
[4] Shimizu, T., Torres, M. P., Chakraborty, S., Souchek, J. J., Rachagani, S., 2013, Holy Basil leaf extract decreases tumorigenicity and metastasis of aggressive human pancreatic cancer cells in vitro and in vivo: potential role in therapy, Cancer Lett, 336, pp. 270-280.
[5] Balachandran, P.; Govindarajan, R. Cancer - An ayurvedic perspective. *Pharmacol. Res*., 2005, *51*, 19-30.
[6] Khare CP. Encyclopedia of Indian medicinal plants 1982:419–21.
[7] Chadha: The Wealth of India-Raw Materials, National Institute of Science Communication and Information Resources (CSIR), New Delhi 1989; 10:251–252.
[8] Kirtikar KR and Basu BD. Indian Medicinal Plants. Bishen Singh Mahendra Pal Singh, Dehradun, India, International book distributor 1975; Vol.2, 2nd edn: 894- 895.
[9] Naveen Kumar AD, Bevara GB, Laxmikoteswramma K, Malla RR. Antioxidant, cytoprotective and anti-inflammatory activities of stem bark extract of Semecarpus anacardium. Asian J Pharm Clin Res. 2013; 6:213–9.
[10] A.D.Naveen Kumar, Ganesh Babu Bevara, K.Laxmi Koteswaramma, Anil Badana and Rama Rao Malla. Protective effect of 3-O- methyl quercetin and Kaempferol from *Semecarpus anacardium* against H2O2induced Cytotoxicity in Lung and Liver cells. BMC Complementary and Alternative Medicine. 2016; 16:376.
[11] Cuendet M, Hostettmann K, Potterat O. Iridoid glucosides with free radical scavenging properties from Fragrea blumei. Helv Chim Acta. 1997; 80: 1144–1151.
[12] Wong SP, Lai PL, Jen H W K. Antioxidant activities of aqueous extracts of selected plants. Food Chem 2006; 99: 775783.
[13] Marcocci I, marguire JJ, Droy – lefaiz MT, packer L. The nitric oxide scavenging properties Ginkgo biloba extract. Biochemical and biophysical Research communication 1994; 201:748-755.
[14] Mallick N, Washim K, Mhaveer S, Zeeshan NM, Mohammad K, Sayeed A, Syed AH. In vitro anticancer potential of Semecarpus anacardium Linn. Symposium. 2016;7(1):5558.
[15] Mosmann, T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *Journal of immunological methods*, Vol. 65, pp. 55-63, (1983).
[16] Netzel Arnett S, Mallya SK, Nagase H, Birkedal-Hansen H, Van Wart H E: Continuously recording fluorescent assays optimized for five human matrix metalloproteinases. Analytical Biochemistry1991, 195:86–92.
[17] Bajkowski AS, Frankfater A: Redistribution of cathepsin B activity from the endosomal-lysosomal pathway in chick intestine within 3 min of calcium absorption. Analytical Biochemistry 1975, 68: 119-121.
[18] Choi E M, Hwang J K: Effect of some medicinal plants on plasma antioxidant system and lipid levels in rats. Phytother Res 2005,19(5):382-386.
[19] Nam Joo Kang, Seung Ho Shin, Hyong Joo Lee, Ki Won Lee: Polyphenols as small molecular inhibitors of signaling cascades in carcinogenesis. Pharmacology & Therapeutics 2011,130: 310324.
[20] Fernando Holguin, Sumita Khatri, David M Guidot: Antioxidant treatment for lung diseases. 2005, 15:1711-1725.]
[21] Miller DM, Buettner GR and Aust SD. Transition metals's catalysts of "autoxidation" reactions. Free Radic Biol Med 1990; 8:95-108.
[22] Ridnour LA, Isenberg JS, Espey MG, Thomas DD, Roberts DD and Wink DA. Nitric oxide regulates angiogenesis through a functional switch involving thrombospondin1.Proc.Natl.Acad.Sci2005;102:13147-52.
[23] Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y. Scavenging effects of tea catechins and their derivatives on 1,1diphenyl-2-picryl hydrazyl radical. Free Radic Biol Med 1996; 21:895–902.
[24] Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry 2000 48:3396-3402.
[25] Halliwell B, Free radicals and metal ions in health and disease; Proc. Nutr. Soc 1987; 46:13-26.
[26] Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT and Hartzfeld PW, High molecular weight plant polyphenolics(tannins) as biological antioxidants. J Agric and Food Chem 1998; 46: 1887-1892.
[27] Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. Inflammo pharmacology 2007; 15(6): 252-259.
[28] Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Essential oil composition and antioxidant activity of Pterocarya fraxinifolia Pak. J. Biol. Sci 2007; 12(13): 957-963.
[29] Mallick N, Washim K, Mhaveer S, Zeeshan NM, Mohammad K, Sayeed A, Syed AH. In vitro anticancer potential of Semecarpus anacardium Linn. Symposium. 2016;7(1):5558.
[30] Liu KJ, Rosenberg GA: Matrix metalloproteinases and free radicals in cerebral ischemia. Free Radic Biol Med 2005, 39:71–80.