Original Article

Anti-Peroxidative and Anti-Diabetic Activities of Aniseeds (*Pimpinella anisum* l.) and Identification of Bioactive Compounds

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ABSTRACT

Polyphenolic compounds, extensively disseminated in plants, known to be excellent antioxidants, possess the capacity to scavenge free radicals generated due to oxidative processes and protect antioxidant defenses in the body. Also, the polyphenolic compounds present in spices have been used for many years for the effective treatment and management of diabetes mellitus. Aniseed, one of the oldest spices, also used as traditional medicine, possesses carminative, antiseptic, diuretic, and digestive stimulant properties. In this study, lipid peroxidation (marker of oxidative stress) inhibitory effect and anti-diabetic activity of various fractions of aniseed (*Pimpinella anisum* L.) obtained by sequential fractionation of methanolic extract using hexane, benzene, ethyl acetate, n-butanol and water were evaluated using *in vitro* methods and model systems. Though methanolic extract and all the fractions of aniseeds dose dependently exhibited anti-peroxidative and anti-diabetic activities, ethyl acetate fraction exhibited the highest anti-peroxidative effect in linoleic acid model system (IC_{50} 185µg/ml), and liver homogenate model (IC_{50} 199µg/ml) and anti-diabetic activities in terms of α- amylase and α-glucosidase (IC_{50} 0.12mg/ml and 0.15mg/ml respectively) inhibitory activities. Based on the analytical data generated, ethyl acetate fraction of aniseeds being more potent than the other fractions when subjected to flash chromatography followed by thin layer chromatography (TLC) revealed the presence of a number of polyphenolic compounds, apigenin and luteolin being more predominant than the others. Thus, aniseeds are a promising source of natural radical scavengers, anti-peroxidative and anti-diabetic agents such as phenolic compounds that may possess potential applications in combating oxidative stress caused by free radicals.

Keywords: Aniseeds, Anti-peroxidative, Anti-diabetic, Flash chromatography, Apigenin, and Luteolin.

INTRODUCTION

Reactive oxygen species (ROS) are generated in living organisms through many pathways[1]. Accumulation of ROS in aerobic organisms is known as an exacerbating factor in cellular injury and in the ageing process[2]. In addition, ROS induce lipid peroxidation, causing the deterioration of foods[3]. Recently there has been an increased interest in finding natural antioxidants from plants because they attack free radicals, retard the progress of many chronic diseases[4] and also retard the lipid oxidative rancidity in foods. Phenolic compounds, such as flavonoids and phenolic acids, seem to be the most effective of these compounds and can be found in many plant materials, particularly in fruits, seeds, spices and herbs[5]. Many species have been recognized to have medicinal properties viz. antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic effects and hence, possess beneficial impact on health[6].

Diabetes mellitus is an endocrine disorder characterized by hyperglycemia [7] and is mounting, along with an increase in both obesity and ageing in the common population. It is an enormous challenge now, because about 5% of the global population is affected by diabetes mellitus[8,9]. Pancreatic α-amylase (E.C. 3.2.1.1) is a key enzyme in the digestive system and catalyses the initial step in the hydrolysis of starch to a mixture of smaller oligosaccharides consisting of maltose, maltotriose, and a number of α-(l-6) and α-(1-4) oligoglucans which are then acted upon by α-glucosidase and further degraded to glucose which on absorption enters the blood stream. Degradation of the dietary starch proceeds rapidly and leads to elevated post-prandial hyperglycemia (PPHG). It has been shown that activity of human pancreatic α-amylase (HPA) in the small intestine correlates to an increase in post-prandial glucose levels. Hence, one of the therapeutic approaches is to decrease the post-prandial hyperglycemia by retarding the digestion of glucose by α-amylase and α-glucosidase enzymes[10]. From this point of view, many efforts have been made to search for more effective and safe inhibitors of α-amylase and α-glucosidase from natural sources to develop physiological functional foods to treat diabetes[11].

Aniseed (*Pimpinella anisum* L.) is one such spice that contains considerable amounts of phenolic compounds [12] which possess varying degrees of antioxidative activity. Aniseed, a schizocarpic fruit, also called as fennel seed is a native of the Eastern Mediterranean region and is widely cultivated in southern and central Europe. In India, it is grown to a small extent as a culinary herb. Aniseed is one of the oldest spices and is also used as traditional medicine[13]. However, very few reports are available on the anti-peroxidative and anti-diabetic activity of aniseeds. Thus, the main objectives of this work were to investigate the anti-peroxidative as well as the anti-diabetic activity of methanolic extract and various fractions of aniseeds and to identify the bioactive compounds in aniseed extract.

MATERIALS AND METHODS

Preparation of aniseed extract and evaluation of therapeutic potential

Aniseeds (*Pimpinella anisum* L.) were purchased in one lot from the local market, shade dried, powdered and extracted with 80% methanol(Me), thrice (1:1, w/v) at room temperature[14]. The combined extract was concentrated in a vacuum evaporator and the residue was dissolved in water and fractionated successively using the solvents with increasing polarity viz. hexane (He), benzene (Be), ethyl acetate (Ea), n-butanol (Nb) and water (Aq) and each fraction was evaporated to dryness. Before use, a small

amount of each fraction was redissolved in suitable solvents as required at a concentration of 1mg/ml[15].

All the fractions were evaluated for total polyphenolics[16], total flavonoids[17], total flavonols[18] and tannins[19] and for the anti-peroxidative activity in linoleic acid model system[20] and in liver homogenate model[21] and *in vitro* anti-diabetic activity in terms of inhibition of α- amylase[22] and α-glucosidase[23].

ISOLATION OF THE PHYTOCHEMICALS PRESENT IN ANISEED (*PIMPINELLA ANISUM* L.) EXTRACTS

The fraction of methanolic extract having higher polyphenolic content was evaporated in vacuum and used for further purification using column chromatography.

↓

The dark brown solid (1.5g) was adsorbed on silica gel (20g) and transferred to a column of silica gel (150g) equilibrated with hexane.

↓

Elution was performed with I) hexane, II) hexane:chloroform (3:1), III) hexane:chloroform (1:1), IV) hexane: chloroform (1:3), V) chloroform: ethyl acetate (3:1), VI) chloroform: ethyl acetate (1:1), VII) chloroform: ethyl acetate (1:3), VIII) ethylacetate:methanol (3:1), IX) ethyl acetate: methanol (1:1), X) ethyl acetate: methanol (1:3) and XI) methanol.

↓

Eleven sub-fractions

Thin Layer Chromatography (TLC) was carried out for all the eleven sub fractions obtained after elution using various solvent systems from I-XI. For thin layer chromatography, the solvent systems tried for the best performance [(a) 30% ethyl acetate in hexane, b) 20% ethyl acetate in chloroform, c) 5% methanol in chloroform] revealed 5% methanol in chloroform to be the best solvent system for the separation of compounds in ethyl acetate fraction. The fractions (I-XI) were evaporated, concentrated and made up to 10 ml in standard flask.

IDENTIFICATION OF THE PHYTOCHEMICALS PRESENT IN ANISEED (PIMPINELLA ANISUM L.) EXTRACTS

Samples and standards were applied on Aluminium plate (E. Merck) pre-coated with Silica gel 60 F254 of 0.2 mm thickness. During this process many solvent system were developed viz. (a) 30% ethyl acetate in hexane, b) 20% ethyl acetate in chloroform, c) 5% methanol in chloroform. Samples and standards (5µL - 30µL) were manually applied on TLC plates in a band-shape of 1cm. The TLC plates were dried with a blow-dryer and run 8cm. After air drying, the plate was visualized in UV 254 and 366nm and comparison was done using different standards (apigenin, quercetin, rutin and luteolin) with respect to the no. of spots and the length of elution (Rf).

$$\text{Relative front (Rf)} = \frac{\text{Distance travelled by the solute from the origin}}{\text{Distance travelled by the solvent from the origin}}$$

STATISTICAL ANALYSIS

The results obtained were subjected to two-way analysis of variance (ANOVA) using SPSS software and the significant difference between means was calculated. Values expressed are mean of three samples analyzed in triplicate ± standard error of means (SEM)

RESULTS AND DISCUSSION

Phenolic compounds in methanolic extract and other fractions of aniseeds

Phenolic compounds viz. total phenolics, total flavonoids, total flavonols and tannins estimated in methanolic extract, hexane, benzene, ethyl acetate, n-butanol and aqueous fractions of methanolic extract of aniseeds are presented in **Table 1**.

Total phenolics

Ethyl acetate fraction had the highest phenolic content followed by benzene and aqueous fractions, methanolic extract, hexane and n-butanol fractions.

Total flavonoids and flavonols

Flavonoids were concentrated in ethyl acetate fraction followed by hexane fraction, methanolic extract, benzene, aqueous and n-butanol fractions. Similarly, flavonol content was more in ethyl acetate fraction followed by methanolic extract, benzene, n-butanol, hexane and aqueous fractions.

Tannins

n-butanol had maximum tannin content followed by ethyl acetate, aqueous, hexane, and benzene fractions and methanolic extract.

Ethyl acetate fraction was found to possess maximum amount of phenolics, flavonoids and flavonols than methanolic extract and all the other fractions which is in second position in tannin content. The variation in the phytochemicals in methanolic extract and other fractions is due to the variation in their solubility in the solvents of varying polarity.

Inhibition of lipid peroxidation in linoleic acid model system

Fig.1 shows inhibition of lipid peroxidation in linoleic acid emulsion system by methanolic extract of aniseeds and various fractions. Though all the fractions showed dose dependant and significantly ($p<0.001$) high inhibitory effect, ethyl acetate (IC_{50} value 185 µg/ml) fraction displayed the highest activity at all the concentrations. Benzene (IC_{50} value 295µg/ml) and aqueous fractions (IC_{50} value 311µg/ml) though lesser than ethyl acetate fraction, also showed better inhibitory effect. The IC_{50} values of other fractions i.e. hexane, n-butanol and methanol are 378 µg/ml, 362µg/ml and 542µg/ml respectively, the lowest being for methanolic extract.

The inhibition of lipid peroxidation in linoleic acid emulsion system also called as ferric thiocyanate (FTC) method measures the amount of peroxide in the beginning of the reaction, where ferric ion was formed upon reaction of peroxide with ferrous chloride. The ferric ion will then unite with ammonium thiocyanate producing ferric thiocyanate, a red colored substance. The darker the color, the higher will be the absorbance[24].

Inhibition of oxidation of linoleic acid in a reaction system is a reflection of the complexity of the extract (aqueous /hydrophobic nature of compounds) as well as potential interaction between the extract and emulsion component, oil, water or lipid: air interfaces as reported by Koleva *et al*. [25] for antioxidant activity of *Camellia sinensis* L. O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. Maximum inhibition of lipid peroxidation (LPO) in the reaction system exhibited by ethyl acetate fraction in the present study is a result of highest phenolic content present in the fraction (flavonoids and flavonols) (**Table 1**). More or less, other fractions also exhibited inhibition of peroxidation of lipids in proportion to their polyphenolic content conforming that polyphenols are efficient in preventing peroxidation of lipids.

Inhibition of lipid peroxidation in liver homogenate model

The methanolic extract and various fractions of methanolic extract of aniseeds significantly ($p<0.001$) inhibited $FeSO_4$-induced lipid peroxidation (LPO) in the liver homogenate as shown in **Fig. 2**. A highly significant inhibitory activity was shown by all the fractions and methanolic extract of aniseeds among which, ethyl acetate fraction exhibited highest per cent inhibition of lipid peroxidation with IC_{50} value of 199µg/ml and the lowest per cent inhibition was exhibited by the methanolic extract with IC_{50} value of 506µg/ml. The IC_{50} value of other fractions viz. hexane, benzene, n-butanol and aqueous fractions ranged from (272µg/ml to 506µg/ml). Though lesser than ethyl acetate, benzene and n-butanol fractions also showed quite good per cent inhibition of lipid peroxidation.

Lipid peroxidation is initiated by reactive oxygen species (ROS). Some typical ROS are superoxide (O_2•), singlet oxygen $^1O_2$, triplet oxygen ($^3O_2$), ozone (O^3), hydroxyl radical (•OH), alkoxyl radical (RO•), and peroxyl radical (ROO•). Once these free radicals are formed, lipid peroxidation progresses and, consequently, lipids produce various so-called secondary oxidation products, some of which have been used as biomarkers to investigate their role in the diseases such as cancer, diabetes, arthritis etc. In biological systems, oxidative degradation of PUFA in cell membranes generates a number of degeneration products, such as malondialdehyde (MDA), which is found to be an important cause of the cell membrane destruction and cell damage[26].

In the present investigation, iron-induced lipid peroxidation in liver homogenate, used as a model system to examine anti-lipid peroxidative effect of various aniseed fractions, is a well validated system for generating ROS as reported by Gutteridge[27]. Although a number of other *in vitro* assays are useful to assess antioxidant potential of plant extracts in terms of inhibition of LPO, only this assay involves biological tissue which has a considerable amount of historical control data. In the present study, ethyl acetate fraction of aniseed extract exhibited potential inhibition (80%) of $FeSO_4$-induced lipid peroxidation, a marker of oxidative stress. Iron, a transition metal is capable of generating free radicals from peroxides by the Fenton reaction and is implicated in many human diseases[28]. Fe^{2+} has also been shown to produce oxyradicals and lipidperoxides, so the decrease of Fe^{2+} concentration in the Fenton reaction would protect against oxidative damage. The results of the present study reveal the presence of potent antioxidant compounds such as polyphenols, flavonoids, flavonols etc. in ethyl acetate fraction that could efficiently inhibit $FeSO_4$-induced lipid peroxidation in liver homogenate since polyphenols are also known for their ability to prevent peroxidation of fatty acids and provide a defence against oxidative stress caused by oxidizing agents and free radicals[29].

In vitro anti-diabetic activity

α-amylase and α-glucosidase inhibitory activities

The different fractions of methanolic extract of aniseeds (hexane, benzene, ethyl acetate, n-butanol, aqueous) tested for α-amylase and α- glucosidase inhibitory effects showed significant ($p<0.001$) and concentration dependant inhibition of α-amylase and α- glucosidase activities (**Fig.3 &4**). However, among the different fractions, ethyl acetate fraction displayed the highest α-amylase (IC_{50}- 0.12mg/ml) and α-glucosidase (IC_{50}-0.15mg/ml) inhibitory effects. At the concentration of 500µg/ml, the sequence of inhibitory effects on α-

amylase and α- glucosidase activities respectively had the order as follows:

Ethyl acetate (94% and 87%) > hexane (93% and 86%) > benzene (91% and 85%) > methanol (84% and 83%) > aqueous (81% and 79%) > n-butanol (75% and 77%).

The treatment goal of diabetes patients is to maintain near normal levels of blood glucose, in both the fasting and post-prandial states. Many natural resources have been investigated with respect to suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine[30]. α- amylase catalyses the hydrolysis of α-1,4-glucosidic linkages of starch, glycogen and various oligosaccharides and α-glucosidase further breaks down the disaccharides into simpler sugars, readily available for the intestinal absorption. The inhibition of the activity of these enzymes in the digestive tract of humans is considered to be effective to control diabetes by diminishing the absorption of glucose hydrolyzed from starch by these enzymes[31].

Many medicinal plants have been reported to possess anti-diabetic activity when assessed using presently available experimental techniques[32]. For example, polyphenols from tea[33], sweet potato[34], berry[35] and Vietnamese edible plants such as *Syzygium zeylanicum*, *Cleistocalyx operculatus*, *Horsfieldia amygdalina* and *Careya arborea*[32] exhibited an inhibitory effect on α-glucosidase and/or α-amylase. Since various compounds have been generally accepted as antioxidants, it has been shown that the activity of α-glucosidase and α-amylase is effectively inhibited by flavonoids, such as naringenin, kaempferol, luteolin, apigenin, (+)-catechin/(-)-epicatechin, diadzein and epigallocatechin gallate[36], indicating that polyphenolic compounds are able to inhibit the activities of carbohydrate- hydrolyzing enzymes, due to their ability to bind with proteins[37]. Khan *et al*.,[38] reported that the improvement in glucose metabolism was apparently due to the phenolic compounds in the extracts of common culinary herbs and spices.

It has been proved that aniseed contains various phenolic compounds. As ethyl acetate fraction of methanolic extract of aniseeds contains a larger amount of phenolic compounds, the higher inhibitory effect shown by ethyl acetate fraction (**Fig 3&4**) could be due to the higher phenolic compounds present in the fraction. Although, further experiments are required to identify the bioactive compounds from aniseed, it could be proposed that rutin, caffeic acid and luteolin, which are the major phenolic compounds of aniseed[12], are possible active compounds linked to α-glucosidase and α-amylase inhibitory activities. Insights from a number of studies suggest that these phenolic compounds from a large number of various plants including crop, medicinal and ornamental plants[37,39] could have potential in the prevention of type 2 diabetes as part of a dietary strategy.

Identification of bioactive compounds in the extracts of aniseeds

In the trial thin layer chromatography conducted for I to XI fractions, no spots were observed with I to III sub-fractions. The remaining eight sub-fractions, multi-component methanolic extract and ethyl acetate fraction subjected to thin layer chromatography, revealed very interesting information by means of development of spots in thin layer chromatograms as shown in Fig. 5, 6 and 7.

Chromatogram 1 (Fig. 5) shows development of spots for methanolic extract, ethyl acetate fraction with apigenin (A), rutin (R) and luteolin (L) as standards. From this chromatogram it can be evidenced that ethyl acetate fraction had eight compounds, among which apigenin and luteolin could be

identified from the Rf values. Methanolic extract showed many spots indicating that it is a multi-component extract.

Chromatogram 2 (Fig.6) developed for ethyl acetate fraction, sub-fractions IV-VII and standards viz. quercetin (Q), rutin and apigenin revealed the presence of apigenin in ethyl acetate fraction and sub-fraction V and VI.

Chromatogram 3 (Fig.7) developed for ethyl acetate fraction, sub-fractions XIII-XI and standards (quercetin, apigenin and rutin) confirmed the presence of apigenin abundantly in ethyl acetate fraction and in minute quantities in sub-fractions XIII, IX and X. Sub fraction XIII-XI showed few more compounds comparable to that present in ethyl acetate fraction which could be other polyphenolic compounds.

CONCLUSIONS

Methanolic extract and the fractions of methanolic extract of aniseeds contained phenolic compounds, ethyl acetate being very rich in phenolics viz. flavonoids and flavonols. Though methanolic extract and all the fractions exhibited potent anti-peroxidative and anti-diabetic activities, ethyl acetate fraction ranked first owing to the presence of phenolic compounds viz. apigenin and luteolin as evidenced by chromatographic analysis. In light of these effects, it can be concluded that aniseed is a potent anti-peroxidative and anti-diabetic agent and thereby, possesses broad prospects for potential applications and exploitations for the food and drug industry.

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REFERENCES

1. Fridovich I. Superoxide radical and superoxide dismutase. *Annu Rev Biochem* 1995; 64: 97-112.
2. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerantol* 1956; 11: 298-300.
3. Duthie GG. Lipid peroxidation. *Eur J Clin Nutr* 1993; 47: 759-764.
4. Pryor WA. The antioxidant nutrients and disease prevention- What do we know and what do we need to find out? *Am J Clin Nutr* 1991; 53: S391-S393.
5. Caragay AB. Cancer-preventive foods and ingredients. *Food Technol* 1992; 56: 65-68.
6. Aaby K, Hvattum E, Skrede G. Analysis of flavonoids and other phenolic compounds using high-performance liquid chromatography with couloumetric array detection: Relationship to antioxidant activity. *J Agric Food Chem* 2004; 52: 4595-4603.
7. Chandra A, Singh R K, Tewari L. Antioxidative potential of herbal hypoglycemic agents in diabetes - an overview. *SFRR- India Bull* 2004; 3: 24-26.
8. WHO. Traditional medicine strategy 2002-2005.WHO Publications; 2002:1-6
9. Wang H, Du Y, Song H. α-glucosidase and α-amylase inhibitory activities of guava leaves. *Food Chem* 2010; 123: 6-13.
10. Bhandari MR, Nilubon JA, Gao H, Kawabata J. α-glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata* Haw.). *Food Chem* 2008; 106: 247–252.
11. Kim YM, Wang MH, Rhee HI. A novel α-glucosidase inhibitor from pine bark. *Carbohydr Res* 2004; 339: 715–717.
12. http://www.ars-grin.gov/duke// (accessed on December, 2011).
13. Parry JW. Spices: their morphology, histology and chemistry, In: The wealth of India. XIII; 1962: 60-64.

14. Petra M, Britta T, Macki K, Eckart E. Flavonoid sulfates from the Convolvulaceae. *Phytochem* 1999; 50: 267–271.
15. Hashim MS, Lincy S, RemyaV, Teena M, Anila L. Effect of polyphenolic compounds from *Coriandrum sativum* on H_2O_2-induced oxidative stress in human lymphocytes. *Food Chem* 2005; 92: 653-660.
16. Singleton VL and Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Ecol Viticul* 1965; 37: 144–158.
17. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Analysis* 2002; 10: 178-182.
18. Kumaran A, Karunakaran J. *In vitro* antioxidant activities of methanol extract of five phyllanthus species from India. *LWT* 2006; 40:344-352.
19. Price MC, Suoyoe VS, Butler LG. A critical evaluation of the vanillin reaction and assay for tannins and sorghum gravies, *J Agric Food Chem* 1978; 26: 1214-1218.
20. Kikuzaki H, Nakatani N. Antioxidant effects of some ginger constituents. *J Food Sci* 1993; 58: 1407–1410.
21. Yue TL, Gu J L, Lysko P G, Sponer G, Cheng HY, Ruffolo RJ. Antioxidant activity of a *β*- adrenoceptor antagonist, carazolol, caparison with propranolol and carvedilol. *Pharmacol Commun* 1995; 7: 11-19.
22. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959; 31: 426-428.
23. McCue P, Kwon YI, Shetty K. Anti-amylase, anti-glucosidase and anti-angiotensin I-converting enzyme potential of selected foods. *J Food Biotech* 2005; 29: 278–294.
24. Manian R, Anusuya N, Perumal S, Manian S. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. *Food Chem* 2008; 107: 1000-1007.
25. Koleva II, Van Beck TA, Linsen JPH, de Groot A, Evstatieva IN. Screening of plant extract for antioxidant activity: A comparative study on three testing methods. *Phytochem Anal* 2002; 13: 8-17.
26. Yoshikawa T, Naito Y, Kondo M. Food and diseases. In, Hiramatsu M, Yoshikawa T and Inoue M (eds). Free Radicals and Disease. New York: Plenum Press; 1997: 11-19.
27. Gutteridge JMC. Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin Chem* 1995; 41: 1819–28.
28. Halliwell B, Gutteridge JMC. Role of free radicals and catalytic metal ions in human diseases: An overview. Methods Enzymol 1990; 186**:** 1-86.
29. Slusarczyk S, Hajnos M, Skalicka-Wozniak K, Matkowski A. Antioxidant activity of polyphenols from *Lycopus lucidus* Turcz. *Food Chem* 2009; 113:134–138.
30. Matsui T, Tanaka T, Tamura S, Toshima A, Tamaya K, Miyata et al. Alpha glucosidase inhibitory profile of catechins and the aflavins. *J Agric Food Chem* 2007; 55: 99-105.
31. Hara Y, Honda M. The inhibition of α-amylase by tea polyphenols. *Agric Biol Chem* 1990; 54: 1939–1945.
32. Mai TT, Thu NN, Tien PG, Chuyen NV. Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. *J Nutr Sci Vitaminol* 2007; 53: 267–276.
33. Honda M and Hara Y. Inhibition of rat small intestinal sucrase and α-glucosidase activities by tea polyphenols. *Biosci Biotech Biochem* 1993; 57: 123–124.
34. Matsui T, Ueda T, Oki T, Sugita K, Terahara N, Matsumoto K. Alpha-glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity. *J Agric Food Chem* 2001; 49: 1948–1951.
35. McDougall GJ, Stewart D. The inhibitory effects of berry polyphenols on digestive enzymes. Biofactors 2005; 23: 189–195.
36. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. *J Nutr Sci Vitaminol* 2006; 52: 149–153.
37. Shobana S, Sreerama YN, Malleshi NG. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: mode of

inhibition of α-glucosidase and pancreatic amylase. *Food Chem* 2009; 115: 1268–1273.
38. Khan A, Bryden NA, Polansky MM, Anderson RA. Insulin potentiating factor and chromium content of selected foods and spices. *Biol Trace Elem Res* 1990; 24: 183–188.
39. Yao Y, Sang W, Zhou M, Ren G. Antioxidant and α-glucosidase inhibition activity of colored grains in China. *J Agric Food Chem* 2010; 58: 770–774.

Table 1. Phenolic compounds in methanolic extract and various fractions of methanolic extract of aniseeds

Sample extract	Total phenolics (mg/100g) GAE	Total flavonoids (mg/100g) RE	Total flavonols (mg/100g) RE	Tannins (mg/100g) CE
Methanol	0.48±2.3	0.22±0.9	0.23±1.9	0.005±0.8
Hexane	0.36±2.9	0.30±0.5	0.04±0.5	0.06±1.3
Benzene	0.60±0.6	0.12±1.4	0.19±0.3	0.05±0.8
Ethyl acetate	0.77±0.3	0.45±1.6	0.55±0.1	0.07±0.2
n-butanol	0.23±1.7	0.05±1.1	0.07±0.9	0.08±0.6
Aqueous	0.55±2.1	0.07±1.7	0.03±1.4	0.06±1.6

Values are mean ± SEM of three replicates $p<0.01$

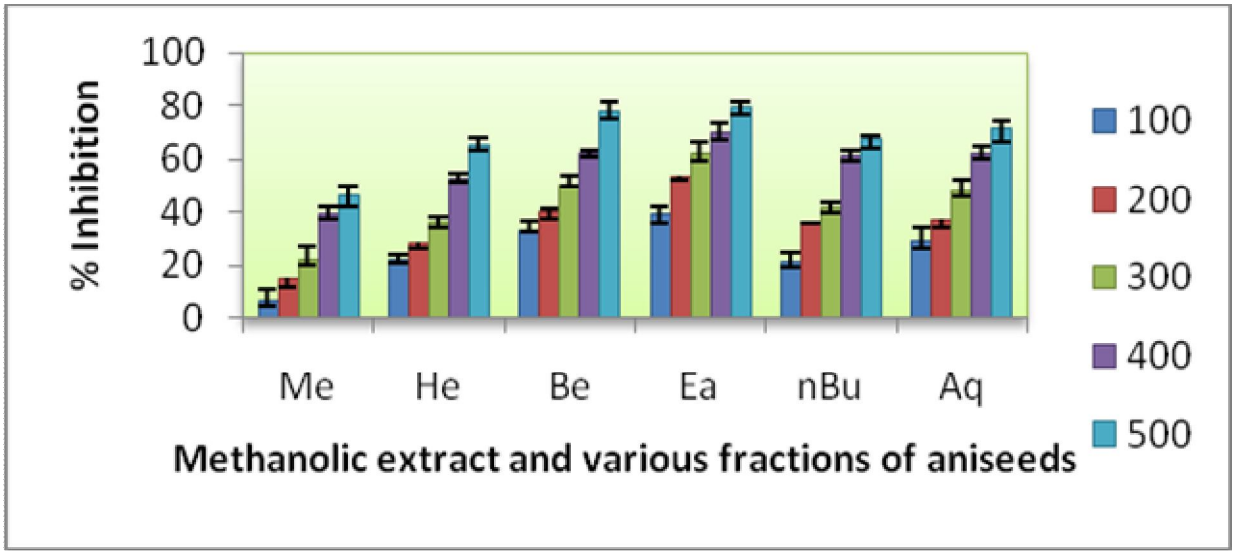

Figure.1. Anti-peroxidative effect of methanolic extract and various fractions of aniseeds in linoleic acid model system ($p<0.001$) Values are mean±SEM of three replicates

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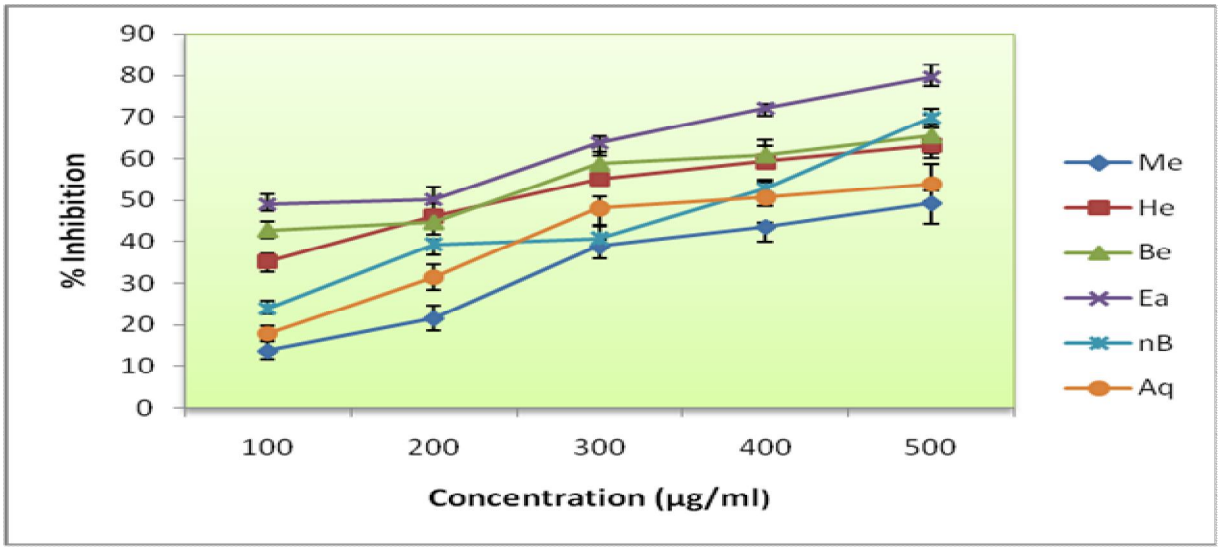

Figure.2. Anti-peroxidative effect of methanolic extract and various fractions of aniseeds in liver homogenate model ($p<0.001$) Values are mean±SEM of three replicates.

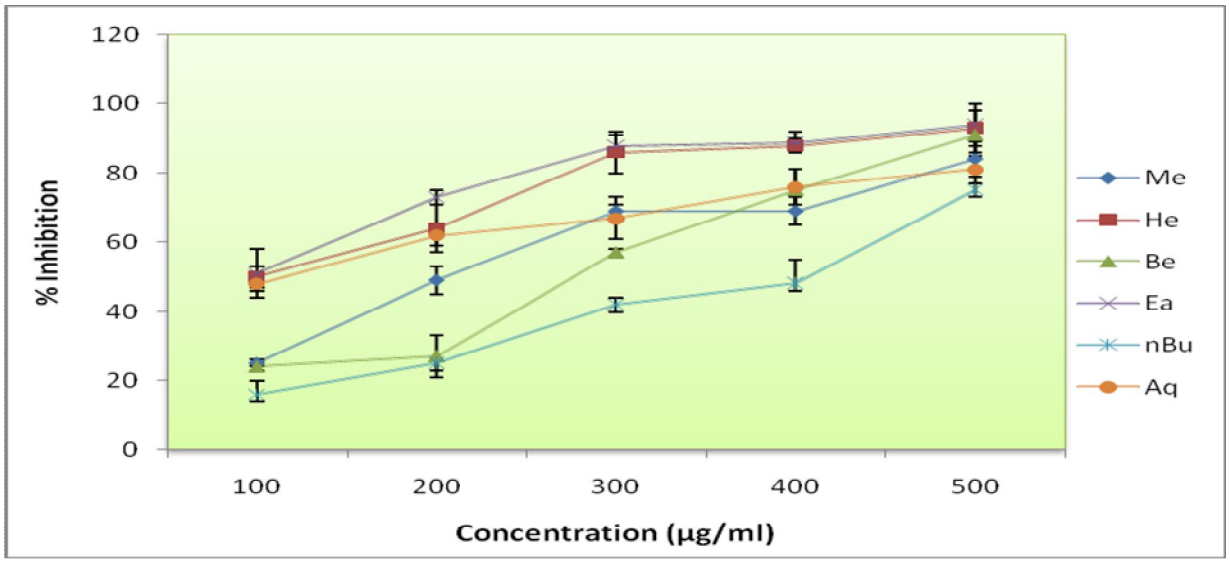

Figure.3. α-amylase inhibitory effectof methanolic extract and various fractions of aniseeds ($p<0.001$) Values are mean±SEM of three replicates.

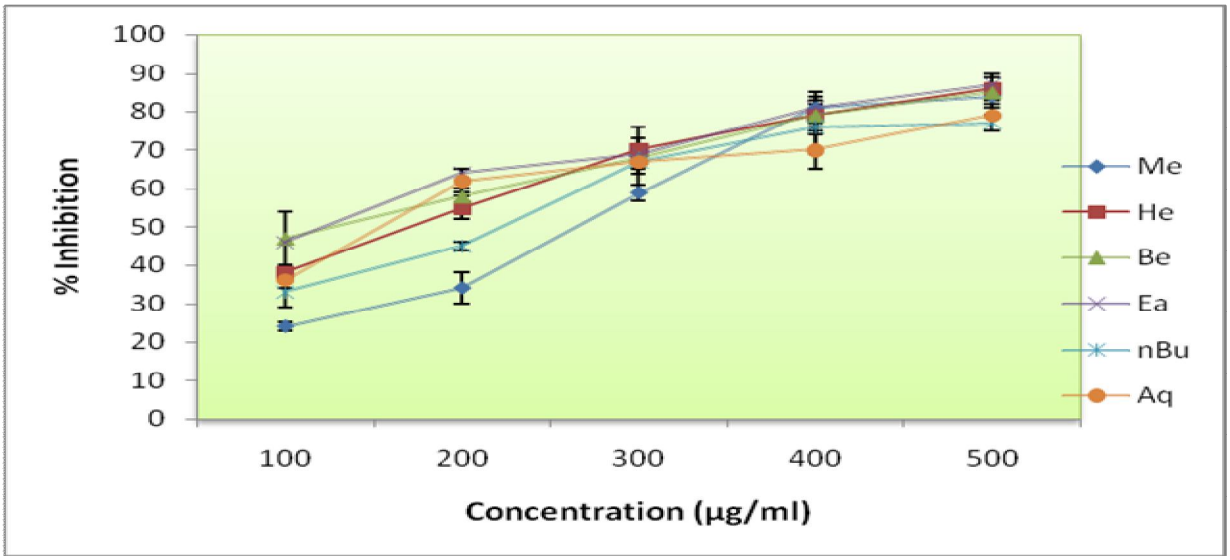

Figure.4. α-glucosidase inhibitory effectof methanolic extract and various fractions of aniseeds ($p<0.001$) Values are mean±SEM of three replicates.

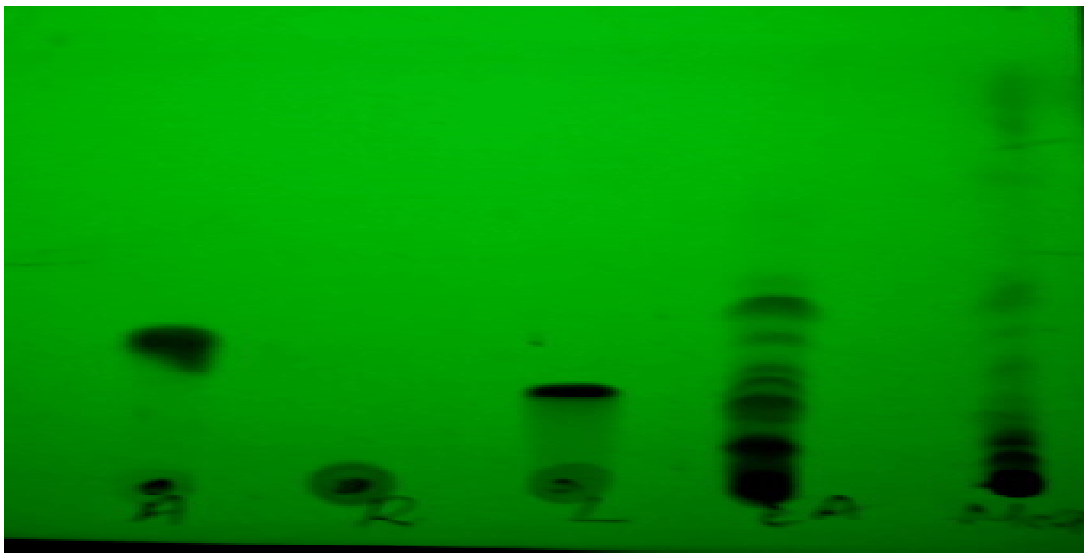

Figure.5. Chromatogram of methanolic extract and ethyl acetate fraction of aniseeds and standards apigenin (A) , rutin (R) and luteolin (L)

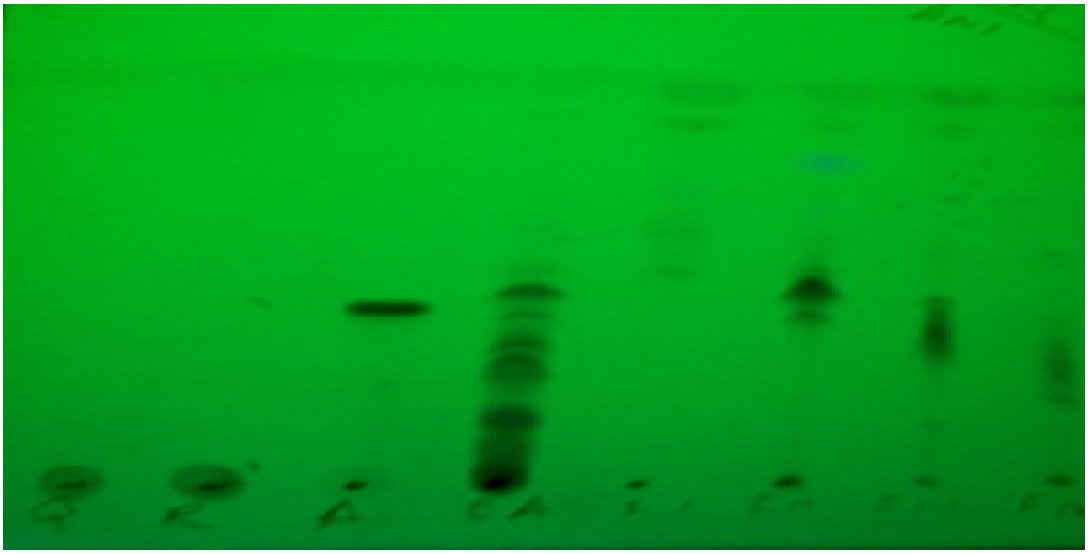

Figure.6. Chromatogram of ethyl acetate fraction and sub fractions (IV-VII) of aniseeds and standards quercetin (Q), rutin (R) and apigenin (A)

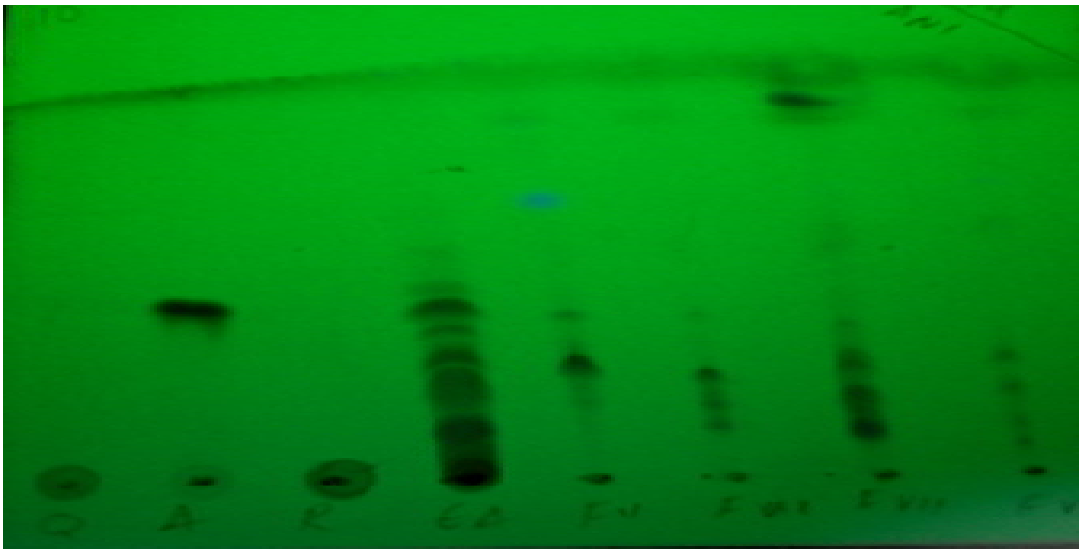

Figure.7. Chromatogram of ethyl acetate fraction and sub-fractions (VIII-XI) of aniseeds and standards quercetin (Q), rutin (R) and apigenin (A)