Research Article

Study of Anti-Cancer Properties of Crude Seed Extracts of *Vitis vinifera, Nigella sativa* and *Ocimum basilicum* by *in-vitro* and *ex-vivo* Studies

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ABSTRACT

Cancer is a disease that is increasingly affecting people globally, in recent times. In this study, we have considered methanolic, ethyl acetate and aqueous extracts of three seed samples viz. seeds of *Vitis vinifera, Nigella sativa* and *Ocimum basilicum*. The extracts were screened for their protection capacity against hemolysis, inhibition of lipid peroxidation (<15% by all extracts), and cellular toxicity using MTT assay (>60%). Further, the potential of the extracts to inhibit angiogenesis and migration of leucocytes were studied, as these two mechanisms play a significant role in proliferation tumors. It was seen that the seeds exhibited desirable results in all the mentioned assays. Extracts of *O. basilicum* and *V. vinifera* seeds showed better results compared to the seeds of *N. sativa.* Hence, it can be concluded that the seeds possess potential chemotherapeutic properties.

Keywords: Cancer, leucocyte migration, lipid peroxidation, anti- hemolytic, cell viability, angiogenesis.

INTRODUCTION

Cancer is a disease that has become the second leading cause of death globally. 5–10% of cancer cases can be attributed to genetic defects and the remaining 90–95% has its roots in the environment and lifestyle. Diet, obesity, and metabolic conditions are linked to various cancers and account for as much as 30–35% of cancer deaths. Hence, dietary changes can help in prevention of cancer. Our project involves studies of dietary sources like seeds of grape, basil and nigella to assess their properties that can be effective in suppression or prevention of cancer[1,2].

Chemotherapy is the main treatment method for certain types of cancer. It is important to monitor and ensure that these chemotherapeutic drugs are potent and effective prior to administration to patients [3].

ROS also causes lysis of red blood cells (hemolysis) by changing the structure of haemoglobin [4]. The damage caused by this ROS to the erythrocytes can be inhibited by some of the antioxidants present in the plant extracts. The destruction of the RBC membrane is the major factor for hemolysis. Various antioxidant mechanisms together form the reason for the anti-hemolytic activity [5].

Lipid peroxidation is often low in tumor tissue when compared to normal tissue. In the paper, various contributory factors controlling the rate of microsomal lipid peroxidation in normal rat liver and in the Novikoff hepatoma were analyzed. The low rate of lipid peroxidation in the hepatoma seemed to be due to low levels of polyunsaturated fatty acids and of cytochrome P-450 and elevated levels of lipid-soluble antioxidant, α-tocopherol. However, low rates of inhibition led to increase in cell division. The mechanism is still controversial and under study [6].

In vitro cell-based cytotoxicity assay can be said to be useful, reliable and rapid method for demonstrating chemotherapeutic drug activity [3].

Tumor cells are seen to utilize leukocytes for their extravasation as linkers to the endothelium [7]. Macrophage Migration Inhibitory Factor (MIF) is an overexpressed inflammatory cytokine in many types of cancer, correlating with tumor aggressiveness and poor patient outcomes. It was discovered that depletion of MIF in the 4T1 model results in delayed tumor growth and impaired metastasis. MIF-expressing tumor cells showed decreased markers of immunogenic cell death. Thus, MIF expression in the primary tumor dampens the anti-tumor immune response, promoting tumor growth [8].

CAM assays have been widely used to study angiogenesis, tumor cell invasion and metastasis. The CAM assay is carried out as a closed system, the half-life of many experimental molecules such as small peptides tends to be much longer in comparison to animal models, allowing experimental study of potential anti-metastatic compounds that are only available in small quantities [9].

Vitis vinifera

Vitis vinifera is a perennial woody, climbing plant that belongs to family Vitaceae. It is commonly known as grape vine. The grapevine is native to Southern Europe and Western Asia[10]. Grapes have been used for winemaking since ancient Greek and Roman civilizations[11]. In recent years, grape seed extract has become increasingly popular on the market as a nutritional supplement [12]. Phenolics of grape seed may help to inhibit enzyme systems that are responsible for production of free radicals and are associated with inflammatory reactions [13].

In vitro, proanthocyanidins have been shown to exhibit strong antioxidant activity and scavenge reactive oxygen and nitrogen species, modulate immune function and platelet activation, and produce vasorelaxation by inducing nitric oxide (NO) release from endothelium[14]. Grape seed extracts have also exhibited anti-tumor properties. Promising results have been shown by several studies performed in human colorectal carcinoma, prostate cancer cells[15], head and neck squamous cell carcinoma[16], skin and breast cancer[17].

Nigella sativa

Nigella sativa is also commonly known as black caraway, black cumin and kalonji is an annual flowering plant [18]. Black cumin has been used as a spice and food preservative for thousands of years. It is a native plant found in south and southwest Asia[19]. Black cumin seeds also contain other compounds in trace amounts. The seeds contain two different types of alkaloids; *i.e.* isoquinoline alkaloids and pyrazole alkaloids. It also contains terpenes (e.g. - α-hederin) and saponins which are found to be a potential anticancer agent[20].

In various studies by the researchers, it has been shown that thymoquinone present in the Nigella seed is involved greatly in anti-cancer activity and therefore, called as an anti-cancer agent. Thymoquinone has been shown to alter genes which control angiogenesis (the growth of new blood vessels), tumor suppression, inflammation and apoptosis (natural cell death). It has also been shown to suppress osteosarcoma, bladder, pancreatic, breast, lung, skin and colon cancers [21].

The anti-cancer properties of N. sativa have been mainly attributed to its ability to exert potent anti-proliferative, pro-apoptotic, anti-oxidant, anti-mutagenic, and anti-metastatic roles. Experimental findings suggest that N. sativa extracts can potentially be employed in the development of effective therapeutic agents that can be employed in the regulation of various stages of tumorigenesis and treatment of many types of cancer[22].

Ocimum basilicum

Ocimum basilicum is commonly known as basil which is native plant of tropical regions. It is one of endemic plant which is used as pharmaceutical plant and also as culinary herb. Basil seeds have been traditionally used for the treatment of dyspepsia, ulcer, diarrhoea and other illness. It has also been used for preparation of beverages, cosmetics, perfumes and food industry. They are a good source of dietary fibres[23-25].

Basil seeds are shown to offer multiple benefits. They are proven to have good anti-microbial, antioxidant and anticancer activities. Bioactivity of *O.basilicum* seeds has projected the great importance of functional foods[26]. They are also used in treatment of acidity, diabetes, constipation, migraine, depression, cholesterol, arthritis and so on. They are shown to improve memory and reduce risk of heart diseases [27].

Thus, the aim of the current study is to compare the antioxidant properties of the seeds of *Vitis vinifera, Nigella sativa and Ocimum basilicum.*

METHODS AND MATERIALS

Sample preparation

10% methanolic, ethyl acetate and aqueous extracts of each seed sample was freshly prepared and was used for the assays.

Phytochemical analysis

Phytochemical examinations were carried out for all the seed extracts as per the standard methods.

Alkaloids were detected by Mayer's test [28], Wagner's test and Dragendroff's test.

Carbohydrates were detected by Molisch's test, Benedict's test and Fehling's test.

Glycosides were detected by Keller-Kiliani test.

Saponins were detected by Foam test [29].

Flavonoids were detected by Lead acetate test [30].

Phenols were detected by Ferric chloride test [31, 32].

Coumarins were detected by adding 10% Sodium Hydroxide to 1mL of the extract making it alkaline. Appearance of blue green fluorescence shows the presence of coumarin glucosides [33].

Fixed oils were detected by adding 0.5N Potassium Hydroxide to the sample extract and heated in a water bath after the addition of few drops of phenolphthalein. Appearance of pink color indicates the presence of fixed oils or fats [31, 32]

Tannins were detected by boiling 1ml of each sample was separately with 10ml distilled water for 5 minutes in a water bath and was filtered hot. 1ml of cool filtrate was diluted to 5ml with distilled water and a few drops of 10% Ferric Chloride were observed for any formation of precipitate and for any color change. A reddish blue black or brownish green precipitate indicated the presence of tannins [34].

Triterpenes and terpenoids were detected by Salkowski's test [31, 32, 35].

Proteins and amino acids were detected by Xanthoproteic test [36].

Anti-hemolytic activity

5mL of blood from a healthy person was collected in EDTA vials and centrifuged for 5 min at 1000 rpm. Supernatant was removed and pellet was washed thrice with PBS (0.2 M, pH 7.4) before re-suspending in saline solution (0.5 %). 0.5 ml of the extract was dispensed to 1 ml of erythrocyte suspension and incubated at room temperature for 20 min. Next add 0.5 ml of H_2O_2 solution made in buffered saline to the reaction mixture for provoking oxidative degradation of the membrane lipids.

Subsequently, the samples were centrifuged at 1000 rpm for 10 min and the absorbance of supernatant was noted spectrophotometrically at 540 nm.

The relative hemolysis was assessed in comparison with the hemolysis using phosphate buffer saline as a control. Each set of experiments was performed in duplicates and inhibitory activity of different fractions was calculated and expressed as percent inhibition of hemolysis.

Hemolysis (%) = (Sample abs/ control abs)*100

Protection (%) = 100 - % Hemolysis

Lipid peroxidation inhibition

Standard solution of MDA (1,1,3,3-tetramethoxypropane) was prepared in distilled water. 1-5mL aliquots of the standard were made up to 5mL with distilled water. 5mL glacial acetic acid and 0.5mL TBA (0.5%) were added and the mixture was incubated in boiling water bath for 45min. After cooling, 0.5mL of 5M HCl was then added to each tube and absorbance was read at 535nm against a suitable blank.

Lipid peroxidation inhibition of the extracts was estimated by using 1.5mL of MDA and 0.5mL of sample in each tube. The volume was made up to 5mL in each case and processed as mentioned above. Percent inhibition of the sample extracts were calculated relative to the control.

Leucocyte migration assay

Whole blood sample was freshly collected from a volunteer and was centrifuged at 1500rpm for 15min.White buffy layer of leucocytes was collected. 1% solution of agar was poured on to glass plates, allowed to solidify and two wells were punched out (opposite to one another) on each plate. Sample extract was loaded into one well and the collected leucocytes were loaded into the other. This creates a chemical gradient for migration of leucocytes. Migrated cells were observed under light microscope after 24hr incubation at 37^{o}C.

MTT assay

MTT assay is a quantitative assay that assesses the metabolic activity of the cells. This assay involves the conversion of yellow tetrazolium MTT to insoluble purple formazan by the enzyme, NAD(P)H-dependent cellular oxidoreductase [37].The formed formazan can be dissolved to yield a purple colour, the intensity of which is directly proportional to the cell number and indicates cell viability.

The liver tissues were weighed (250mg) and cultured in 1x PBS for 24 hrs, at 37^{o}C. After 24 hrs, the culture medium was replaced. The cells were treated with 0.5mL of the sample extracts and incubated for 24hrs at 37^{o}C. The culture medium was again replaced with fresh medium. Subsequently, 20µL of standard MTT (5mg/mL in phosphate buffer) was added to each well and incubated for 4hrs. 0.04N HCl (in isopropanol) was then added to stop the reaction and solubilize the crystals. The viability of the cells were then determined spectrophotometrically at 570nm. Relative cell viability with respect to a viable cell control was calculated as follows:

Cell viability (%) = (abs. sample/ abs control)*100

Chick Chorioallantoic Membrane (CAM) Assay

The CAM assay was used to study antiangiogenic activity of the aqueous extracts of the seed samples according to the method proposed by Ribatti *et al*., 1997 [38] . Fertilized chicken eggs were obtained from a local breeder in Kolar, Karnataka, India. The eggs were checked for damage and were cleaned with 70% ethanol. The eggs were then incubated at 37°C, under constant humidity. The eggs were rotated three times a day.

Extract administration was carried out as per the development of CAM and vitelline veins. The development of CAM and vitelline veins were observed under the light. A small window was made on the narrow end of the egg after 3 days of incubation using sterile scalpel and needle. A combination of sample extracts i.e., GW, BW and NW were used to determine the synergistic effect on angiogenesis.

Sterile gelatin sponges were dipped in 1mL mixture of GW, BW and NW samples. These sponges were then placed inside the egg next to the blood vessels. A sterile adhesive tape was then used to seal the window on the egg. The eggs were then incubated for 3 days under constant humidity at 37°C.

The adhesive tape was removed carefully and the eggs were checked for anti-angiogenic effects, i.e., blood coagulation or breakage in blood vessels.

All the above steps were carried out in the laminar air flow hood.

RESULTS AND DISCUSSION

Phytochemical screening

The qualitative phytochemical analysis of methanol, ethyl acetate and aqueous extracts of Grape, Nigella and Basil seeds showed the results as tabulated in Table 1.

The phytochemical screening of the sample extracts showed the presence of alkaloids, flavonoids, phenols, coumarins, tannins, terpenes, proteins, carbohydrates, cardiac glycosides, saponins, fixed oils, sterols and phytosterols. Each of the experiments were performed in duplicates and the results obtained are represented as + for positive results and – for negative results.

The tests revealed that all the extracts of the seeds showed the presence of alkaloids, flavonoids, phenols, coumarin, tannins, terpenes and proteins. Carbohydrates are present in aqueous extracts of all the seeds and in methanolic extracts of Nigella and Basil seeds. Cardiac glycoside is present in methanolic and ethyl acetate extracts of Grape and Nigella seeds. Triterpenes are present only in GM and GE. Saponin is found to be present in all the extracts of Nigella seeds. Fixed oils are present in all the extracts of the seeds except in ethyl acetate extracts of Grape and

Basil seeds. Sterols and Phyto steroids are present only in methanolic and ethyl acetate extracts of Nigella seeds.

Phytochemical properties aid in detection of secondary metabolites constituents. They are natural compounds produced by plants and show biological significance by playing an essential role in the plants to defend themselves against various pathogenic microbes, predators or competitors. The secretion of these compounds varies from plant to plant. Laboratory studies have shown the evidence that these phytochemicals possess antioxidant activity, which reduces the risk of cancer and various other diseases.

Table 1: Results of qualitative phytochemical screening

Test	GM	GE	GW	NM	NE	NW	BM	BE	BW
Alkaloids									
Mayers	+	+	+	+	+	+	+	+	+
Wagners	+	+	+	+	+	+	+	+	+
Dragendroff	+	+	+	+	+	+	+	+	+
Carbohydrates									
Molisch	-	-	+	+	-	+	+	-	+
Benedict	-	-	-	-	-	+	-	-	-
Fehling	-	-	-	+	-	-	-	-	-
Cardiac Glycoside	+	+	-	+	+	-	-	-	-
Saponin		+	-	+	+	+	-	+	+
Flavonoid	+	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	+	+	+	+	+
Coumarin	+	+	-	+	+	+	+	+	+
Fixed Oils	+	-	+	+	+	+	+	-	+
Tannins	+	+	+	+	+	+	+	+	+
Sterols & Phytosteroids	-	-	-	+	+	-	-	-	-
Salkowski									
Triterpenes	+	+	-	-	-	-	-	-	-
Terpenoids	-	-	+	+	+	+	+	+	+
Protein									
Xanthoproteic	+	+	+	+	+	+	+	+	+

Anti-hemolysis assay

Erythrocytes are considered as the major target for the free radicals due to the presence of both high membrane concentration of polyunsaturated fatty acids (PUFA) and hemoglobin molecules which are redox active oxygen transport molecules and potent promoters of activated oxygen species [39].

The magnitude of hemolysis was appeared to be much more immense, when red blood cells were exposed to any toxicant like hydrogen peroxide [40]. The main purpose of this experiment was to assess whether different extracts of seed samples prevented oxidative damage to the human erythrocyte membrane or not.

The principle of this assay is that H_2O_2 mediated oxidation of lipid in human blood erythrocytes membrane induces membrane damage and eventually leads to hemolysis. It is a peroxyl radical initiator that generates free radicals by its thermal decomposition and will attack erythrocytes to cause the chain oxidation of lipid and protein, disturbing the membrane organization and eventually leading to hemolysis [41].

When red blood cells were treated with extracts along with H2O2, marked reduction in hemolysis was found. Maximum inhibition of hemolysis was exhibited by NE, NW and NM (>90% RBC membrane stabilization). The anithemolytic activity of Basil seed methanolic extract (BM) was significantly higher (95%) than the other solvent extracts.

GM showed the highest percentage (~70%) protection against hemolysis in comparison to the other solvent extracts of the Grape seed.

This high activity may due to the presence of secondary metabolites in these sample seed extracts.

The presence of phenolic compounds in the sample seed extracts was revealed by preliminary phytochemical screening which is strongly correlated with anti-hemolytic activity [42].Since phenolic compounds appear to function as good electron and hydrogen atom donors, they are able to cease radical chain reaction by converting free radicals and reactive oxygen species to more stable products [43]. Therefore, our findings were in agreement with the studies showing that phenolic compounds protect RBCs form oxidative stress or increase their resistance to oxidative damage (Fig 1).

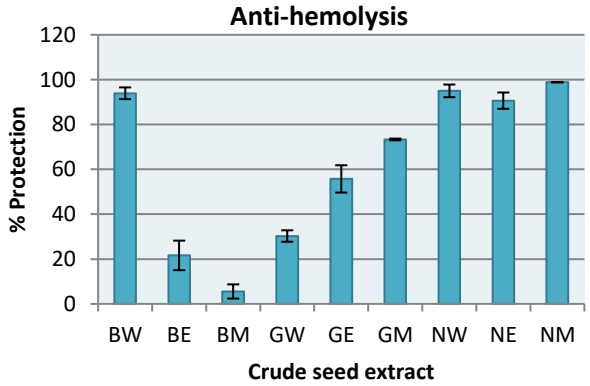

Figure 1: Anti-haemolysis % protection exhibited by various extracts of the seed samples.

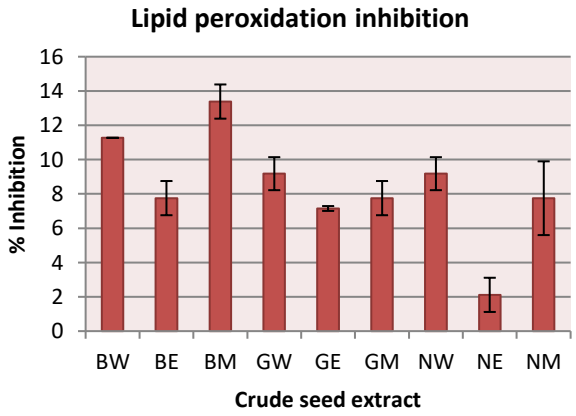

Figure 2: Lipid peroxidation inhibition by seed extracts.

Lipid peroxidation inhibition assay

Lipid peroxidation is the oxidative degradation of lipid cell membranes, resulting in cell damage. The radicals procure electrons from the membrane. It mostly affects polyunsaturated fatty acids[44]. Malondialdehyde (MDA) and 4-Hydroxynonenal (HNE) are generally the final products of polyunsaturated fatty acids peroxidation in the cells. Malondialdehyde level is commonly known as a marker of oxidative stress and the antioxidant status in cancerous patients [45].

Lipid peroxidation products control cell proliferation and induce differentiation, maturation, and apoptosis. Lipid peroxidation and ROS are triggers and mediators of apoptosis, which eliminates precancerous and cancerous, virus-infected and otherwise damaged cells. Lipid peroxidation, also facilitates mitochondrial permeabilization, leading to cell death. This mechanism can be prominently seen in breast cancer and skin cancer[46]. However, at low concentrations of ROS and peroxidation products, cancer cell growth and proliferation can be observed [47].

The crude extracts of seeds showed a low percentage (<15%) of inhibition of lipid peroxidation. NE showed almost no inhibition of lipid peroxidation (Fig 2). This indicates that the samples do not cause significant decrease in oxidative stress and aids in driving the cells to apoptosis.

Leucocyte migration assay

Leukocytes and their soluble mediators play a critical role during primary tumor development and metastasis. Chronic infiltration of tissue by leukocytes, aka, chronic inflammation, is associated with predisposition to cancer[48]. These cells release growth factors, including members of epidermal growth factor, which directly influence the behaviour of tumor cells. They could provide chemotactic cues to promote the egress of carcinoma cells from the tumor [49].

Most carcinomas contain infiltrates of diverse leukocyte subsets including both myeloid- and lymphoid-lineage cells [50], whose complexity and activation status vary depending upon the stage of malignancy[51, 52]. Monocytes and macrophages are a subset of leukocytes that play distinct roles in tissue homeostasis and immunity. Direct communication between macrophages and tumor cells leads to invasion and intravasation[53]. Overall, macrophages contribute to invasion, intravasation, angiogenesis, and extravasation equally [54].

Therefore, inhibition of leucocyte and macrophage movement can restrict the migration and metastasis of carcinoma cells.

Crude seed extracts showed varied effect on cell migration (Fig 3). NE, BM and GE showed maximum inhibition of cellular migration; most cells were seen to be retained at the region of loading. Ethyl acetate extracts of seed showed to have maximal activity. The methanolic extracts-NM and GM, and BW showed minimal inhibition, thereby acting as a chemo attractant for the cells (Fig 4).

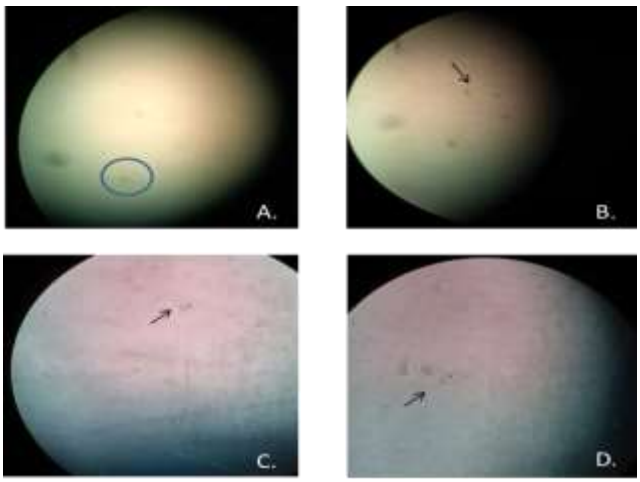

Figure 3: Leucocytes as observed under the microscope. A,B-> show the migrating cells and macrophages. They are seen to have an amoeboid shape. C,D ->cells after the completion of migration, after 24hr incubation.

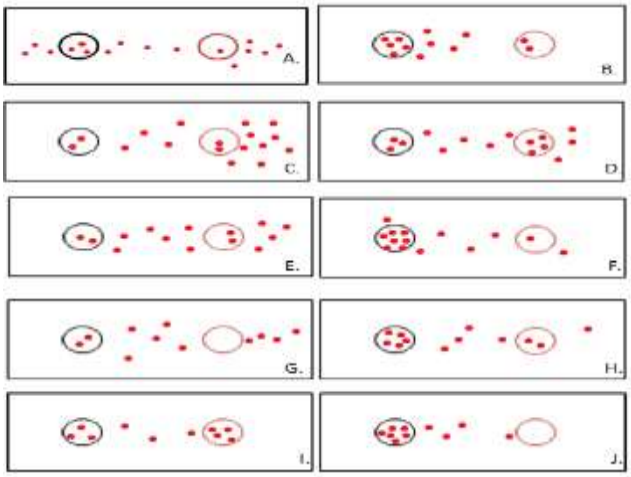

Figure 4: Representation of leucocyte migration on the gel matrix. Black circle represents the well containing leucocytes and brown circle is the well containing crude seed extract (A -> J: control {PBS}, GW, BW, NW, GM, BM, NM, GE, BE, NE). The migrated leucocytes are indicated by red dots

MTT assay

MTT assay is a cell viability assay used for the assessment of metabolic activity of the cell, cytotoxicity and cytostatic activity. Cytotoxic potential of different extracts of the samples was studied and compared[55]. The liver tissue of Gallus gallus domesticus was treated with the crude seed extracts and formation of violet-coloured formazan crystals were observed and compared with the control.

Cells with active metabolism are viable and have the ability to convert MTT to formazan. The non-viable cells lose their ability to cause the conversion. Hence, this assay serves as marker to detect cell viability. The MTT assay is one of the methods used to predict the drug response in malignancies[56].

A decrease in absorbance at 540nm was observed in all cases, indicating that the crude seed extracts induced cytotoxicity. NM treated cells showed a comparatively higher absorbance, indicating that NM maintains mitochondrial activity and cell viability to a large extent. The other extracts decreased the cell viability by more than 60%. GE extract showed the greatest cytotoxicity, making the cells almost non-viable. Thus, the crude seed extracts act as a potential chemotherapeutic agents (Fig 5).

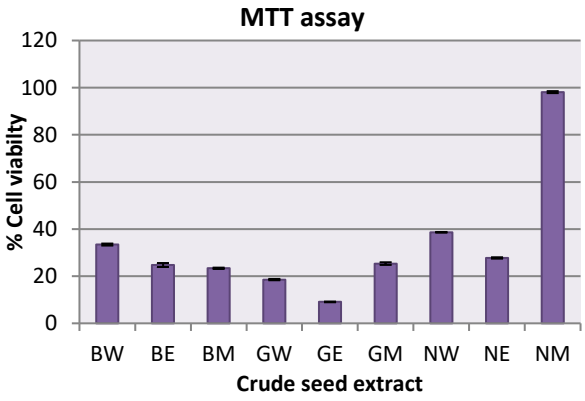

Figure 5: Ability of various crude seed extracts to preserve cell viability

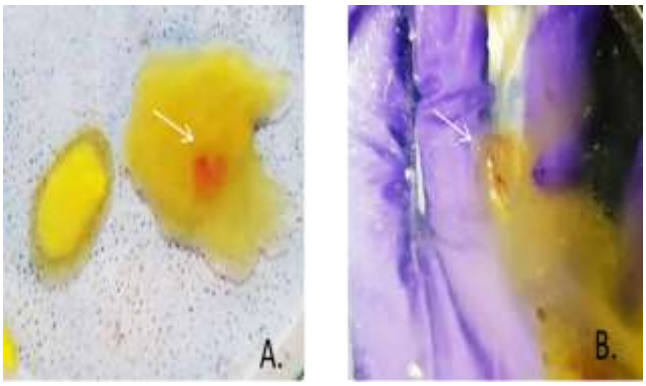

Figure 6: blood vessels of the chick embryo observed after the incubation period. A: control- showed intact vasculature. B: treated- coagulation and degeneration of blood vessels were observed.

Chick chorioallantoic membrane assay

Angiogenesis is an important step in the tissue expansion which when get enhanced leads to the formation of tumors and haematological malignancies[57]. Anti-angiogenic activity is considered to be a promising approach for treatment of cancer[58]. Angiogenesis is an important factor in the biology of solid neoplasms. Solid tumors display full malignant potential only after they have elicited new vascular channels from the host for their own use. If this vascular growth is prevented, then small tumor populations tend to remain dormant[59].

For the assay, aqueous extracts of seeds were used to determine their synergistic effect on angiogenesis. Expected endpoints included reduction in number of blood vessels, mild haemorrhage, vasoconstriction, dilation or disintegration of vessels.

After the period of incubation, it was observed that the eggs treated with the seed extracts showed coagulation and disintegration of blood vessels. The control showed no significant change in the morphology of the blood vessels (Fig 6). This indicated that the aqueous extracts of seed, i.e., BW, GW and NW work in a synergistic manner to prevent the proliferation of blood vessels, thereby exhibiting a good anti- angiogenetic property.

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REFERENCES

1. Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell, 144, 2011, 646–674.
2. Anand P, Kunnumakkara A.B, Sundaram, C, Harikumar K.B, Tharakan S.T, Lai O.S, Sung B, Aggarwal B.B: Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical research, 25(9), 2008, 2097-2116.
3. Florento L, Matias R, Tuaño E, Santiago K, Dela Cruz F and Tuazon A: Comparison of Cytotoxic Activity of Anticancer Drugs against Various Human Tumor Cell Lines Using In Vitro Cell-Based Approach. International journal of biomedical science, 8(1), 2012, 76-80.
4. Karou SD, Tchacondo T, Ouattara L, Anani K, Savadogo A: Antimicrobial, anti-plasmodial, haemolytic and antioxidant activities of crude extracts from three selected Togolese medicinal plants. Asian Pacific Journal of Tropical Medicine, 4(10), 2011, 808-813.
5. Adwaid Manu K, Ambili Narikot, Lokesh Ravi and Sarveswari S: Anti-haemolytic and Antioxidant Activity of Piper Longum Seed. Advances in Biotechnology & Microbiology, 4(4), 2017, 555642.
6. Kevin H. Cheeseman., Graham W. Burton, Keith U. Ingold and Trevor F. Slate: Lipid Peroxidation and Lipid Antioxidants in Normal and Tumor Cells. Toxicologic Pathology, 12(3), 1987.
7. Strell C and Entschladen F: Extravasation of leukocytes in comparison to tumor cells. Cell communication and signaling 6: 10, 2008. doi:10.1186/1478-811X-6-10
8. Balogh K. N, Templeton D. J and Cross J. V: Macrophage Migration Inhibitory Factor protects cancer cells from immunogenic cell death and impairs anti-tumor immune responses. PloS one; *13*(6), 2018, e0197702.
9. Noor A Lokman, Elder A.S, Ricciardelli C and Oehler M.K: (2012) Chick chorioallantoic membrane (CAM) assay as an *in vivo* model to study the effect of newly identified molecules on ovarian cancer invasion and metastasis. International journal of molecular sciences, 13(8), 2012, 9959-70.
10. Jean-Frédéric Terral ,Elidie Tabard, Laurent Bouby, Sarah Ivorra, Thierry Pastor, Isabel Figueiral, Sandrine Picq, Jean-Baptiste Chevance, Cécile Jung, Laurent Fabre, Christophe Tardy, Michel Compan, Roberto Bacilieri, Thierry Lacombe and Patrice This: Evolution and history of grapevine (*Vitis vinifera*) under domestication: New morphometric perspectives to understand seed domestication syndrome and reveal origins of ancient European cultivars, Annals of botany, 105(3), 2010, 443-55.
11. Estreicher SK, Wine. *The Encyclopedia of Ancient History*-Bagnall, R.S., Brodersen, K., Champion, C.B., Erskine, A., Huebner, S.R., Eds.; John Wiley & Sons, Inc.: Oxford, UK, 2013: pp. 1–5. ISBN 9781444338386.
12. Yamakoshi J, Saito M, Kataoka S, Kikuchi, M, Safety evaluation of proanthocyanidin-rich extract from grape seeds. *Food Chemistry Toxicology*, 40, 2002, 599–607.
13. Amella AL, Inhibition of mast cell histamine release by flavonoids and bioflavonoids. *Plant medicine*, 51, 1985, 16-21.

14. Varzakas T, Zakynthinos G, Verpoort F, Plant food residues as a source of nutraceuticals and functional foods. *Foods*, 5, 2016, 88.

15. Kaur M, Singh RP, Gu M, Agarwal R., Agarwal C, Grape seed extract inhibits in vitro and in vivo growth of human colorectal carcinoma cells. *Clinical Cancer Research*, 20, 2006, 6194–6202.

16. Sun Q, Prasad R, Rosenthal E, Kativar SK, Grape seed proanthocyanidins inhibit the invasive potential of head and neck cutaneous squamous cell carcinoma cells by targeting EGFR expression and epithelial-to- mesenchymal transition. *BMC Complementary and Alternative Medicine*., 11, 2011, 134.

17. Kaur M, Agarwal C, Agarwal R, Anticancer and cancer chemopreventive potential of grape seed extract and other grape-based products. *The Journal of nutrition*, 139(9), 2009, 1806S-12S.

18. Hyam R and Pankhurst R.J: Plants and their names: a concise dictionary. Oxford: Oxford University Press, First edition 1995.

19. SKL Karna, Phytochemical Screening and Gas Chromatography - Mass Spectrometry and Analysis of Seed Extract of *Nigella sativa* Linn. International Journal of Chemical Studies, 1(4), 2013, 183-188.

20. Al-Jassir MS, Chemical composition and microflora of black cumin (*Nigella sativa* L.) seeds growing in Saudi Arabia. Food Chemistry, 45, 1992, 239–242.

21. Rahmani AH, Alzohairy MA, Khan MA, Aly SM, Therapeutic Implications of Black Seed and Its Constituent Thymoquinone in the Prevention of Cancer through Inactivation and Activation of Molecular Pathways.2014, PMID: 24959190, DOI: 10.1155/2014/724658.

22. Amin F Majdalawieh, Muneera W. Fayyad, Recent advances on the anti-cancer properties of Nigella sativa, a widely used food additive. *Journal of Ayurveda Integrative Medicine,* 7(3), Jul-Sep 2016, 173–180.

23. M. Munir, A. Qayyum, S. Raza, N.R. Siddiqui, A. Mumtaz, N. Safdar, S. Shible, S. Afzal and S. Bashir: Nutritional assessment of basil seed and its utilization in development of value added beverage. Pakistan Journal of Agricultural Research, 30(3), 2017, 266-271.

24. Naghibi F, M. Mosaddegh, S.M. Motamed and A. Ghorbani: Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. Iranian journal of pharmaceutical Research, 2, 2005, 63–79.

25. S H Hosseini-Parvar, L Matia-Merino, K.K.T. Goh, S.M.A. Razavi and S.A. Mortazavi: Steady shear flow behaviour of gum extracted from basil seed (*Ocimum basilicum* L.): Effect of concentration and temperature. Journal of Food Engineering, 101(3), 2010, 236–243.

26. Anudurga Gajendiran, Vidhya Thangaraman, Suji Thangamani, Dhatchayani Ravi and Jayanthi Abraham: Antimicrobial, Antioxidant and Anticancer Screening of *Ocimum Basilicum* Seeds. Bulletin of Pharmaceutical Research, 6(3), 2016, 114-9.

27. Kelvin Bucktowar, Mili Bucktowar, Luchmee Devi Bholoa: A review on sweet basil seeds: *Ocimum basilicum*. World journal of pharmacy and pharmaceutical sciences, 5(12), 2016, 554-567.

28. Masih N., Singh B, Phytochemical Screening of Some Plants Used in Herbal Based Cosmetic Preparations. Khemani L., Srivastava M., Srivastava S. (eds) Chemistry of Phytopotentials: Health, Energy and Environmental Perspectives. Springer, Berlin, Heidelberg, Chapter-24, 2012, 111-112

29. Devmurari VP, Phytochemical screening study and antibacterial evaluation of *Symplocos racemosa* Roxb. *Scholars Research Library*, 2 (1), 2010, ISSN 0975-508X: 354-359.

30. Harborne JB, Willem CA, The flavonoids: Advances in research science, 1982. ISBN 978-1-4899-2915-0.

31. Sofowora AO, Medicinal Plants and Traditional Medicine in Africa. University of Ife Press, 2nd Ed, 1993, pp 320.

32. Trease GE and Evans WC, Pharmacognosy. 11th Edition, Bailliere Tindall, London, 1989, 45-50.

33. Mojab F, Kamalinejad M, Ghaderi N, Vahidipour HR, Phytochemical screening of some species of Iranian plants. *Iranian journal of pharmaceutical Research*, 2, 2003, 77-82.

34. Harborne JB, Willem CA, (1971) The flavonoids: Advances in research science,1982. ISBN 978-1-4899-2915-0.

35. Vimalkumar CS, Hosagaudar VB, Suja SR, Vilash V, Krishnakumar NM, Latha PG, Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of Olea dioica Roxb., infected with the rust fungus Zaghouania oleae (E.J.Butler) Cummins and non-infected plants. *Journal of Pharmacognosy and Phytochemistry ,* 3(4), 2014, 69-72.

36. Muthuboopathi G, Padmalatha K, Sundar S, Poojitha K and Addala VH, Phytochemical screening study and constipation activity of *Spermacoce hispida L*. (Rubiaceae) by using albino rats. *International Journal of Pharmacological Scienes and Research*, 4(12), 2013, 4669-74. doi: 10.13040/IJPSR. 0975- 8232.4(12).4669-74.

37. Mosmann T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *The Journal of Immunological Methods*, 65, 1983, 55-63

38. Ribatti Domenico, Gualandris Anna, Bastaki Maria, Vacca Angelo, Iurlaro Monica, Roncali Luisa and Presta Marco: New Model for the Study of Angiogenesis and Antiangiogenesis in the Chick Embryo Chorioallantoic Membrane: The Gelatin Sponge/ Chorioallantoic Membrane Assay. Journal of Vascular Research, 34, 1997, 455-63.

39. Shobana S and Vidhya R: Evaluation of in vitro hemolytic activity of different parts of abutilon indicum (linn.). World journal of pharmacy and pharmaceutical sciences, 5(5), 2006, 1182-1196

40. Naim M, Gestetner B, Bondi A, Birk Y, Analysis of Foods and Beverages: Modern Techniques Book, 1976

41. Joshan D S and Rawal S: Comparative evaluation of antioxidant and anti-hemolytic capacities of plants of Indian origin using multiple antioxidant assays. International Journal of Phytopharmacology, 2, 2012, 107-115.

42. Khaili M, Ebrahimzadeh MA and Safdari Y 2014, Antihemolytic activity of thirty herbal extracts in Mouse red blood cells, ArhHig Rada Toksikol, 65, 2014, 399-406.

43. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Eslami B and Rahmani ZP: Antioxidant and antihemolytic activities of the leaves of Kefe cumin (Laser trilobum L) Umbeliiferae. Trop J Pharm Res, 9, 2010, 441-449.

44. Antonio Ayala., Mario F. Muñoz., and Sandro Argüelles., (2004) Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Oxidative Medicine and Cellular Longevity, 6, 2014, DOI: 10.1155/2014/360438.

45. Stefan Gawel, Maria Wardas, Elzbieta Niedworok and Piotr Wardas: Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomości lekarskie (Warsaw, Poland: 1960), 57, 2004, 453-5.

46. Manuela GagoDominguez, J.Esteban Castelao, MalcolmC. Pike, Alex Sevanian and Robert W. Haile: Role of Lipid Peroxidation in the Epidemiology and Prevention of Breast Cancer. Cancer Epidemiol Biomarkers Prev, 14(12), 2005, 2829–39.

47. Barrera G.: Oxidative stress and lipid peroxidation products in cancer progression and therapy. ISRN Oncol. 2012; 2012: 137289. doi: 10.5402/2012/137289.

48. Erez N and Coussens LM: Leukocytes as paracrine regulators of metastasis and determinants of organ-specific colonization. International journal of cancer, 128(11), 2011, 2536-44.

49. Wyckoff J, Wang W, Lin E, Wang Y, Pixley F, Stanley ER and Condeelis J: A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Research 64*(19), 2004, 7022-7029.

50. Thea D. Tlsty and Lisa M. Coussens: Tumor stroma and regulation of cancer development. Annual Review of Pathology: Mechanisms of Disease 1(1), 2006, 119-150.

51. Alberto Mantovani, Paola Allavena, Antonio Sica and Frances Balkwill: Cancer-related inflammation. Nature, 454, 2008, 436–444., doi: 10.1038/nature07205.

52. Ruffell B, Au A, Rugo HS, Esserman LJ, Hwang ES & Coussens LM: Leukocyte composition of human breast cancer. Proceedings of the National Academy of Sciences of the United States of America, 109(8), 2011, 2796-801.

53. John Condeelis and Jeffrey W Pollard: Macrophages: Obligate Partners for Tumor Cell Migration, Invasion, and Metastasis. Cell, 124, 2006, 263-6. 10.1016/j.cell.2006.01.007.

54. Douglas Hanahan and Robert Weinberg: The Hallmarks of Cancer. Cell, 100, 2000, 57-70. 10.1016/S0092-8674(00)81683.

55. Ashutosh Bahuguna, Imran Khan, Vivek K Bajpai and Sun Chul: MTT assay to evaluate the cytotoxic potential of a drug. Bangladesh Journal of Pharmacology, 12, 2017. Doi: 8.10.3329/bjp.v12i2.30892.

56. Tamar Hayon, Alexander Dvilansky, Ofer Shpilberg and Ilana Nathan: Appraisal of the MTT-based Assay as a Useful Tool for Predicting Drug Chemosensitivity in Leukemia. Journal of Leukemia and Lymphoma, 44 (11), 2003, 1957-1962. DOI: 10.1080/1042819031000116607

57. Chen H, Wang CS, Li M, Sanchez E, Li J, Berenson A and Berenson JR:(2010) A novel angiogenesis model for screening anti-angiogenic compounds: The chorioallantoic membrane/feather bud assay. International Journal of Oncology, 37, 2010, 71-79.

58. Moonmun Dhara, Lopamudra Adhikari and Raja Majumder:(2018) Chorioallantoic Membrane (CAM) Assay of Different Extracts of Rhizome and Inflorescence of Heliconia rostrata. Indian Journal of Pharmaceutical Education and Research, 52(4s) [Suppl 2], 2018.

59. Folkman J: Tumor Angiogenesis. Adv. Cancer Res., 19, 1974, 331-358.

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