



EVALUATION OF ANTI-ANGIOGENIC AND ANTIOXIDANT PROPERTIES OF NATURAL EXTRACTS - GARLIC, HONEY AND GREEN TEA

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ABSTRACT

Tumour growth and metastasis are angiogenesis dependent and hence, blocking angiogenesis is one of the strategies to arrest tumour growth. It is being increasingly proposed that reactive oxygen species (ROS) play a key role in human cancer development. This study aimed to explore the anti-angiogenic and anti-oxidant activities of Indian honey, *Allium sativum* (Garlic) and *Camellia sinensis* (Green tea catechin). Antioxidant properties were evaluated by Total phenol content, FRAP and DPPH assays and anti-angiogenesis was detected by *in ovo* Chick Chorioallantoic Membrane (CAM) Assay and was correlated by estimation of haemoglobin by Drabkin's Assay. All the extracts showed good anti-oxidant and anti-angiogenesis properties but among the three, green tea showed better inhibition of angiogenesis which could be correlated to better antioxidant levels.

KEYWORDS- phytochemicals, cancer, CAM, naturopathy.

INTRODUCTION

Cancer has become an important public health problem with over 8, 00,000 new cases occurring every year. At any point of time, it is estimated that there are nearly 2.5 million cases in India with nearly 4,00,000 deaths occurring due to cancer^[1]. One of the important factors responsible for the tumour growth and progression is the process of angiogenesis. Cancer is a multistep process. It starts as an onset from a single transformed cell. Its genesis is characterized by the swift proliferation, invasion, and metastasis^[2] which also requires the sprouting, splitting and remodelling of the existing blood vessels. This dynamic process is activated by various carcinogens, tumour promoters, and inflammatory agents. Hence, blocking angiogenesis is one of the strategies to arrest tumour growth.

Free radicals produced by cells via various enzymatic and non-enzymatic reactions like respiratory chain reaction, oxidative phosphorylation *etc.* initiates autocatalytic reactions, damage different bio molecules^[3] and may help cancer development. Cells exhibit defence system against oxidative damage. This defence system consists of antioxidants or oxidative protective agents such as catalase, superoxide dismutase, peroxidase, ascorbic acid, tocopherol, and polyphenols. Antioxidants acting as free radical scavengers may inhibit the cancer process *in vivo*^[4] and evidence is growing that antioxidants may prevent or delay the onset of some types of cancer^[5]. Endogenous ROS also plays an important role for cancer cells to induce angiogenesis and tumour growth^[6]. The main treatment for cancer is by using chemotherapy and radiotherapy which themselves are toxic to other viable cells of the

body. Recently, there are many studies focusing on the use of natural products for cancer prevention and treatment. Naturopathy or naturopathic medicine is a form of alternative medicine that employs an array of practices branded as "natural", "non-invasive", and as promoting "self-healing". According to Naturopathy, "Food is only the Medicine", no external medications are used^[7].

In the present study the three natural foodstuffs namely Garlic, Green Tea and Honey was evaluated for the potential antioxidant and antiangiogenic properties. Garlic consist of many active organo sulphur compounds (OSC) such as alliin, alliinase, allicin, S-allyl cysteine (SAC), diallyl disulphide (DADS), diallyltrisulphide (DATS) and methylallyl sulphide. Green tea is rich in green tea polyphenols (GTPs). Honey is composed of various sugars, flavonoids, phenolic acids, enzymes, etc. It has been shown to have anti-inflammatory^[8], antimicrobial^[9], antimutagenic^[10], antioxidant^[11], and antitumor^[12] effects.

MATERIALS & METHODS

1. Sample preparation

▪ **GARLIC-** Whole garlic was procured from local organic farms. For assay purposes, 2g of macerated garlic was weighed and added to 10ml of sterile distilled water. The tubes were plugged and incubated at room temperature. After 3 days, the mixture was filtered and the resulting garlic extract solution was diluted with distilled water to obtain various concentrations. Garlic extract was evaluated at 5 different concentrations *viz.*, 20,40,60,80 and 100mg/ml for all the assays.

- **HONEY**- Pure honey was obtained from forest in Sahyadri Hills, Khopoli. A stock of 5% honey was made from the crude sample and was used for various assays. Honey was evaluated at 5 different concentrations viz., 1,2,3,4 and 5% v/v for all the assays.
- **GREEN TEA**- Organic green tea leaves were obtained from Wayanad District of Kerala. A decoction of green tea leaves was made by weighing 10g of green tea leaves in 100ml of sterile distilled water. From this stock solution, various concentrations viz., 0.5, 1.0, 1.5, 2.0 and 2.5mg/ml were prepared and used for evaluation of green tea extract in all assays.

2. Phytochemical screening^{13,14}

Phytochemical screening of all three natural extracts was carried out by known standard qualitative tests to detect the presence of different phytochemicals like flavonoids, phenols, glycosides, tannins, terpenoids and saponins.

3. Total phenolic content¹⁵

The total phenolic content was determined by using the Folin-Ciocalteu assay. An aliquot of 1 ml of extracts or standard solution of Gallic acid (100, 200, 300, 400, and 500µg/ml) was added to 25 ml volumetric flask containing 9 ml of distilled water. Reagent blank using distilled water was prepared. 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes, 10 ml of 7% Na₂CO₃ solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV-Visible spectrophotometer. Total phenolics content was expressed as mg Gallic acid Equivalents (GAE) using the standard graph.

4. Estimation of antioxidant activity

- **FRAP ASSAY**¹⁶- An aliquot of 0.5 ml of different concentrations of the extract samples were added to 0.5 ml of 1% potassium ferricyanide [K₃Fe(CN)₆] solution. The reaction mixture was then incubated at 50°C for 20 min in boiling water bath. At the end of the incubation, 0.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. 0.5 ml of the supernatant was mixed with 0.5 ml of deionised water and 0.5 ml of 0.1% ferric chloride. The colored

solution was read at 700 nm against the blank with reference to standard using UV Spectrophotometer. Here, ascorbic acid was used as a reference standard. The reducing power of the samples was expressed as mg of ascorbic acid equivalence using standard graph.

- **DPPH ASSAY**¹⁷-The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of natural extract solution of varying concentrations. Corresponding blank sample were prepared and L-Ascorbic acid (10-70 µg/ml) was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control. The decrease in absorbance was measured at 517nm after 30 minutes incubation in dark using UV-Vis spectrophotometer. The % Radical scavenging activity was calculated using the following formula. Inhibition % = $\frac{Ac-As}{Ac} \times 100$

Where Ac = absorbance of the control,

As = absorbance of the sample.

The radical scavenging capacity of the sample was comparable with the reference standard.

5. Estimation of anti angiogenic activity

CAM ASSAY¹- Chick Chorio-allantoic membrane assay was performed using 3 day old embryonated chicken eggs that were obtained from a local poultry farm. After candling, 200µl of different concentrations of samples and positive control (1% SDS) were inoculated under sterile condition and the eggs were then incubated at 37°C for 48 hrs. After incubation, CAM sections were dissected & were observed for the pattern of vasculature, growth of secondary blood vessels.

6. Estimation of haemoglobin content

Drabkin's assay^{1, 18} - The dissected CAM sections were homogenized in 5ml of chilled normal saline and centrifuged at 7000 rpm for 30 minutes. 10 ml of Drabkin's reagent was added to 3ml of supernatant. Reaction mixture was incubated at room temperature for 20 minutes and absorbance was measured at 540 nm on UV spectrophotometer. All above assays were performed in triplicates in order to ensure reproducibility of the result.

RESULTS

TABLE 1:- Phytochemical analysis

1. Phytochemical screening

Sr. no.	Phytochemicals	Garlic	Green tea	Honey
1.	Flavanoids	-	+	+
2.	Terpenoids	-	-	-
3.	Saponins	+	+	+
4.	Glycosides	+	+	+
5.	Phenolics	-	+	+
6.	Tannins	-	+	-

Key: + present - absent

1. Total Phenolic Content

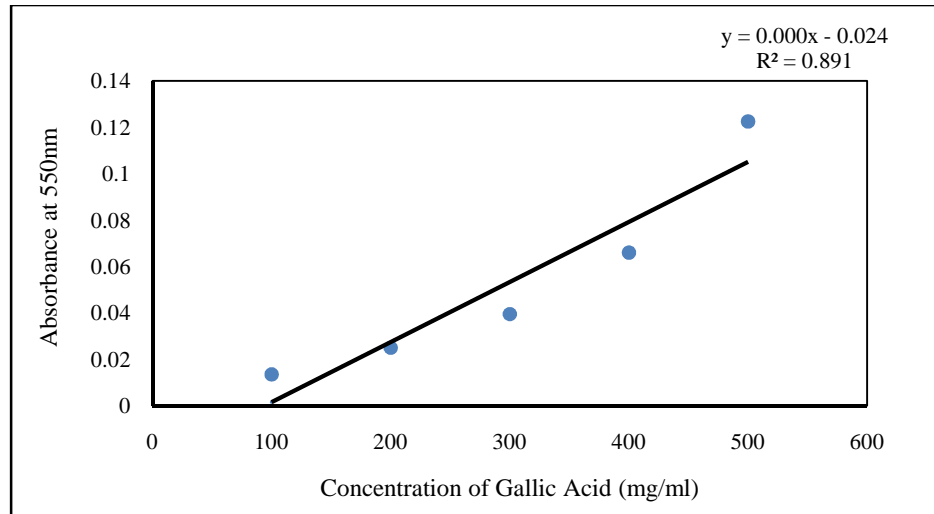


FIGURE 1A :- Standard Gallic Acid Graph

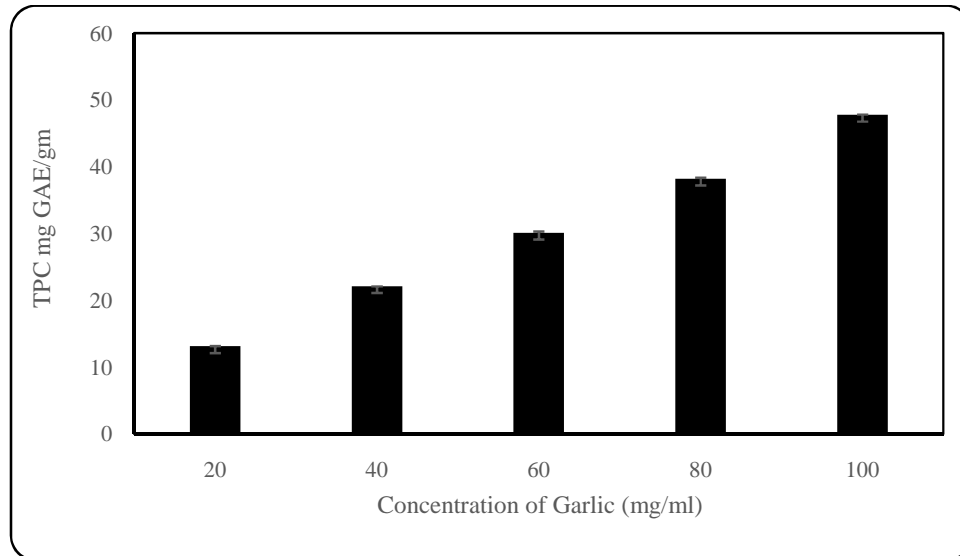


FIGURE 1B :- Total Phenolic Content of Garlic

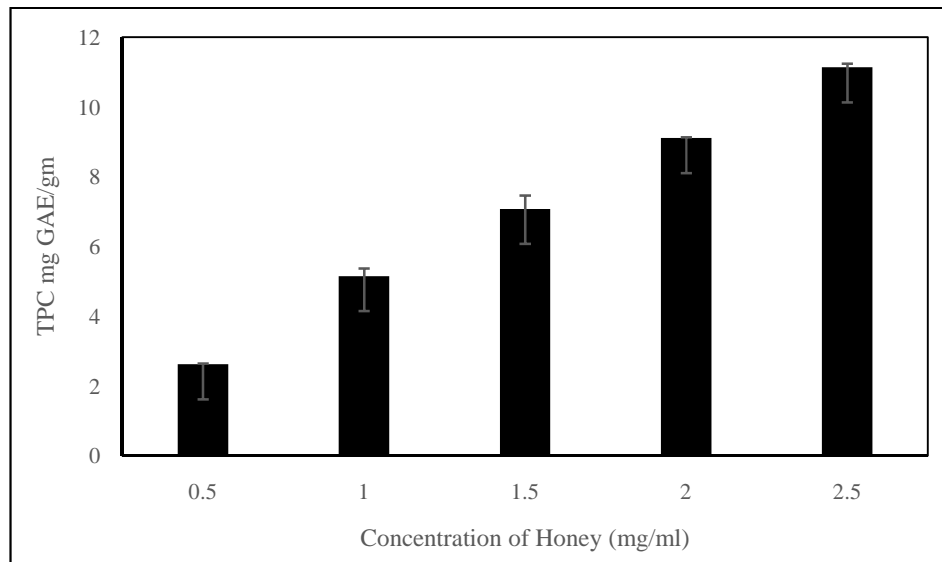


FIGURE 1C: Total Phenolic Content of Green tea

Antiangiogenic potential of three natural extracts

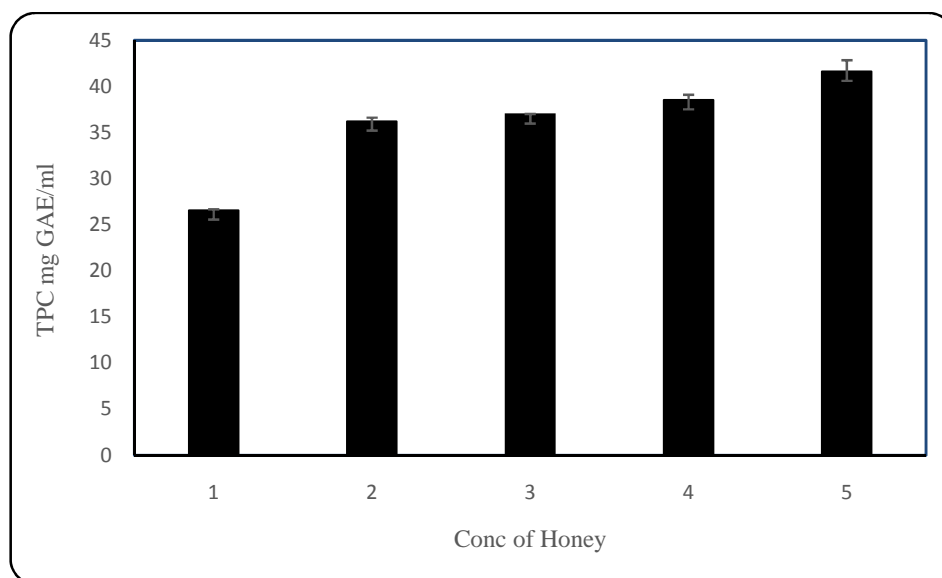


FIGURE 1D : Total Phenolic Content of Honey

The Total Phenolic content of three natural extracts was estimated by Folin Ciocalteu method. Gallic acid was used as a standard for the Folin Ciocalteu assay (Fig.1). The total phenolic content was calculated for the three extracts and a graph was plotted as concentration of extracts v/s TPC as Gallic Acid Equivalence (GAE) mg/ml. From the graph plotted, it can be seen that all the three natural extracts viz. honey, garlic, and green tea showed the presence of phenolic compounds and as it can

be clearly observed that as conc. of the extracts was increased there was gradual increase in the total phenolic content of the extracts. As compared to honey and garlic, green tea showed the presence of phenolic compounds even at lesser concentrations. Phenolic compounds and its derivatives are responsible for the functional and medicinal properties of honey, garlic and green tea. Hence, the total phenolic content in general reflects the therapeutic importance of honey, garlic and green tea.

2. Ferric Reducing Anti-Oxidant Power Assay (FRAP)

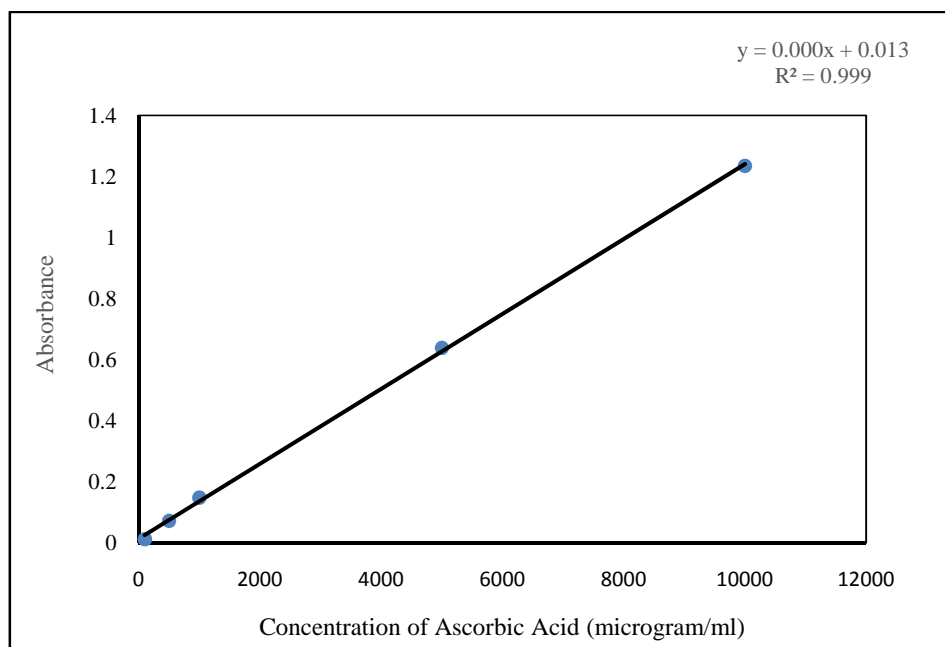


FIGURE 2A :- Standard Ascorbic Acid Graph

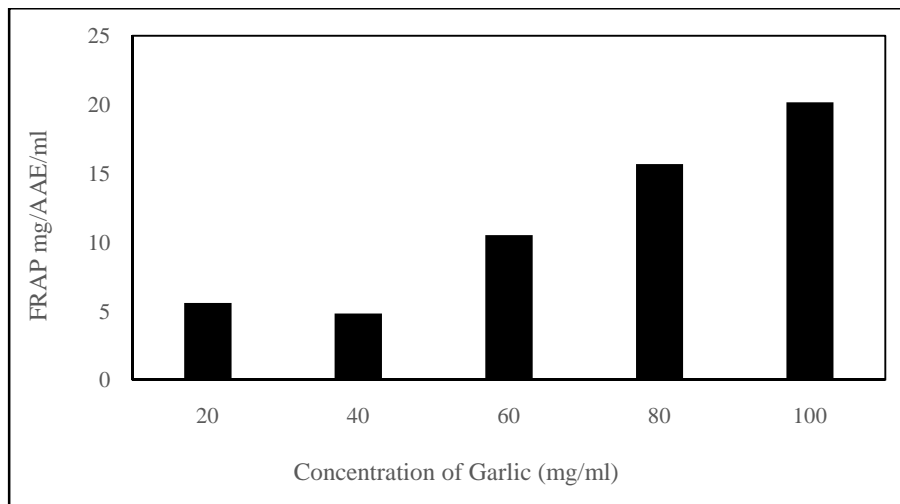


FIGURE 2B :- Ferric Reducing Activity of Garlic

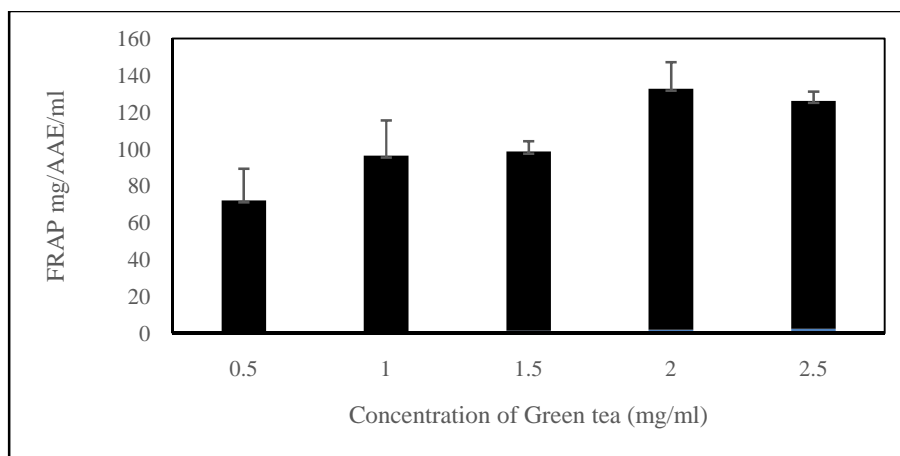


FIGURE 2C :- Ferric Reducing Activity of Green tea

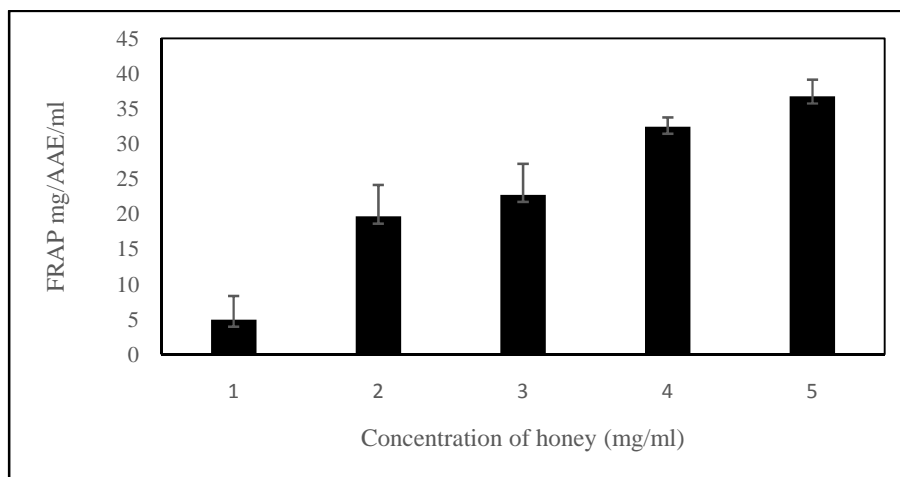


FIGURE 2D :- Ferric Reducing Activity of Honey

Ferric Reducing Antioxidant Power (FRAP) assay was performed for the three natural extracts. Ascorbic acid was used as standard for the FRAP assay (fig. 2). A graph was plotted using different concentrations of the extracts V/S the FRAP as Ascorbic Acid Equivalence (AAE). As it can be clearly observed from the graph below that all the three extracts showed the presence of ferric reducing antioxidant

activity. Green Tea as compared to honey and garlic showed the presence of maximum antioxidant activity at even lower concentrations than that of honey and garlic. Also all the three extracts showed gradual increase in the antioxidant activity as there was increase in the concentration of the extracts.

3. DPPH Assay:

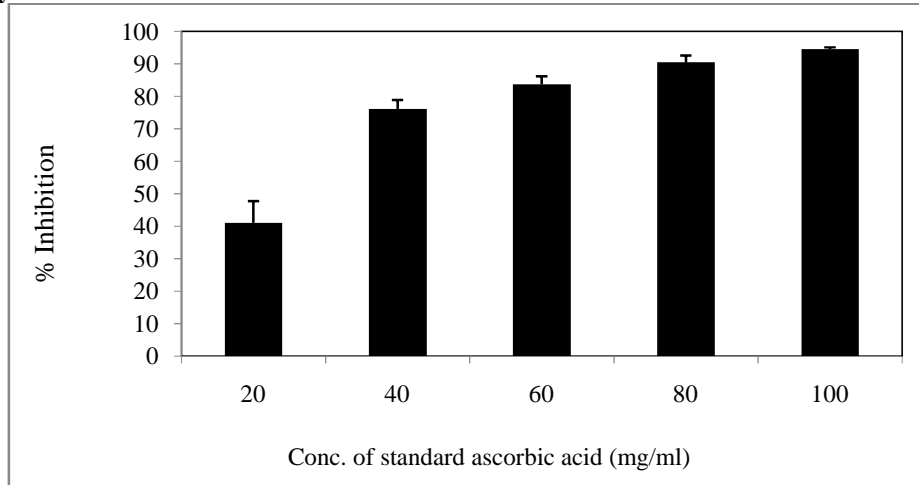


FIGURE 3A:- Standard Ascorbic Acid Graph

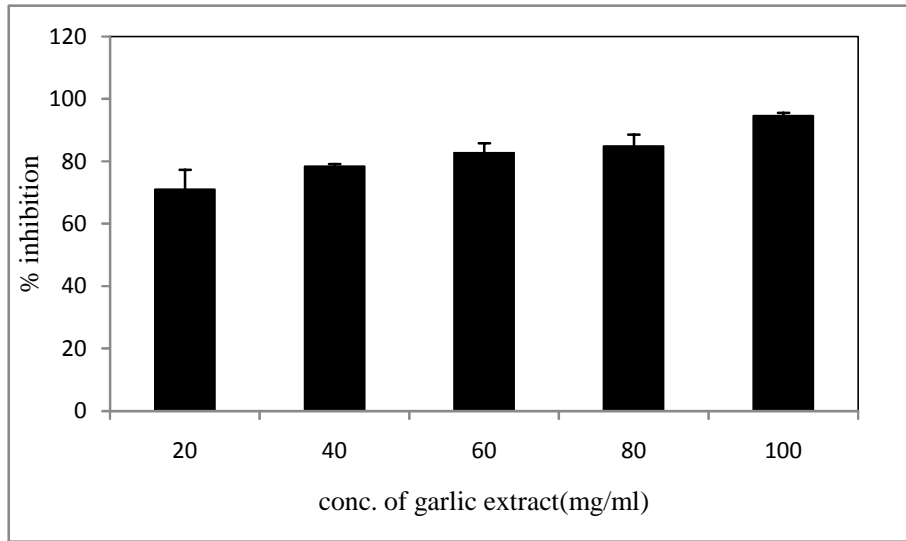


FIGURE 3B:- DPPH Scavenging Activity of Garlic

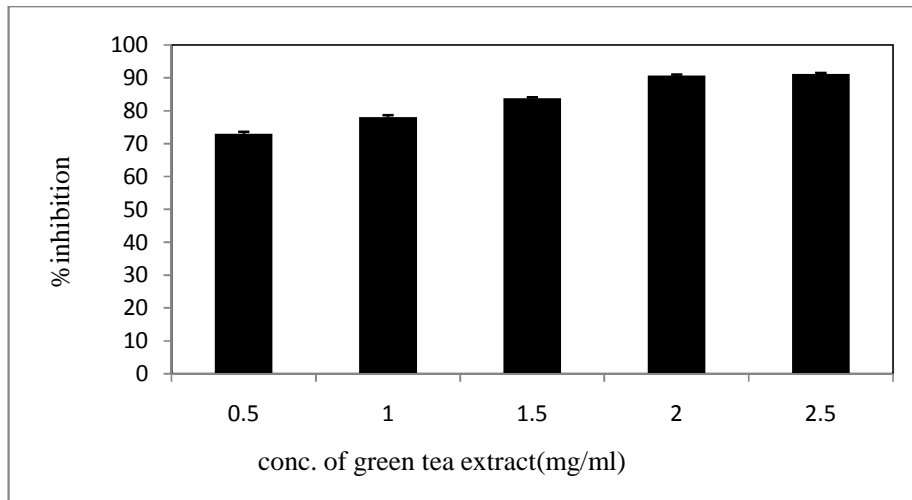


FIGURE 3C :- DPPH Scavenging Activity of Green tea

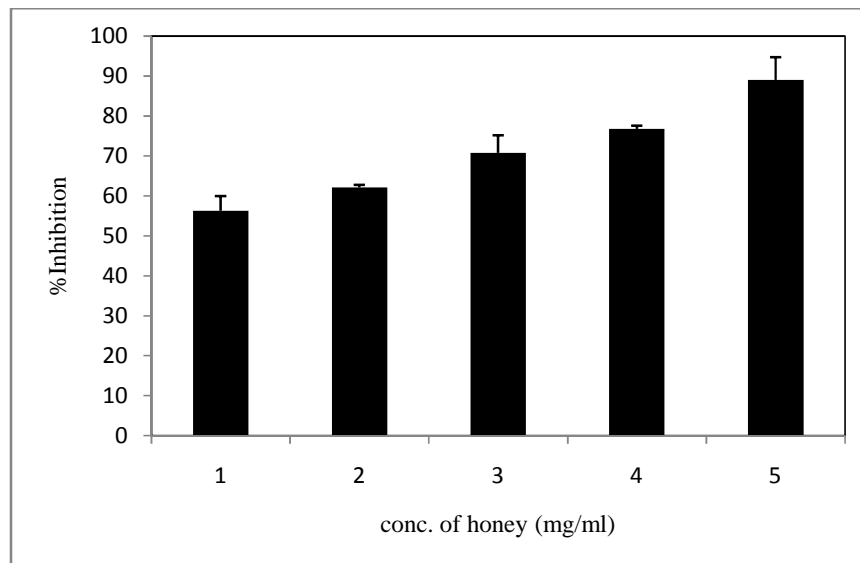


FIGURE 3D:- DPPH Scavenging Activity of Honey

Ascorbic Acid was used as standard for DPPH assay (fig. 3). Fig. 3B, 3C and 3D shows the dose dependent increase in antioxidant activity of all three extracts of garlic, green tea and honey respectively. Potent anti-oxidant activity was shown by green tea sample even at lower

concentration as compared to garlic and honey. The antioxidant potential of a molecule is a reflection of the amount of phytochemicals and phenolic compounds present in it.

4. Chorioallantoic Membrane Assay (CAM Assay):



FIGURE 4A CONTROL

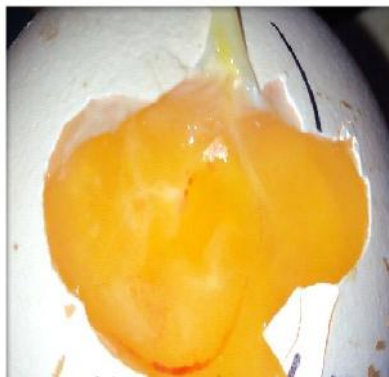


FIGURE 4B. Garlic Und.
(Und- Undiluted)



FIGURE 4C. Honey Und



FIGURE 4D. Green tea Und.

GREEN TEA CAM DILUTED:

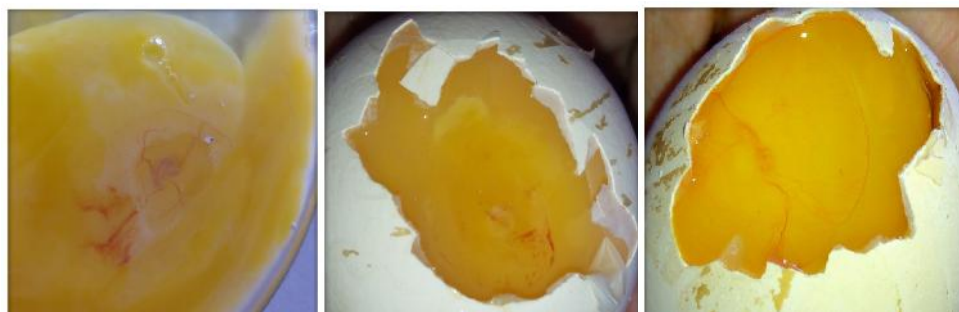


FIGURE 4E. 0.5 mg/ml

FIGURE 4F. 1.5 mg/ml

FIGURE 4G. 2.5 mg/ml

As shown in Fig. 4B, the vasculature of the CAM sections after treatment with the three samples in the varying concentration and also of the undiluted extracts indicates decrease in the branching blood vessels and also fragmentation of blood vessels as compared to the control (Fig 4A). Comparing the CAM sections of the three

undiluted samples, green tea showed maximum damage of the blood vessels compared to garlic and honey.

Fig. 4 E, F and G, demonstrates that treatment with increasing concentrations of green tea shows decrease in total number of sprouts on blood vessels in dose dependent manner.

5. Drabkins Assay:

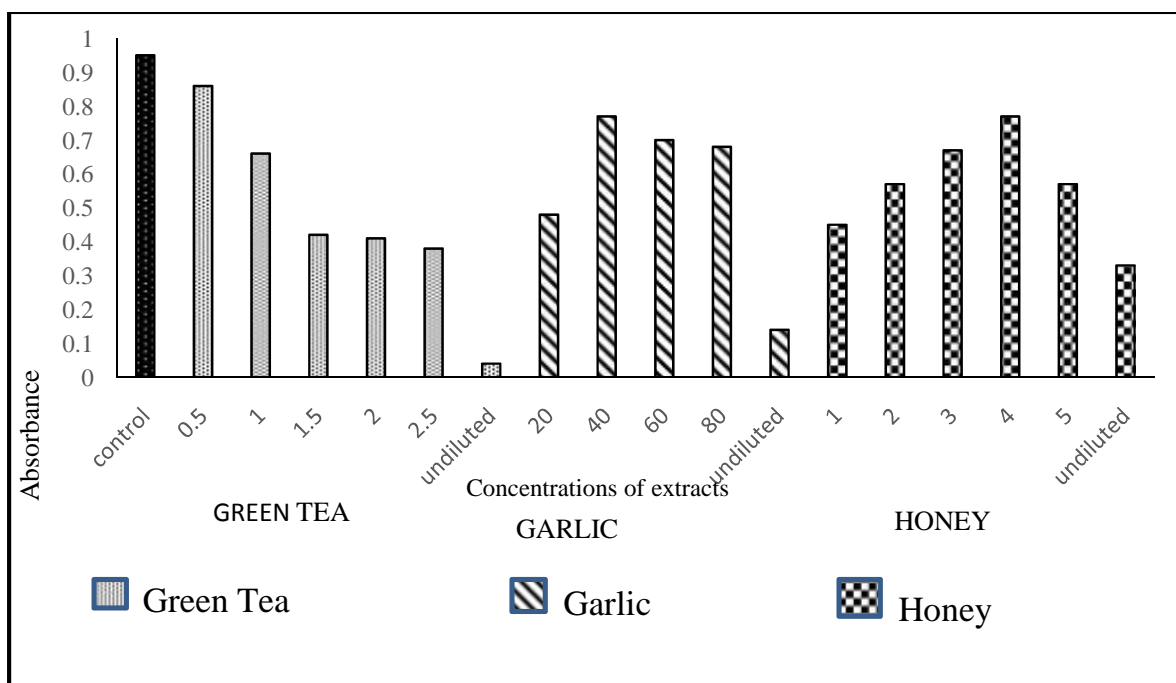


FIGURE 5: Drabkin's Assay Result Of Garlic , Green Tea , Honey.

In order to find out haemoglobin content of the CAM sections treated with the three extracts drabkin's assay was performed. It was carried out to confirm the dose dependent anti-angiogenic behaviour of the three natural extracts in the CAM assay. As shown in Fig. 10, dose dependent decrease was observed in haemoglobin content of CAM sections treated with increasing concentrations of the three extracts which confirms the results of CAM assay.

DISCUSSION

Garlic

Garlic cloves used in this study were obtained from the local market. Garlic extract prepared for the analysis

purpose was done using distilled water so as to preserve the active component allicin^[2]. Allicin and other thiosulfinates are somewhat unstable, but dilution and dissolving in water can greatly improve their stability.

For processing and phytochemical screening of the extract, the reagents and solvents used in this study were of analytical grade. Among the various phytoconstituents that were tested for, garlic extract showed the presence of saponins and glycosides. Though this is contrary to the findings^[3], the difference could be attributed to the raw material sourcing and processing involved in extraction. Garlic extract, having been determined by DPPH assay, showed good anti oxidant activity and the results are in keeping with other studies^[2]. Further, FRAP assay was

carried out that confirmed antioxidant potential of the extract. Total phenolic content of garlic extract was determined as well using standard methods.

Anti angiogenic activity of garlic extract was determined using a CAM model—the extract showed inhibition of angiogenesis at all concentrations tested. These findings are consistent with the study done by Namrata H. Bhogani *et al* (2013)^[4] who found that allicin inhibits FGF2 and VEGF induced angiogenesis in a dose dependent manner in chick chorioallantoic membrane. Aged garlic extract could prevent tumour formation by inhibiting angiogenesis through the suppression of endothelial cell motility, proliferation, and the tube formation and thus acts as a good chemopreventive agent for colorectal cancer when consumed on a daily basis^[19].

Green tea

Green Tea leaves used in this study were organic so as to prevent any interference that could arise from additives that are present in commercially marketed tea leaves. Green tea extract was qualitatively analysed for its phytochemical constituents using standardised methods. Presence of flavonoids, saponins, glycosides, phenolics and tannins were determined and these results are consistent with the findings of other investigators^[20, 21].

Anti oxidant potential of green tea was determined using DPPH assay and FRAP assay. The extract showed high antioxidant potential at all concentrations tested. Also, the total phenolic content was estimated as well. The flavonoid and phenolic compound presence might have contributed in high free radical scavenging activity of GT extracts. Highly positive and significant relationship of phenolics and flavonoids with antioxidant potential of GT has been reported^[21].

For estimating the anti angiogenic potential of green tea extract, CAM assay was carried out. The extract showed maximal distortion of blood vessels which indicated its potent inhibition of angiogenesis. The CAM assay was supplemented by Drabkins assay to estimate the haemoglobin content—the assay also revealed lowered haemoglobin content that relates to the higher antiangiogenic effect of green tea. These results are in correlation with the findings of Namrata H. Bhogani *et al.* (2013)^[4] who demonstrated that Epigallocatechin gallate (EGCG) from green tea has powerful anti-angiogenic properties as EGCG significantly inhibited the development of experimental endometriosis through anti-angiogenic effects.

Honey

Pure, unbranded honey obtained from local sources was used as study sample. The raw honey was diluted using distilled water and the dilutions were further used for all analysis. Phytochemical analysis revealed presence of flavonoids, saponins, glycosides and phenolics. The findings are in keeping with the study of S. Bhuvaneshwari *et al.* (2014) who demonstrated that unbranded honey had higher amount of phytoconstituents than branded honey^[5]. The present study also found that honey has good antioxidant potential by two assays- DPPH and FRAP assay as reported by workers^[22, 23]. The antioxidant potential had positive correlation with concentration. Total phenolic content of honey was estimated using Folin – Ciocalteu method, and it was found that honey showed

dose dependent increase in phenolic content. Phenolic compounds and its derivatives are responsible for the functional and medicinal properties of honey and hence, the total phenolic content in general reflects the therapeutic importance of honey^[11].

The anti angiogenic activity of honey was analysed by the CAM model. Honey showed distortion and inhibition of blood vessel growth at higher concentrations thus indicating that honey has significant antiangiogenic potential in concentrated raw form. This was further confirmed by carrying out Drabkins assay which showed dose dependent decrease in haemoglobin content of CAM sections treated with increasing concentrations of honey samples. The antiproliferative, antitumor, antimetastatic and anticancer effects of honey are mediated via diverse mechanisms, including cell cycle arrest, activation of mitochondrial pathway, induction of mitochondrial outer membrane permeabilization, induction of apoptosis, modulation of oxidative stress, amelioration of inflammation, modulation of insulin signaling, and inhibition of angiogenesis in cancer cells^[6]. Thus by integrating honey in daily diet, its protective action against cancer can be exploited.

CONCLUSION:

All three extracts garlic, green tea and honey showed a very good dose dependent antioxidant activity and anti-angiogenic potential. Among all the extracts, Green tea exhibited higher anti-oxidant properties at lower concentration. This helped it to better inhibit the angiogenesis process in chorioallantoic membrane of the embryonated chicken eggs. The CAM assay was supplemented with Drabkin's assay. Herein, Green tea showed good decrease in the haemoglobin content with decrease in concentration of the extracts. Further, the active ingredients from these extracts could be isolated and their targets in inhibiting the angiogenesis could be identified. The combined effect of these extracts could also be evaluated.

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REFERENCES

- [1]. Naik, A.A., Juwekar, O.D., Ansulkar, P.V., Deshpande, S.D. and Chowdhary, A.S. (2015) Evaluation of the Anti-Angiogenic and Antioxidant activity of Indian Honeys in Comparison with Manuka Honey from New Zealand, *Int J Pharm Sci Res.* 6(4): 1813-18.
- [2]. Rahman, M.M., Fazlic, V. & Saad, N.W. (2012) Antioxidant properties of raw garlic (*Allium sativum*) extract, *International Food Research Journal* 2012, 19(2): 589-591.
- [3]. Divya, B.J., Suman, B., Venkataswamy, M., Thyagaraju, K. (2017) A Study On Phytochemicals, Functional Groups And Mineral Composition of

- Allium Sativum* (Garlic) Cloves , International Journal Of Current Pharmaceutical Research 2017 Issn- 0975-7066 Vol 9 (3):42-45.
- [4]. Namrata H. Boghani, Meonis A. Pithawala, Bhakta, C.G. Anti-angiogenic property of some plants, ISSN : 0974 – 7508, Vol.9(7): 294-297.
- [5]. Bhuvaneswari, S., Ashwin Karthick, N., Deepa, S. Hima Aishwarya and UdayaPrakash, N.K. (2014) Quality Analysis of Phytocomposition of Branded and Unbranded Honey procured from the Markets of Chennai, India, International Journal of Chem Tech Research CODEN (USA) 2014: IJCRGG ISSN : 0974-4290 Vol.6 (9):4070-4077
- [6]. Omotayo, O. Erejuwa, Siti, A. Sulaiman and Mohd, S. Ab Wahab *Molecules* 2014, 19, 2497-2522; doi:10.3390 molecules19022497. Effects of Honey and Its Mechanisms of Action on the Development and Progression of Cancer Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
- [7]. Matsuura, N, Miyamae, Y., Yamane, K., Nagao, Y., Hamada, Y., Kawaguchi N., Katsuki T., Hirata K., Sumi, S., Ishikawa, H. (2006) Aged garlic extract inhibits angiogenesis and proliferation of colorectal carcinoma cells. *Journal of Nutrition* Vol. 136(3):842S-846S.
- [8]. Govindappa, M., Naga Sravya, S., Poojashri, M.N., Sadananda T.S. and Chandrappa C.P., Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedeliatrilobata* (L.) Hitchc, *Journal of Pharmacognosy and Phytotherapy*, 3(3), 2011, 43-51
- [9]. Haque, N., Salma, U., Nurunnabi, T.R., Haque, A.K., Mukti, I.J., Pervin, S., Nahar, R. (2010) Lifestyle related causes of cancer and chemoprevention through phytonutrients, *Pak J Biol Sci*, 13(19): 916-26.
- [10]. Jose Miguel, Alvarez Suarez, Sara Tulipani, Stefania Romandini, Enrico Bertoli, Maurizio Battino, Contribution of honey in nutrition and human health: a review, *Mediterr J Nutr Metab*, 3, 2010, 15–23.
- [11]. Hamzaoglu, I., Saribeyoglu, K., Durak, H. (2000) Protective covering of surgical wounds with honey impedes tumour implantation, *Arch Surg*, 135–142.
- [12]. Xia, C., Meng, Q., Liu, L.Z., Rojanasakul, Y., Wang, X.R., Jiang, B.H. (2007) Reactive Oxygen Species Regulate Angiogenesis and Tumour Growth through Vascular Endothelial Growth Factor (2007). *Cancer Research*, Volume: 67, Issue: 22, 10823–30.
- [13]. Egwaikhide, P.A. and Gimba, C.E. (2007) Analysis of the Phytochemical Content and Anti-microbial Activity of *Plectranthusglandulosus* Whole Plant, *Middle-East Journal of Scientific Research*, 2 (3-4):135-138.
- [14]. Edeoga, H.O. Okwu, D.E. & B.O Mbaebie (2005) Phytochemical constituents of some Nigerian medicinal plants, *African Journal of Biotechnology*, 4 (7):685- 688.
- [15]. Biju John, Sulaiman, C.T., Satheesh George & Reddy, V.R.K. (2014) Total Phenolics And Flavonoids In Selected Medicinal Plants From Kerala, *International Journal of Pharmacy And Pharmaceutical Sciences* , 2014, Issn- 0975-1491 Vol 6(1):23-27.
- [16]. Vijaylaxmi M., Ruckmani K. Ferric Reducing Antioxidant Power in Plant Extracts. *Bangladesh Journal of Pharmacology*. 2016; Volume: 11, 570-572.
- [17]. Rajani Kanta Sahu, Manoranjan Kar, Rasmirani Routray (2013) DPPH Free Radical Scavenging Activity of Some Leafy Vegetables used by Tribal's of Odisha, *Journal of Medicinal Plants Studies*, Vol. (4):21-27.
- [18]. Drabkin, D.L., and Austin, J.H, Spectrophotometric studies - II. Preparations from washed blood cells; nitric oxide haemoglobin and sulfhemoglobin, *J. Biol. Chem.*, 112, 1935, 51.
- [19]. Nariaki, M., Yumiko, M., Kyoko, Y., Yukinori, N., Yoshinosuke, H., Naomasa, K., Takefumi, K., Keiji, H., Shin-Ichiro, S., Hideki, I. (2006) Aged Garlic Extract Inhibits Angiogenesis and Proliferation of Colorectal Carcinoma Cells. *The Journal of Nutrition*, Volume 136, Issue 3, Pages 842S–846S.
- [20]. Avneet, K., Milandeep, K., Prabhjot, K., Harpreet, K., Sarbjeet, K., Khushwinderjit, K. (2015) Estimation and comparison of total phenolic and total antioxidants in green tea and black tea. *Global Journal of Bio-Science and Biotechnology*, vol.4 (1): 116-120.
- [21]. Hajjimaahmoodi, M., Hanifeh, M., Oveisi, M.R., Sadeghi, N., Jannat, B. (2008) Determination of total antioxidant capacity of green teas by the ferric reducing/antioxidant power assay. *Iran. Journal of Environmental Health Science and Engineering*, Volume 5, No. 3, 167-172.
- [22]. Sumitra, M., Ajeet, K., Sunita, S., Gurdip, S. (2014) An overview on antioxidative potential of honey from different flora and geographic origins. *Indian Journal of Natural Products and Resources*. Vol. 5 (1):9-19.
- [23]. Jaromir, L., Alena, H., Jan, S., Jindrich, K., Matyas, O. and Barbora, R. (2010) Contents of Major Phenolic and Flavonoid Antioxidants in Selected Czech Honey. *Czech Journal of Food Science*. Volume 28, No. 5: 412–426.