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Antidiabetic potential of different parts of the Psidiumguajava, Foeniculumvulgareand Nigella sativa extractsusing various solvents systems through GC-MS and human pancreatic β-cell lines (RIN-5F cells) model

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Abstract

Background: Diabetes mellitus is a major cause of mortality and the most common metabolic disorder characterized by hyperglycemia due to lack of insulin production by the pancreas or the inability of the insulin produced to control blood glucose. Medicinal plants are one of the main resources of therapeutic agents. Indeed, 80% of the world's population uses plants in health care.Despite progress in the management of diabetes mellitus by synthetic drugs most of these drugs have side effects in the long run.

Aim: To study the anti- diabetic enzyme namely alpha amylase and alpha glucosidase in aqueous and methanol extract of combinations of *Psidiumguajava, Foeniculumvulgare and Nigella sativa*.

Methods: In order to find the antidiabetic efficacy *in vitro,* alpha amylase and alpha glucosidaseinhibitory analysis was done as per the standard methods in the aqueous and methanolic extract of *Psidiumguajava, Foeniculumvulgare and Nigella sativa* and combination of both.Preliminary screening of the plant extracts such as hexane, chloroform, ethyl acetate, ethanol, methanol and aqueous extracts of *P.guajava, F.vulgare*and*N.sativa* were studied as per the standard protocols.Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed to find out the bioactive compounds. To check the cytotoxicity of plant extracts human pancreatic β-cell line (RIN-5F cells).

Results: Results of the study showed that methanol extract of *P.guajava, F.vulgare* and *N.sativa* exhibited answered positive for major secondary metabolites. Alpha amylase and alpha glucosidase inhibitory Effect of combination of *P.guajava, F.vulgare* and *N.sativa* extracts on the activities of α- amylase and α-glucosidase was evaluated. The plant extract showed potent inhibition of alpha amylase and glucosidase activity. Results of cell viabliltity of aqueous and methanol extract of combinations of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* the most effective in protecting RIN-5F cells.

Conclusion: Present findings clearly indicate that differentialextracts of*Psidiumguajava, Foeniculumvulgare*and *Nigella sativa*have strong alpha amylase and alpha glucosidase inhibitory activity and showed no toxicity in RIN-5F pancreatic beta cells. Hence, our study concludes that*Psidiumguajava, Foeniculumvulgare*and *Nigella sativa*can be considered as potential antidiabetic natural drugs for the treatment of diabetes mellitus and associated complications.

Key words:Psidiumguajava, Foeniculumvulgareand Nigella sativa, antidiabetic activity, RIN-5F cells, GC-MS analysis.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis by

disturbances of carbohydrate, fat and protein metabolism. The world prevalence of diabetes among adults is expected to be 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7%. The number of diabetes mellitus cases has been increasing worldwide in recent years. In 2000, the world health organization estimated a total of 171 million of people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030. Type II DM is the common most form of diabetes that constitutes 90-95% of the diabetic population. It was estimated to be at 2-3% of the world population and is increasing at a rate of 4-5% per year (Wild *et al*., 2004). With a long course and serious complications often resulting in high death rate, the treatment of diabetes spent vast amount of resources including medicines, diets, physical training and so on in all countries (Syamsudin,2010).

An exocrine function that helps in digestion regulates the level of blood sugar. Pancreatic production of insulin, somatostatin, gastrin, and glucagon plays an important role in maintaining the sugar and salt balance in our bodies and therefore any problem in the production or regulation of these hormones will manifest itself with problems in blood sugar and fluid or salt imbalance. They are small, highly vascularized masses of cells scattered throughout the pancreas. Like all endocrine glands, they secrete their hormones into the blood stream; pancreatic islets are surrounded by small blood vessels. The islets of langerhans contain four types of secretory cells. They are alpha cells, beta cells, delta cells and F cells (Quesada et al., 2006). The pancreas is a soft, elongated, flattened gland 12 to 20 cm in length. The adult gland weighs between 70 and 110 g. The head lies behind the peritoneum of the posterior abdominal wall and has a lobular structure. The pancreas is covered with a fine connective tissue but does not have a true capsule. The head of the pancreas is on the right side and lies within the curvature of the duodenum. The neck, body, and tail of the pancreas lie obliquely in the posterior abdomen, with the tail extending as far as the gastric surface of the spleen (Bockman, 1993).

Insulin is a key player in the control of glucose homeostasis. Lack of insulin affects carbohydrate, fat and protein metabolism (Rajiv Gandhi and Sasi Kumar,2012). It is characterized by chronic hyperglycemia that produces multiple biochemical impairments and oxidative stress especially an increased susceptibility to lipid peroxidationthatplayroleintheprogressionofthesymptomsofdiabetes.

Despite progress in the management of diabetes mellitus by synthetic drugs most of these drugs have side effects in the long run. So, the search for improved and safe natural antidiabetic agents is ongoing and World Health Organization has also recommended the development of herbal medicine in this concern (Ho-dac-Pannekeet et al., 1999; Brownlee, 2000; Philippe and Raccah, 2009). A traditional component of food that can reduce appetite, glucose absorption in intestine, hepatic gluconeogenesis, blood glucose level, body weight, and can stimulate glucose induced secretion of insulin from β-cells in pancreas, may prove to be useful for prevention and control of diabetes mellitus. A traditional component of food that can reduce appetite, glucose absorption in intestine, hepatic gluconeogenesis, blood glucose level, body weight, and can

stimulate glucose inducedsecrertion of insulin from β-cells in pancreas, may prove to be useful for prevention and control of diabetesmellitus.

Most of the medicinal plants are scientifically validated for their therapeutic efficacy and safety. In modern medicine no satisfactory and effective therapy is available to cure diabetes mellitus, which is a syndrome resulting from a variable interaction of hereditary and environmental factors and characterized by abnormal insulin secretion or insulin receptor or post receptor evens affecting metabolism involving carbohydrates, proteins and fats in addition to damaging β-cells of pancreas, liver and kidney in some cases. Hence in the present study *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* were investigated.

α -amylase and α-glucosidase are key enzymes involve in carbohydrates breakdown and intestinal absorption, respectively. Inhibition of these enzymes hamper blood glucose level increased after a carbohydrates diet and can be an important strategy in the management of non-insulin-dependent diabetes mellitus (NIDDM) (Al-Zuhair, 2010). Alpha-amylase are distributed all over various organisms and show diverse substrate specificities, while possessing a common topology formed from three domain, one of which being a typical alpha-beta barrel. Inhibition of insectsalphaamylase is a proposed method of crop protection. On the other hand, inhibition of mammalian alpha- amylase is a proven therapeutic approach in diabetes and related disorders (Mahmoud, 2010).

In general there is very little biological knowledge on the specific modes of action in the treatment of diabetes but most of the plants have been found to contain substance secondary plant metabolites like glycosides, alkaloids, terpenoids, flavonoids etc., that are frequently implicated as having antidiabetic effects. Since time immemorial,

patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. In India a number of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions known as “madhumeha” and some of them have been experimentally evaluated and the active principles isolated (Singh,2001).

Several α-glucosidase inhibitors, such as acarbose, trestatin, amylostatin and valiolamine have been isolated from microorganisms. Natural α-amylase and α- glucosidase inhibitors from food-grade plant sources offer an attractive strategy to control post-prandial hyperglycaemia. Natural inhibitors from plants, which have been shown to have a low inhibitory effect against a-amylase activity and a strong inhibition activity against α-glucosidase, can be used as an effective therapy for postprandial hyperglycaemia with minimal side effects (Afonne et al., 2000). Widespread

species of plants have been described in the scientific and popular literature as having hypoglycemic activity. Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown (Daisy et al., 2009; Shirwaikar et al., 2005 Inrecent years, many efforts have been made to identify effective α-glucosidase inhibitors from natural sources in order to develop a physiologic functional food or lead compounds for use against diabetes. Many α-glucosidase inhibitors that are phytoconstituents, such as flavonoids, alkaloids, terpenoids, anthocyanins, glycosides, phenolic compounds, and so on, have been isolated from plants. Predominantly herbal drugs have been widely used globally for diabetic treatment over thousands of years due to their traditional acceptability and lesser side effects. Therefore, screening of α-amylase and α-glucosidase inhibitors in medicinal plants has received muchattention. The present study was aimed to assess the anti-diabetic potential of aqueous and methanol extract of combinations of *Psidiumguajava, Foeniculumvulgare and Nigella sativa*by alpha amylase and alpha glucosidase inhibitory activity and in RIN-5F pancreatic β-cells.

2.MATERIALS AND METHODS

Collection of plant materials

The seeds of *Nigella sativa* and *Foeniculumvulgare*(Fig 2) were purchased from nearby local store,Aminjikarai, Chennai - 600 030, India. The leaves of *Psidiumguajava*(Fig 3) took from my garden.

Plant extraction

A solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber (Fig 4) is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch ofsolventisrecycled.Afterextractionthesolventisremoved,typically by means of a rotary evaporator, yielding the extracted compound. The non- soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Preliminary phytochemical screening of *P.guajava, F.vulgare andN.sativa*extracts

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
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Qualitative tests were performed to assess the nature of phytochemicals present in various extracts of ***Psidiumguajava, Foeniculumvulgare***and ***Nigella sativa*** namely Hexane, Chloroform, Ethyl acetate, ethanol, methanol and aqueous extracts.

Quantitativeestimation

Determination of totalPhenols:

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds with slight modifications. One hundred µL of ethanol and methanol plant extracts of nigella sativa,foeniculumvulgare and psidiumguajava (1 mg/mL was mixed with 900 µL of distilled water and 1 mL of FolinCiocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of ethanol solution of Na_2CO_3 (20%) was added. Themixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured by UV-VIS spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common referencecompound.

Determination of totalflavonoids:

The total flavonoid content of ethanol and methanol plant extracts of *nigella sativa, foeniculumvulgare and psidiumguajava*was determined using aluminium chloride colorimetric method with slight modification as described by Liu *et al* One mL of extract (1mg/mL) was mixed with 0.5 mL of 5% sodium nitrite solution and incubated for 5 min at room temperature. Then, 0.5 mL 10% aluminium chloride solution was added and incubated for further 5 min at room temperature followed by 1 mL of 1 M NaOH solution was added. The total volume was made up to 5 mL with distilled water. Absorbance was measured at 510 nm using spectrophotometer. The result was expressed as (µg/mg of extract) quercetinequivalent.

Analysis of the combined plant extracts of *nigella sativa, foeniculumvulgare*and *psidiumguajava*by gas chromatography-mass spectrometry(GC-MS)

GC-MS technique was used in this study to identify the phytocomponents. GC-MS analysis of the fractions was performed using GC-MS-QP (Shimadzu) 2010 and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with Elite -1 fused silica capillary column (Length : 30.0 m, Diameter : 0.25 mm, Film thickness : 0.25 µm composed of 100 % Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51 ml/min and an injection volume of 1 µl was employed (split ratio: 10), Injector temperature 240 °C; Ion-source temperature 200 °C. The oven temperature was programmed from 70 °C (isothermal for 3 min), with an increase of 300 °C for 10 min. Mass spectra were taken at 70 eV; a scan interval of 0.5 sec with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC-MS solution ver.2.53.

In vitro*antidiabetic activity of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
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Alpha amylase inhibitory activity

The assay mixture was prepared containing 200 µl of sodium phosphate buffer, 20 µl of enzyme and 20 µl of extracts and incubated for 10 minutes at room temperature followed by the addition of 200 µl of starch in all the tubes. The reaction was terminated with the addition of 400 µl of DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any extracts. The % inhibition was calculated by following theformula.

Inhibition (%) = Abs.control – Abs.sample/ Abs.control x 100.

Alpha glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity of extracts was carried out according to method of with slight modification. Reaction mixture containing 50 µl phosphate buffers,10 µl alpha-glucosidase and 20 µl of varying concentrations of extracts was pre- incubated at37oCfor30min. The reaction was stopped by adding 50 µl sodiumcarbonate. The yellow color produced was read at 405nm. Each experiment was performed along with appropriate blanks. Acarbose at various concentration (20-100 µg/ml) was included as a standard. Negative control without extracts was set up in parallel. The result is expressed as percentage inhibition.

Inhibition (%) = Abs.control – Abs.sample/ Abs.control x 100.

Antidiabetic activity of* Psidiumguajava, Foeniculumvulgare*and* Nigella sativa*on RIN-5F pancreatic β cell line

Briefly, the RIN-5F cells (1 x 10^5/well) were plated in 24-well plates and incubated in 37^0C with 5% CO_2 condition. After the cell reaches the confluence, the varies concentration of the extracts (7.8, 15.6, 31.2,, 62.5, 125, 250, 500 and 1000 µg/mL doses of the extract were tested) were added and incubated 24 hours. After incubation the sample was removed from the well and washed with phosphate-buffered saline (Ph 7.4) or DMEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl— tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with uv- spectrophotometer.

Statistical analysis

All the data obtained in the present study were statistically analyzed using the statistical software SPSS version 16.0. One-way ANOVA using Bonferroni test was appliedto find out the significant difference between the different concentrations of plantextracts.

3. RESULTS AND DISCUSSION

Preliminary phytochemical screening of *P.guajava, F.vulgare andN.sativa extracts*

The present elucidated the antidiabetic potential of *Psidiumguajava*, *Foeniculumvulgare and Nigella sativa in vitro.* DM is one of the most common metabolic diseases; it is known as an increase of the blood glucose level and impaired metabolism of proteins and lipids. Currently, diabetes is considered as one of the most critical issues in the world. Much research performed more efforts to seek new natural antioxidant molecules, which are considered to be relatively safer and with less or without side effects. Recently, the attention is focused on plants which contain high concentrations of

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

phytochemical compounds because of their potential health-promoting effects.Preliminary screening of the plant extracts such as hexane, chloroform, ethyl acetate, ethanol, methanol and aqueous extracts of *Psidiumguajava,Foeniculumvulgare*and *Nigella sativa* were found to contain various phytochemical constituents which are shown in Table 1 - 4. Methanol extract of *Psidiumguajava*, *Foeniculumvulgare*and *Nigella sativa* answered positive for major secondary metabolites namely steroids, flavonoids, triterpenoids, coumarin, sugars, alkaloids, saponins, acids and tannins and phenols. Combination extract of *Psidiumguajava*, *Foeniculumvulgare*and *Nigella sativa* also exhibited steroids, flavonoids, triterpenoids, coumarin, sugars, alkaloids, saponins, acids and tannins and phenols. Phytochemical analysis of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* extracts revealed the presence of bioactive constituents which are known to exhibit medicinalproperties.

The quantitative analysis shows the concentration which is present in methanol and aqueous extracts of *Psidiumguajava*, *Foeniculumvulgare*and *Nigella sativa.* Combination of methanol and aqueous extracts of *Psidiumguajava*, *Foeniculumvulgare*and *Nigella sativa* showed the phenol and flavonoid concentration (Table 5- 8)Bioactive compounds in plants are of natural origin and serve as secondary metabolites (Kanimozhi and Bai, 2012). Biologically active components have always been of huge interest to scientists working on infectious diseases (PerumalSamy and Ignacimuthu, 2000). Chemical constituents from natural sources have contributed significantly to the development of new drugs from medicinal plants (Cox and Balick, 1994). Medicinal plants have therapeutic properties due to the presence of various complex chemical substances of different composition which are found as secondary plant metabolites. These plant metabolites according to their composition are grouped as alkaloids, glycosides, corticosteroids, essential oils etc. The preliminary phytochemical studies are important because the crude extracts posses varied composition of secondary metabolites (Balandrin et al., 1985; Wink, 1999). Medicinal plants are the major source of many primary and secondary metabolities. These biodynamic compounds are of therapeutic value.

Medicinal plants are the main source of organic compounds such as polyphenols, tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These organic compounds represent a source for the discovery and development of new types of antidiabetic molecules. Many compounds isolated from plant sources have been reported to show antidiabetic activity (Firdous, 2014). Our findings indicated that the methanol extract of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* was rich in flavonoid and phenolic contents, which could be the main contributor to their antioxidative properties as many studies affirmed thatflavonoids and phenols offered the highest ability of scavenging activity in medicinal plants (Sharififar et al., 2009 and Miceli et al.,2016).

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

GC – MS analysis of aqueous and methanol extract of combinations of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* (PFN)

Phytochemicals present in methanol extract of PFN revealed n-hexadecanoic acid, 9,12-octadecadienoic acid (z,z)-, 9-octadecyne and oleyl alcohol. Similarly, aqueous extracts of PFN n-hexadecanoic acid, 9,12-octadecadienoic acid, methyl ester, methyl 13-octadecenoate, oxacycloheptadec-8-en-2-one and 9-eicosyne respectively (Table 9 -10). Similarly, Kanthal et al. (2014) who studied the GC-MS analysis of bio-active compounds in methanolic extract of *Lactucaruncinata*. GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in (Figure). The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. The presence of various bioactive compounds confirms the applications of *Psidiumguajava*, *Foeniculumvulgare*and *Nigella sativa* for anti diabetic activity.

The medicinal actions of the plants unique to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct. The spectrometric and chromatographic screening method could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations. The determination of phytoconstituents is largely performed by the relatively expensive and often laborious techniques

such as gas (GC) and liquid chromatography (LC) combined with specific detection schemes. In the last few years, GCeMS has become firmly established as a key technological metabolic profiling in both plant and non-plant species (Kell et al., 2005; Janakiraman et al., 2012).

Anti-diabetic enzyme namely alpha amylase and alpha glucosidase on aqueous and methanol extract of combinations of *Psidiumguajava, Foeniculumvulgare*and *Nigellasativa*

In the present study aqueous and methanol extract of combinations of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* were evaluated for their inhibitory effect on α-amylaseand α-glucosidase enzymes by *in-vitro* method. Maximum level of α-glucosidase inhibition was observed at 100 µg/ml of both methanol and aqueous extracts of *Psidiumguajava*, *Foeniculumvulgare*and *Nigella sativa* of it was found to be 43.79 and 45.09 Similarly in alpha amylase, significant inhibition activity was observed in methanolic and aqueous extracts of combination of *Psidiumguajava*, *Foeniculumvulgare*and *Nigella sativa* at all the concentartiona($P<0.05$). Values are expressed as mean±sd of triplicates.

The use of herbal drugs as complementary approaches in existing medications for the treatment of diabetes and its complications is growing worldwide and many plants in different countries are known to have antidiabetic effects. The ancient Indian literature reports more than 800 plants with antidiabetic properties while ethnopharmacological surveys indicate that more than 1200 plants can be used for hypoglycemic activity. Mainly two carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) are responsible for postprandial hyperglycemia. α- amylase begins the process of carbohydrate digestion by hydrolysis of 1, 4-glycosidic linkages of polysaccharides (starch, glycogen) to disaccharides and α-glucosidase catalyzes the disaccharides to monosaccharides, which leads to postprandial hyperglycemia. Hence, inhibitors of α-amylase and α-glucosidase are useful in the control of hyperglycemia as they delay carbohydrate digestion, which consequently reduce the post prandial plasma glucoselevel (Hasani-Ranjbar*et al*., 2008; Mishra *et al*., 2010; Hara and Honda, 1990, Matsui *et al*., 2007). Inhibitors of -glucosidase delay the breaking down of carbohydrate in the small intestine and diminish the postprandial blood glucose excursion in a person suffering from diabetes One of the strategies and methods adopted to cure diabetes mellitus involves the inhibition of carbohydrate digesting enzymes such as -amylase and -glucosidase in the gastrointestinal glucose absorption thereby lowering postprandial glucose level (Kwon *et al*., 2007; Matsui *et al*., 2006; Matsuda *et al*., 2002; Ogunwande*et al*., 2007). This is an attempt to search for alternative drugs from medicinal plants with increased potency and lesser adverse effects than existing drugs.

In this study, the effect of combination of *Psidiumguajava*, *Foeniculumvulgare*and *Nigella sativa* extracts on the activities of α- amylase and α-glucosidase was evaluated. The plant extract showed potent inhibition of alpha amylase and glucosidase activity. This result is in agreement with previous reports which indicated that hypoglycaemic activity through either increased secretion of the insuline from pancrease or similar action to the insulin. Several plant species have been described as hypoglycaemic such as *Opuntiastreptacantha*, *Trigonellafoenumgraecum*, *Momordicacharantia*, *Ficusbengalensis*, *Polygala senega*,

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
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*Gymnemasylvestre, Allium sativum, Citrulluscolocynthis*and *Aloe vera*(Bnouham*et al*., 2006). *Annonasquamosa*commonly called custard apple plant possesses antidiabetic activity. It acts by promoting insulin release from the pancreatic islets, increasing utilization of glucose in muscle and inhibiting the glucose output from liver (Malviya*et al*.,2010).

Cell viability of aqueous and methanol extract of combinations of *psidiumguajava, foeniculumvulgare*and *nigella sativa*

To evaluate the effect of aqueous and methanol extract of combinations of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* extract against the cytotoxic action on RIN-5F ells, 7.8, 15.6, 31.2,, 62.5, 125, 250, 500 and 1000 µg/mL doses of the extract were tested . It is evident from the results that extract is most effective when used at 1000 µg/mL even though other concentrations are also effective in protecting RIN-5F cells. These results showed that extract not only is non-toxic but, in fact, protected RIN-5F cells against the cytotoxic action. It was observed that both aqueous and methanol extract of combinations of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* schedules employed in the present study the most effective in protecting RIN-5Fcells.

4. CONCLUSION

In the present study, we have investigated the antidiabetic potential of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* which is used in traditional medicine for the treatment of several diseases. The present study provides the first pharmacological insight into the antidiabetic potential of the combination of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa*. These combined medicinal plant extracts also reduced significantly α-amylase and α-glucosidase activities indicating that the secondary metabolites present in the extracts have potential to reduce postprandial hyperglycemia by delaying the carbohydrate digestion. The antidiabetic ability to inhibit α-amylase, α-glucosidase enzymes and free radical scavenging activities are needed to be further explored using *in vivo* experimental models to validate the findingsinthepresentstudy.Hence, our study concludes that *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* can be considered as potential antidiabetic natural drugs for the treatment of diabetes mellitus and associated complications.Thepresentfindingwouldbeusefulforfutureresearchdirectionson the application of traditional medicinal plants in the development of nutraceuticals and pharmaceuticals.

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Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

Table 1 Phytochemical screening of leaf extract of psidium*guajava*

S.no	Phytochemical tests	Hexane	Chloroform	Ethylacetate	Ethanol	Methanol	Aqueous
1	Liebermann - Burchad test(Steroid)	-	-	-	+	+	-
2	Noller's test(Triterpenoid)	-	+	-	+	+	-
3	Shinoda test(Flavonoid)	-	+	-	+	+	-
4	Furan test	-	-	-	-	+	-
5	Coumarin test	-	-	-	-	+	-
6	Sugar test	-	-	-	+	+	+
7	Quinone test	-	-	+	+	+	-
8	Saponin test	-	-	-	-	+	-
9	Acid test	-	-	-	-	-	-
10	Tannin test	-	+	-	+	+	-
11	Phenol test	-	-	+	+	+	-
12	Alkaloid test	-	+	-	+	+	-

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

Table 2 Phytochemical screening of seed extract of foeniculum*vulgare*

S.no	Phytochemical tests	Hexane	Chloroform	Ethylacetate	Ethanol	Methanol	Aqueous
1	Liebermann - Burchad test(Steroid)	-	-	-	+	+	-
2	Noller's test(Triterpenoid)	-	-	+	+	+	-
3	Shinoda test(Flavonoid)	-	-	+	+	+	-
4	Furan test	-	-	-	-	-	-
5	Coumarin test	-	-	-	+	+	-
6	Sugar test	-	-	-	+	+	+
7	Quinone test	-	-	-	-	+	-
8	Saponin test	-	-	-	-	-	-
9	Acid test	-	-	-	-	-	-
10	Tannin test	-	-	-	+	+	-
11	Phenol test	-	-	+	+	+	+
12	Alkaloid test	-	-	-	+	+	-

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

Table 3 Phytochemical screening of seed extract of nigella *sativa*

S.no	Phytochemical tests	Hexane	Chloroform	Ethylacetate	Ethanol	Methanol	Aqueous
1	Liebermann - Burchad test(Steroid)	-	-	-	-	+	-
2	Noller's test(Triterpenoid)	-	-	+	+	+	-
3	Shinoda test(Flavonoid)	-	-	-	+	+	+
4	Furan test	-	-	-	-	-	-
5	Coumarin test	-	-	-	-	-	-
6	Sugar test	-	-	-	+	+	+
7	Quinone test	-	-	-	-	+	-
8	Saponin test	-	+	+	+	-	-
9	Acid test	-	-	+	-	-	+
10	Tannin test	-	+	-	+	+	-
11	Phenol test	-	-	-	-	+	-
12	Alkaloid test	-	-	-	-	+	-

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

Table 4 Phytochemical screening of combined plant extracts of psidium *guajava,*foeniculum*vulgare and* nigella *sativa*

S.no	Phytochemical tests	Hexane	Chloroform	Ethylacetate	Ethanol	Methanol	Aqueous
1	Liebermann - Burchad test(Steroid)	-	-	-	+	+	-
2	Noller's test(Triterpenoid)	-	-	+	+	+	-
3	Shinoda test(Flavonoid)	-	-	-	+	+	-
4	Furan test	-	-	-	-	-	-
5	Coumarin test	-	-	-	-	-	-
6	Sugar test	-	-	-	+	-	-
7	Quinone test	-	+	-	+	+	-
8	Saponin test	-	-	+	+	+	-
9	Acid test	-	-	-	-	-	+
10	Tannin test	-	+	-	+	+	-
11	Phenol test	-	-	-	+	+	+
12	Alkaloid test	-	-	-	+	+	-

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

Table 5 quantitative analysis of phytochemical screening of Psidiumguajava.

S.No	EXTRACTS	ESTIMATION OF PHENOL	ESTIMATION OF FLAVONOID
1	METHANOL	0.575	0.354
2	AQUEOUS	0.683	0.500

Table 6 quantitative analysis of phytochemical screening of Foeniculumvulgare.

S.No	EXTRACTS	ESTIMATION OF PHENOL	ESTIMATION OF FLAVONOID
1	METHANOL	0.357	0.469
2	AQUEOUS	0.495	0.694

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

Table 7 quantitative analysis of phytochemical screening of Nigella sativa.

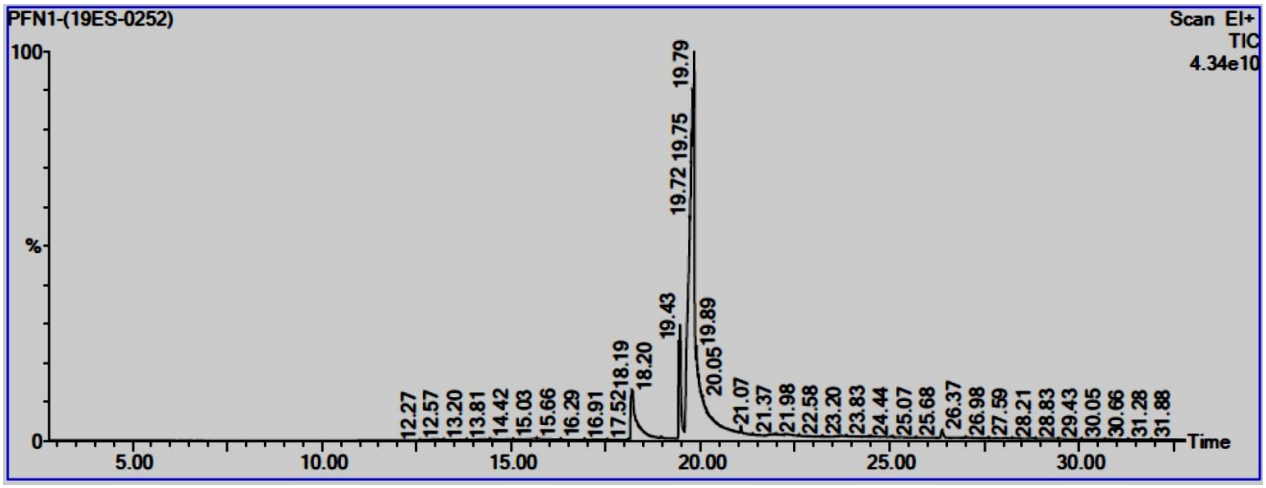
S.No	EXTRACTS	ESTIMATION OF PHENOL	ESTIMATION OF FLAVONOID
1	METHANOL	0.306	0.759
2	AQUEOUS	0.365	0.771

Table 8 quantitative analysis of phytochemical screening of combined plant extracts of Psidiumguajava, Foeniculumvulgare and Nigella sativa.

S.No		ESTIMATION OF PHENOL	ESTIMATION OF FLAVONOID
1	METHANOL	0.435	0.858
2	AQUEOUS	0.629	0.892

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

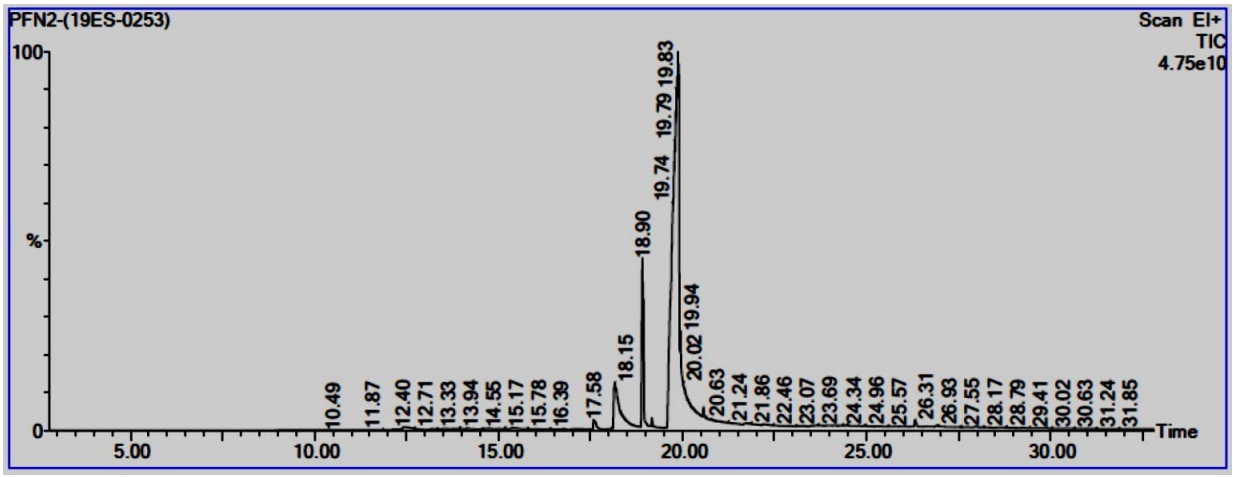
Fig.5 GC-MS CHROMATOGRAM OF PFN (METHANOLIC EXTRACT)



S. No.	Retention Time	Area %
1	18.190	10.192
2	19.425	2.870
3	19.455	5.180
4	19.830	81.757

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

Fig.6 GC-MS CHROMATOGRAM OF PFN2 (AQUAEOUS EXTRACT)



S. No.	Retention Time	Area %
1	18.149	8.977
2	18.905	5.412
3	18.935	2.809
4	19.870	74.618
5	19.940	8.184

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

Table 9 Phytochemicals present in PFN (METHANOL EXTRACT)

S. No.	Name of the Compound	Molecular Formula	Molecular Weight g/mol	Retention Time (min)
1	N-HEXADECANOIC ACID	C16H32O2	256	18.190
2	9,12-OCTADECADIENOIC ACID (Z,Z)-	C18H32O2	280	19.425
3	9-OCTADECYNE	C18H34	250	19.455
4	OLEYL ALCOHOL	C18H36O	268	19.830

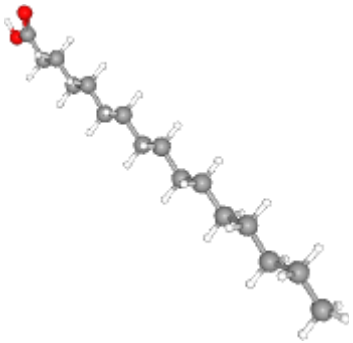
Table 10 Phytochemicals present in PFN2 (AQUEOUS EXTRACT)

S. No.	Name of the Compound	Molecular Formula	Molecular Weight g/mol	Retention Time (min)
1	N-HEXADECANOIC ACID	C16H32O2	256	18.149
2	9,12-OCTADECADIENOIC ACID, METHYL ESTER	C19H34O2	294	18.905
3	METHYL 13-OCTADECENOATE	C19H36O2	296	18.935
4	OXACYCLOHEPTADEC-8-EN-2-ONE	C16H28O2	252	19.870
5	9-EICOSYNE	C20H38	278	19.940

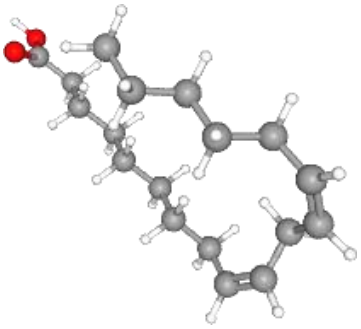
Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

Fig.5A PFN Methanol extract 3d compound structure

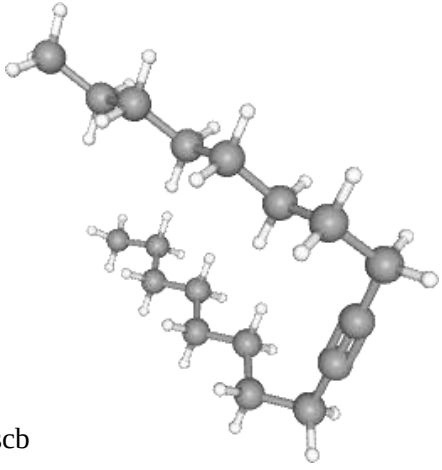
1. N-HEXADECANOIC ACID (Palmatic acid)



2. 9,12-OCTADECADIENOIC ACID (Z,Z)- (Linoleic acid)

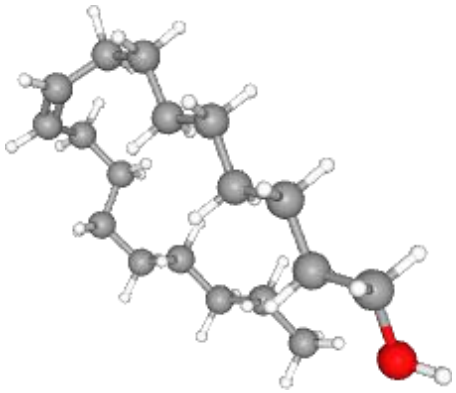


3. 9-OCTADECYNE



Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

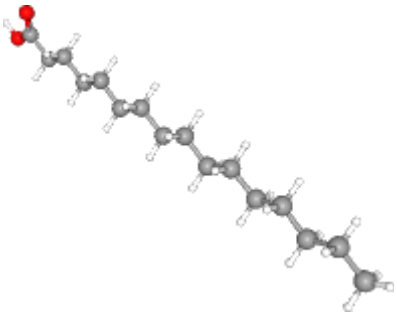
4. OLEYL ALCOHOL



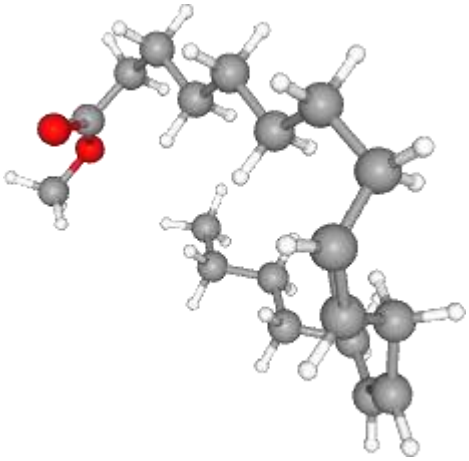
Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

Fig.6A PFN Aqueous extract 3d compound structure

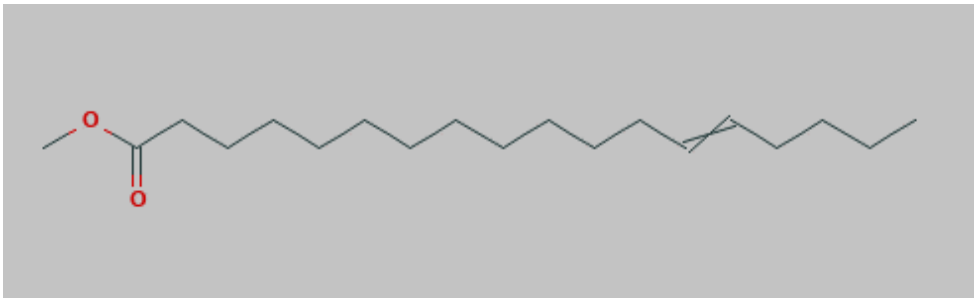
1. N-HEXADECANOIC ACID (Palmitic acid)



2. 9,12-OCTADECADIENOIC ACID, METHYL ESTER (Methyl linoleate)



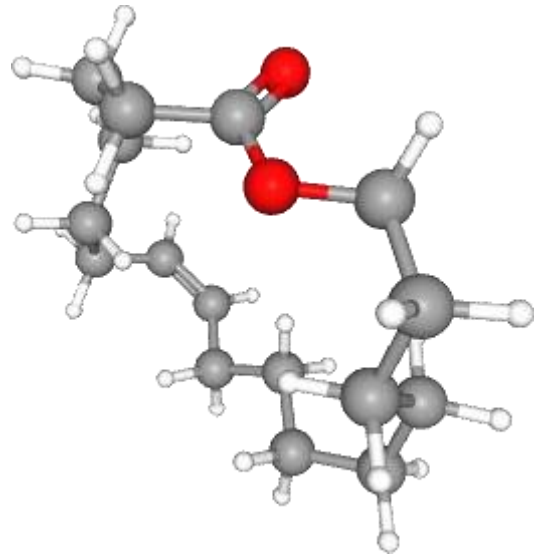
3. METHYL 13-OCTADECENOATE (2D structure)

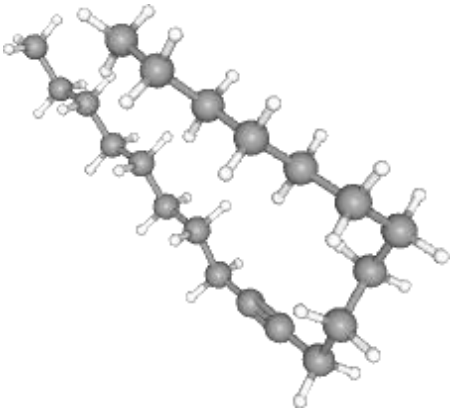


Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

4. OXACYCLOHEPTADEC-8-EN-2-ONE (Ambrettolide compound)

9-EICOSYNE





Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

Table 21 Cell line activity of *Pg*ME+*Fv*ME+*Ns*ME and *Pg*AqE+*Fv*AqE+*Ns*AqE

concentration	*PFN*.methanol	*PFN*. Aqueous
1000	57.48 ± 0.29	55.24 ± 0.34
500	63.26 ± 0.29	61.52 ± 0.35
250	69.16 ± 0.32	67.81 ± 0.37
125	75.59 ± 0.35	72.87 ± 0.39
62.5	81.36 ± 0.33	79.53 ± 0.41
31.2	87.13 ± 0.42	85.45 ± 0.41
15.6	93.43 ± 0.41	91.49 ± 0.43
7.8	99.73 ± 0.45	97.28 ± 0.44

Effect of sample *on RIN 5f* cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	**1000**	**Neat**	**0.438**	**57.48**
2	**500**	**1:1**	**0.482**	**63.26**
3	**250**	**1:2**	**0.527**	**69.16**
4	**125**	**1:4**	**0.576**	**75.59**
5	**62.5**	**1:8**	**0.620**	**81.36**
6	**31.2**	**1:16**	**0.664**	**87.13**
7	**15.6**	**1:32**	**0.712**	**93.43**
8	**7.8**	**1:64**	**0.760**	**99.73**
9	**Cell control**	**1:64**	**0.762**	**100**

Annals of R.S.C.B., Vol. 24, Issue 1, 2020, pp. 744- 771
Received 18April2020; accepted 23June2020

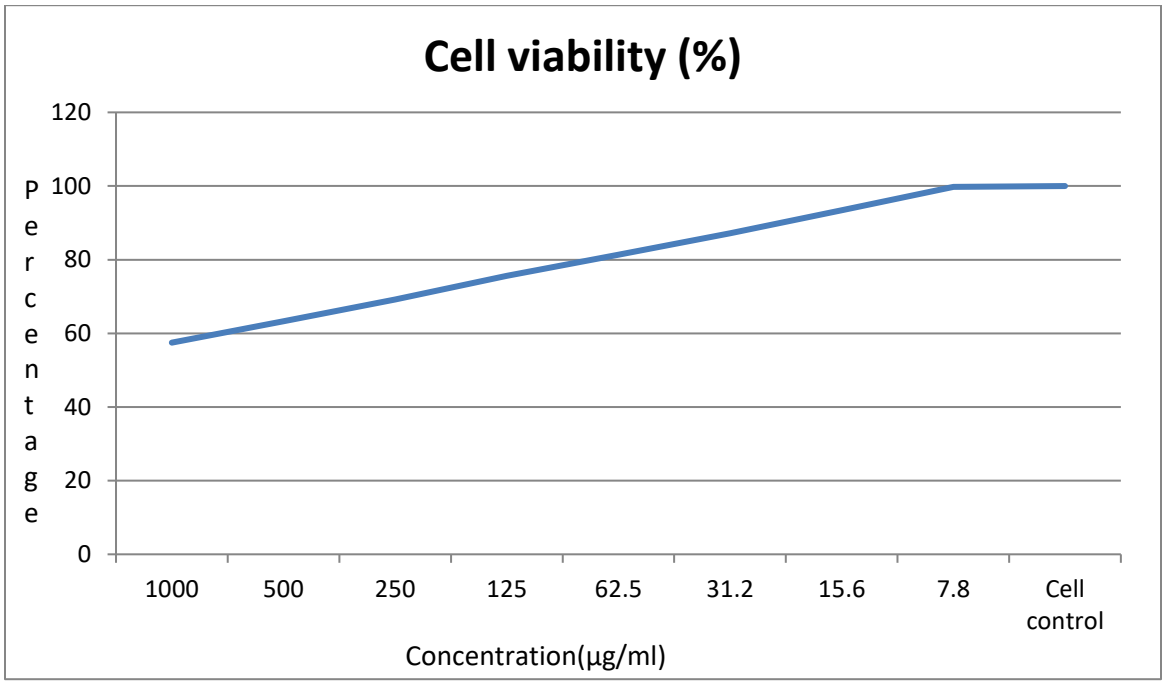
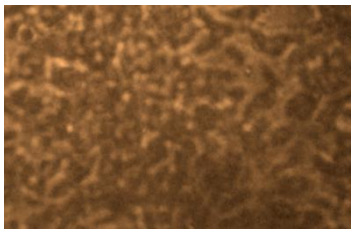
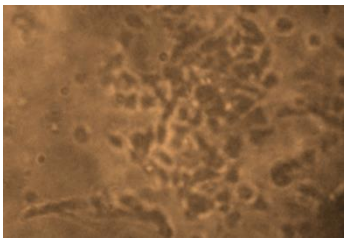


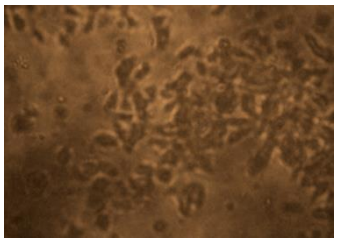
Figure:7Effect of Sample *on RIN 5f* cell line



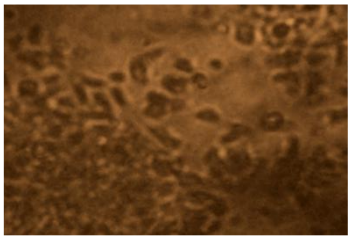
Normal RIN 5f Cell line



Toxicity – 1000 µg/ml



Toxicity –500 µg/ml



Toxicity – 7.8 µg/ml