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# Antioxidant activity in aqueous and methanol extract of combinations of Psidiumguajava, Foeniculumvulgare and Nigella sativa

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**Abstract**

**Background:** Natural antioxidants are widely distributed in food and medicinal plants. These natural antioxidants, especially polyphenols and carotenoids, exhibit a wide range of biological effects, including anti-inflammatory, anti-aging, anti-atherosclerosis and anticancer.
**Objectives:** To study the antioxidantactivity in aqueous and methanol extract of combinations of *Psidiumguajava, Foeniculumvulgare and Nigella sativa*.
**Methods:** The antioxidant activity was done as per the standard methods in the aqueous and methanolic extract of *Psidiumguajava, Foeniculumvulgare and Nigella sativa*by usingDPPH free radical scavenging activity, FRAP assay, Superoxide scavenging assay, phosphomolybdenum complex.
**Results&Discussion:**Our results show high DPPH scavenging activity.Aqueous extract of combination of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* showed the maximum level of inhibition in FRAP assay and it was found to be 97% likewise aqueous extracts of combination of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* also showed the maximum level of inhibition and was found to be 88 % respectively. Aqueous and methanol extract of combination of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* showed a concentration-dependent increase in the inhibition of superoxide generation and the highest scavenging activity for O2 •-, was observed at a concentration of 120µg/ml. Total antioxidant capacity was analysed by using Phosphomolybdenum complex. In our study aqueous and methanol extract of combination of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* showed a maximum level of antioxidant capacity and it was found to be more than 70% This could due to the high contents of total phenolics and flavonoids in these extracts.
**Conclusion:** In the present study, we have investigated the antioxidant potential of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa.* Therefore, it would seem likely that both solvents were able to extract those compounds which are responsible for the antioxidant activity of the plant. Hence, our study concludes that *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa* can be considered as potential antioxidant natural drugs for the treatment of various diseases.
**Keywords:*Psidiumguajava, Foeniculumvulgare,Nigella sativa,Antioxidant activity***

## 1. Introduction

Plants produce an incredible diversity of secondary metabolites, which have multiple

functions throughout the plant's life cycle. Besides their role as mediators in the interaction of the plant with its biotic and abiotic environments, such as plant-microbe, plant-animal and plant– plant interactions, plant secondary metabolites are involved in the fertility and germination of pollen (Schijlen et al., 2004). Secondary metabolites are derived from primary metabolites, like all amino acids and carbohydrates are converted to secondary metabolites through methylation, hydroxylation, and glycosylation biochemical pathways. Up to date, a few thousands of different secondary metabolite structures have been identified in plants; the largest of them are the phenyl- propanoids (synonym, phenylethanoids), then isoprenoids and alkaloids are placed.

Plants normally develop several components of the antioxidant system in response to naturally occurring stresses such as stress at high altitude, chilling, draught, and nutrient deficiencies (Jordan, 2002). More attention has been paid over the past ten years to the effects of UV-B radiation on oxidative stress as well as phenyl propanoids is paying a vital role as antioxidants in plants (Turunen and Latola, 2005).

Plants normally develop several components of the antioxidant system in response to naturally occurring stresses such as stress at high altitude, chilling, draught, and nutrient deficiencies (Jordan, 2002). More attention has been paid over the past ten years to the effects ofUV-B radiation on oxidative stress as well as phenylpropanoids are paying a vital role as antioxidants in plants (Turunen and Latola, 2005). According to Hartmann (1999) alkaloids which are a large family of nitrogen-containing secondary metabolites, are functioned as defense against predators, especially mammals, because of their toxicity and deterrent capabilities. In 2004, Rackova et al. discovered that three alkaloids like berberine, jatrorrhizine, and magnoflorine from Mahoniaaquifolium have potent anti-radical as well as antioxidant capacity. Phenolic alkaloids also serve as a new class of antioxidant agents of various medicinal plants. Antioxidant activities of different phenolic alkaloids (Oleracein A, Oleracein B and Oleracein E) was also reported based on scavenging activity against DPPH radical and inhibitory effect on hydrogen peroxide-induced lipid peroxidation in rat brain homogenates (Zijuan et al., 2009)

In the presence of a hydrogen/electron donor (free radical scavenging antioxidant) the absorption intensity is decreased and the radical solution (the purple chromogen of DPPH radicals) is discoloured to a pale yellow hydrazine according to the number of electrons captured (Locatelli et al., 2009). DPPH works in both electron transfer (SET) and hydrogen transfer (HAT) systems and allows the determination of a substance or a complex mixture that donate either hydrogen atoms or electrons in a homogeneous system (Paixao et al., 2007). Sini et al. (2010) who studied investigated the antioxidant activity of

certain medicinal plants of Attapady, (Palakkad), India using DPPH assay.

The FRAP assay is based on the ability to reduce yellow ferric tripyridyltriazine complex (Fe(III)-TPTZ) to blue ferrous complex (Fe(II)-TPTZ) by electron-donating antioxidants in an acidic medium (Benzie & Strain, 1999; Wojdylo et al., 2007). It is Simple, rapid, inexpensive and robust assay requiring no specialized equipment and can be performed manually or automatically. The literature show that FRAP method is sensitive in the measurement of totalantioxidant power of the fresh biological fluids, such as plant homogenates and pharmacological plant. Wong et al. (2006) studied the antioxidant activity of 30 Chinese medicinal plants by using the ferric reducing antioxidant power assay.

Korycka-Dahl and Richardson (1978) reported that the superoxide anion is also very harmful to cellular components. Robak and Glyglewski (1988) reported that flavonoids are effective antioxidants mainly because they scavenge superoxide anions. Khanom et al. (2000) also reported the superoxide-scavenging and prolylendopeptidase inhibitory activities of Bangladeshi indigenous medicinal plants.

Commonly used synthetic antioxidants like BHT, BHA, ethoxyquinone and propyl gallate can produce beneficial interactions such as antimutagenic activity and antitumorigenic action, radioprotection, protection against acute toxicity of chemicals. Besides the beneficial impacts of synthetic antioxidants, these chemicals also have some adverse side-effects, as they play a key role in radio sensitization, enhanced toxicity from other chemicals, increased tumor yield from chemical carcinogens and increased mutagenic activity (Kahl, 1984; Venkatesh and Sood,2011).

Wilson et al. (1998) stated that there are number of studies which have indicated that oxidative stress is reduced in vitro, by a variety of fruit/plant extracts that contains significant levels of polyphenols, a class of phytochemicals known to have potent antioxidant properties. Eidenberger et al. (2019) studied the dose dependent inhibition of guava leaf ethanol extracts on dipeptidyl-peptidase-W due to the individual flavonoid-glycosides. Similarly, Deguchi and Miya zaki (2010) investigated the in vitro activities of α-glucosidase enzymes in guava extract was a polymerized polyphenol. Oktay et al. (2003) determined the *in vitro* antioxidant activity of fennel (Foeniculumvulgare) seed extracts. Also, Anwar et al. (2009) studied the antioxidant activities of essential oil and extracts of Foeniculumvulgare Mill seeds. Burits and Bucar (2000) studied the antioxidant activity of Nigella sativa essential oil.

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Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter. Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals. The free radicals (oxidants) are species with very short half-life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. In general, the reactive oxygen species circulating in the body tend to react with the electron of other molecules in the body and these also effect various enzyme systems and cause damage which may further contribute to conditions such as cancer, ischemia, aging, adult respiratory distress syndromes, rheumatoid arthritis etc (Chirag et al., 2013).

Natural plants have traditionally been used throughout the world for their anti- oxidant effects. Hence the present study was undertaken to study the antioxidant effects of combined plant extracts namely *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa.*

## 2. MATERIALS AND METHODS

### COLLECTION OF PLANTS

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

### PREPARATION OF EXTRACTS

Ethanol and methanol plant extracts of *Nigella sativa, Foeniculumvulgare and Psidiumguajava*were prepared as per the standard methods.

### IN VITRO ANTIOXIDANT ACTIVITIES

### DPPH˙ RADICAL SCAVENGING ACTIVITY

The antioxidant activity of ethanol and methanol plant extracts of *nigella sativa, Foeniculumvulgare and Psidiumguajava*was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (20-120 μg/mL) of both extracts. The mixture was then allowed to stand for 30 min incubation in dark. One mL methanol and 1 mL DPPH solution was used as the control. The decrease in

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absorbance was measured using UV-Vis Spectrophotometer at 517 nm Khalaf N.A *et.,al*(2008). Ascorbic acid was used as standard reference. The percentage of inhibition was calculated using the following formula:

$$\left[\% \text{ of DPPH}^{\cdot} \text{ radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100\right]$$

**SUPEROXIDE RADICAL (O2●-) SCAVENGING ASSAY**

Superoxide radical scavenging activity was carried out by the method of (Ravishankara*et.,al*) different concentrations of water dissolved ethanol and methanol extracts(20-120µg/mL) of *Nigella sativa, Foeniculumvulgare*and *Psidiumguajava*was mixed with 50mM of phosphate buffer (pH 7.8), 1.5mM of riboflavin, 12mM of EDTA and 50mM of NBT solutions and added in that sequence. The reaction was started byilluminating the reaction mixture for 15min. After illumination, the absorbance was measured at 590nm in UV-Vis Spectrophotometer. Ascorbic acid was used as standard reference.

The percentage of inhibition was calculated as:

% of superoxide radical inhibition = Control– Sample ×100/ Control

**FERRIC (FE 3+) REDUCING POWER ASSAY**

The reducing power of water dissolved ethanol and methanol plants extracts of *Nigella sativa, Foeniculumvulgare*and *Psidiumguajava*was determined by Fe 3+ reduction method with slight modification (Oyaizu.M 1986). One mL of latex extract of different concentrations (20 - 120µg/mL) was mixed with 1mL of phosphate buffer (0.2M, pH 6.6)

and 1mL of potassium ferricyanide [K3Fe (CN)6] (1% w/v). The mixtures were then incubated at 50°C in water bath for 30min. One mL of trichloroacetic acid (10 % w/v) was added to each mixture. Then 1mL of freshly prepared FeCl3 (0.1% w/v) solution was added and the absorbance was measured at 700nm in UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference.

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The percentage of reduction was calculated as:
% of Fe3+ reduction = Sample – Control x 100/ Sample

**PHOSPHOMOLYBDENUM REDUCTION ASSAY**

The antioxidant capacity of water dissolved ethanol and methanol plant extracts of *Nigella sativa, Foeniculumvulgare* and *Psidiumguajava* was assessed by Mo6+ reduction method (Prieto *et., al*). The latex extract with concentrations ranging from 20 t 120µg/mL was combined with 1mL of reagent solution containing ammonium molybdate (4mM), sodium phosphate (28mM) and sulphuric acid (600mM). The reaction mixture was incubated in water bath at 95oC for 90min. The absorbance of the coloured complex was measured at 695nm in UV-Vis spectrophotometer. Ascorbic acid was used as the standard reference.

The percentage of reduction was calculated as:
% of phosphomolybdenum reduction = Sample – Control x 100/ Sample

**STATISTICAL ANALYSIS**

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

## 3. RESULTS AND DISCUSSION

**Antioxidant activity of methanol and aqueous extracts of *Psidiumguajava*, *Foeniculumvulgare*and *Nigella sativa***

**DPPH free radical scavenging activity**

DPPH free radical scavenging activity of the extracts and the standard increased with the concentration elevation; at the maximum concentration (120µg/mL), all the extracts gave inhibition percentages than the Ascorbic acid (95.55 ± 0.17). **Table 1&2.**The methanol extract of *Nigella sativa* had the highest DPPH radical inhibition activity 83.67 ± 0.20 followed by *Psidiumguajava*and *Foeniculumvulgare.* Combination of *Psidiumguajava*, *Foeniculumvulgare*and *Nigella sativa* also exhibits DPPH free radical scavenging activity and it was found to be more than 70% at highest concentrations. Choudhary et al. (2011) studied the DPPH free radical scavenging activity of different medicinal plants in India. . Antioxidants are important substances that play a crucial role in delaying, intercepting, and preventing oxidative reactions catalyzed by free radicals and thus providing protection to humans (Vilioglu et al., 1998). Due to this special ability there is an increased use of antioxidants for the balance of reactive oxygen species. DPPH has been used extensively as a free radical to evaluate reducing substances and is a useful reagent for investigating the free radical scavenging activities of compounds (Duan and Zhang, 2006; Meir and Kanner, 1995). Our results show high DPPH scavenging activity. Many researchers have reported

positive correlation between free radical scavenging activity and total phenolic and flavonoid contents, which also matches with our findings. Medicinal plant tissues are commonlyrich in phenolic compounds such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. Although the DPPH radical scavenging abilities of the extracts were significantly lower than that of control, it was evident that the extracts showed proton-donating ability and this could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The effect of antioxidant on DPPH is believed to be due to their hydrogen-donating ability.

**FRAP assay**

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine ($Fe^{3+}$ -TPTZ) complex and producing a colored ferrous tripyridyltriazine ($Fe^{2+}$ - TPTZ). The free radical chain breaking takes place through donating a hydrogen atom. At low pH of about 3.6, reduction of $Fe^{3+}$ -TPTZ complex to blue colored $Fe^{2+}$ - TPTZ takes place, which has absorbance at 593 nm. **Table 3&4.**Aqueous extract of combination of *Psidiumguajava*, *Foeniculumvulgare* and *Nigella sativa* showed the maximum level of inhibition in FRAP assay and it was found to be 97% likewise aqueous extracts of combination of *Psidiumguajava*, *Foeniculumvulgare* and *Nigella sativa* also showed the maximum level of inhibition and was found to be 88 % respectively. The results obtained are highly reproducible and related linearly with the molar concentration of the antioxidants present. This is in accordance with the results reported by Benzie *et al* (1999) and Jeong *et al* (2001).

**Superoxide scavenging assay**

**Table 5&6.**Aqueous and methanol extract of combination of *Psidiumguajava*, *Foeniculumvulgare*and *Nigella sativa* showed a concentration-dependent increase in the inhibition of superoxide generation and the highest scavenging activity for O2 •-, was observed at a concentration of 120μg/ml. The O2 •- are generated in biological systems during cellular respiration and as such they are less toxic; however, they are converted into highly reactive OH radical in the presence of iron. Moreover, superoxide anions produced as a result of incomplete metabolism of oxygen damage biomolecules directly or indirectly by forming H2O2, •OH and peroxynitrite or singlet oxygen (Lushchak, 2014; Kirkinezosa and Mora, 2011). Therefore, the removal or neutralization of superoxide radicals is necessary to protect the cells from their deleterious effects. Various extracts of S. wallichii inhibited the formation of O2 •- in a concentration-dependent manner. Kaempferol has been found to scavenge O2 •- in an earlier report,(Gandhimathi et al., 2011). Other plant extracts

and certain plant flavonoids including mangiferin, naringin, quercetinmyricetin and rutin have been found to scavenge superoxide free radical in a concentration- dependent manner (Jagetia et al., 2005 and Lalrinzuali et al., 2015).

**Phosphomolybdenum complex (Total antioxidant capacity)**

**Table 7&8.**Total antioxidant capacity was analysed by using Phosphomolybdenum complex. The TAC of the solvent fractions was determined based on the reduction of molybdenum (VI) to molybdenum (V) and the subsequent formation of a green phosphate/molybdenum (V) complex at acidic pH. All the solvent fractions had a steady increase in TAC as the concentrations increased. Total Antioxidant Capacity (TAC) assay by phosphomolybdenum method that based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate/Mo (V) complex at acidic pH, usually detects antioxidants such as some phenolics, ascorbic acid, α-tocopherol and carotenoids (Prieto et al., 1999). In our study aqueous and methanol extract of combination of *Psidiumguajava*, *Foeniculumvulgare*and *Nigella sativa* showed a maximum level of antioxidant capacity and it was found to be more than 70% This could due to the high contents of total phenolics and flavonoids in these extracts. Due to theredox properties of phenolic compounds, they can play an important role in absorbing and neutralizing free radical**s**, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). Therefore, phenolic compounds are considered as good antioxidants (Rice-Evans *et al*., 1995). Although the lowest values of TP and TF contents recorded in the extract of *A. stipularis* underground part; this extract exhibited relatively high antioxidant capacity. This observation can be explained by the phenolic structure and presence of antioxidant compounds unlike phenolics and flavonoids. Javanmardi *et al*. (2003) mentioned that, antioxidant activity of plant extracts is not limited to phenolics. Activity may also come from the presence of other antioxidant compounds such as carotenoids, vitamins and others.

The antioxidative activities observed can be attributed to either the different mechanisms exhibited by different polyphenolic compounds that is, tocopherols, flavonoids and other organic acids and to the synergistic effects of different compounds. Many studies have shown that many polyphenols contribute significantly to the antioxidant activity (Demla and Verma, 2012; Adithya et al., 2012) and act as highly effective free radical scavengers which is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Hasan et al., 2008).

**Table 1.DPPH+ activity of *Pg*ME, *Fv*ME, *Ns*ME and*Pg*ME+*Fv*ME+*Ns*ME**

| Conc. (µg/mL) | Ascorbic Acid | *Psidium* Guajava | *Foeniculum Vulgare* | *Niigella Sativa* | *PFN*.methanol |
|---|---|---|---|---|---|
| 20 | 37.54 ± 0.60 | 28.79 ± 0.12 | 20.42 ± 0.13 | 46.01 ± 0.17 | 53.66 ± 0.13 |
| 40 | 57.22 ± 0.40 | 29.55 ± 0.18 | 30.79 ± 0.14 | 46.38 ± 0.19 | 67.33 ± 0.34 |

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| | | | | | |
|---|---|---|---|---|---|
| 60 | 73.84 ± 0.28 | 33.01 ± 0.21 | 32.01 ± 0.15 | 62.52 ± 0.21 | 73.66 ± 0.27 |
| 80 | 84.18 ± 0.44 | 33.39 ± 0.19 | 44.20 ± 0.17 | 70.50 ± 0.23 | 74.00 ± 0.18 |
| 100 | 95.11 ± 0.44 | 34.54 ± 0.23 | 51.52 ± 0.19 | 73.09 ± 0.18 | 76.66 ± 0.23 |
| 120 | 95.55 ± 0.17 | 42.99 ± 0.22 | 57.62 ± 0.18 | 83.67 ± 0.20 | 79.33 ± 0.37 |

**Table 2. DPPH activity of *Pg*AqE, *Fv*AqE, *Ns*AqE and*Pg*AqE+*Fv*AqE+*Ns*AqE**

| Conc. (µg/mL) | Ascorbic Acid | *Psidium Guajava* | *Foeniculum Vulgare* | *Niigella Sativa* | *PFN*.aqueous |
|---|---|---|---|---|---|
| 20 | 37.54 ± 0.60 | 19.80 ± 0.17 | 07.31 ± 0.12 | 07.53 ± 0.11 | 49.15 ± 0.12 |
| 40 | 57.22 ± 0.40 | 23.00 ± 0.19 | 12.19 ± 0.14 | 14.52 ± 0.09 | 66.77 ± 0.09 |
| 60 | 73.84 ± 0.28 | 28.02 ± 0.22 | 21.64 ± 0.16 | 29.22 ± 0.13 | 71.18 ± 0.15 |
| 80 | 84.18 ± 0.44 | 30.35 ± 0.16 | 42.98 ± 0.21 | 34.74 ± 0.17 | 73.89 ± 0.16 |
| 100 | 95.11 ± 0.44 | 34.18 ± 0.23 | 45.12 ± 0.18 | 36.76 ± 0.14 | 74.91 ± 0.19 |
| 120 | 95.55 ± 0.17 | 47.60 ± 0.26 | 48.47 ± 0.13 | 47.42 ± 0.12 | 75.59 ± 0.18 |

**Table 3. Superoxide radical scavenging activity of *Pg*ME, *Fv*ME, *Ns*ME and *Pg*ME+*Fv*ME+*Ns*ME**

| Conc. (µg/mL) | Ascorbic Acid | *Psidium Guajava* | *FoeniculumVulgare* | *Niigella Sativa* | *PFN*.methanol |
|---|---|---|---|---|---|
| 20 | 47.96 ± 0.31 | 08.23 ± 0.14 | 09.07 ± 0.13 | 21.83 ± 0.14 | 18.94 ± 0.10 |
| 40 | 54.13 ± 0.05 | 17.54 ± 0.18 | 15.56 ± 0.15 | 33.70 ± 0.19 | 28.51 ± 0.12 |
| 60 | 65.07 ± 1.06 | 19.48 ± 0.22 | 23.04 ± 0.19 | 40.64 ± 0.23 | 39.99 ± 0.13 |
| 80 | 77.92 ± 0.27 | 22.13 ± 0.26 | 26.07 ± 0.21 | 54.61 ± 0.26 | 53.64 ± 0.08 |
| 100 | 83.06 ± 0.10 | 25.19 ± 0.31 | 34.01 ± 0.23 | 62.00 ± 0.29 | 66.53 ± 0.14 |
| 120 | 89.29 ± 0.24 | 43.40 ± 0.35 | 40.59 ± 0.25 | 72.28 ± 0.33 | 79.52 ± 0.12 |

**Table 4. Superoxide radical scavenging activity of *Pg*AqE, *Fv*AqE, *Ns*AqE and *Pg*AqE+*Fv*AqE+*Ns*AqE**

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| Conc. (µg/mL) | Ascorbic Acid | *Psidium Guajava* | *Foeniculum Vulgare* | *Niigella Sativa* | *PFN*.aqueous |
|---|---|---|---|---|---|
| 20 | 47.96 ± 0.31 | 16.53 ± 0.09 | 11.44 ± 0.17 | 23.61 ± 0.13 | 32.71 ± 0.06 |
| 40 | 54.13 ± 0.05 | 19.09 ± 0.12 | 21.02 ± 0.19 | 37.70 ± 0.16 | 48.45 ± 0.09 |
| 60 | 65.07 ± 1.06 | 22.43 ± 0.17 | 28.73 ± 0.22 | 43.59 ± 0.19 | 52.65 ± 0.08 |
| 80 | 77.92 ± 0.27 | 28.67 ± 0.19 | 37.12 ± 0.24 | 58.73 ± 0.23 | 58.68 ± 0.10 |
| 100 | 83.06 ± 0.10 | 38.08 ± 0.23 | 49.34 ± 0.28 | 67.46 ± 0.27 | 69.50 ± 0.12 |
| 120 | 89.29 ± 0.24 | 44.24 ± 0.25 | 53.36 ± 0.30 | 8 1.59 ± 0.31 | 93.42 ± 0.11 |

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**Table 5. FRAP activity of *Pg*ME, *Fv*ME, *Ns*ME and *Pg*ME+*Fv*ME+*Ns*ME**

| Conc. (µg/mL) | Ascorbic Acid | *Psidium* Guajava | *FoeniculumV ulgare* | *Niigella Sativa* | *PFN*.methanol |
|---|---|---|---|---|---|
| 20 | 38.31 ± 0.52 | 15.99 ± 0.10 | 11.29 ± 0.15 | 35.73 ± 0.16 | 36.77 ± 0.14 |
| 40 | 51.91 ± 0.94 | 21.39 ± 0.13 | 19.48 ± 0.18 | 40.26 ± 0.19 | 59.56 ± 0.18 |
| 60 | 65.18 ± 0.59 | 29.75 ± 0.15 | 25.72 ± 0.22 | 48.82 ± 0.23 | 63.34 ± 0.24 |
| 80 | 74.10 ± 0.30 | 34.83 ± 0.17 | 31.81 ± 0.25 | 52.19 ± 0.27 | 74.33 ± 0.27 |
| 100 | 75.24 ± 0.58 | 39.98 ± 0.16 | 34.93 ± 0.28 | 59.28 ± 0.21 | 79.25 ± 0.32 |
| 120 | 77.40 ± 0.82 | 42.63 ± 0.19 | 38.34 ± 0.31 | 61.49 ± 0.25 | 88.13 ± 0.35 |

**Table 6. FRAP activity of *Pg*AqE, *Fv*AqE, *Ns*AqE and *Pg*AqE+*Fv*AqE+*Ns*AqE**

| Conc. (µg/mL) | Ascorbic Acid | *Psidium* Guajava | *FoeniculumV ulgare* | *Niigella Sativa* | *PFN*.aqueous |
|---|---|---|---|---|---|
| 20 | 38.31 ± 0.52 | 39.59 ± 0.12 | 25.78 ± 0.12 | 50.45 ± 0.13 | 59.56 ± 0.17 |
| 40 | 51.91 ± 0.94 | 44.78 ± 0.14 | 29.87 ± 0.14 | 55.12 ± 0.17 | 63.34 ± 0.24 |
| 60 | 65.18 ± 0.59 | 49.23 ± 0.17 | 33.34 ± 0.16 | 62.99 ± 0.23 | 89.65 ± 0.28 |
| 80 | 74.10 ± 0.30 | 53.29 ± 0.21 | 35.66 ± 0.18 | 67.13 ± 0.22 | 91.12 ± 0.33 |
| 100 | 75.24 ± 0.58 | 58.02 ± 0.25 | 39.81 ± 0.20 | 67.24 ± 0.27 | 93.05 ± 0.38 |
| 120 | 77.40 ± 0.82 | 60.01 ± 0.29 | 42.13 ± 0.19 | 68.85 ± 0.29 | 97.86 ± 0.37 |

**Table 7. Phosphomolybdenum activity of *Pg*ME, *Fv*ME, *Ns*ME and *Pg*ME+*Fv*ME+*Ns*ME**

| Conc. (µg/mL) | Ascorbic Acid | *Psidium* Guajava | *FoeniculumV ulgare* | *Niigella Sativa* | *PFN*.methanol |
|---|---|---|---|---|---|
| 20 | 45.15 ± 0.38 | 07.06 ± 0.20 | 05.48 ± 0.14 | 15.98 ± 0.23 | 22.75 ± 0.13 |
| 40 | 67.97 ± 0.33 | 13.49 ± 0.17 | 11.63 ± 0.18 | 24.57 ± 0.28 | 31.68 ± 0.34 |
| 60 | 77.99 ± 0.79 | 18.62 ± 0.22 | 15.49 ± 0.24 | 37.44 ± 0.19 | 45.83 ± 0.27 |
| 80 | 88.98 ± 0.33 | 24.88 ± 0.26 | 21.87 ± 0.27 | 44.77 ± 0.30 | 57.78 ± 0.18 |
| 100 | 99.21 ± 0.98 | 29.88 ± 0.19 | 25.08 ± 0.23 | 51.65 ± 0.17 | 62.59 ± 0.23 |
| 120 | 99.36 ± 0.04 | 35.43 ± 0.19 | 33.01 ± 0.28 | 67.01 ± 0.34 | 70.28 ± 0.37 |

**Table 8. Phosphomolybdenum activity of *Pg*AqE, *Fv*AqE, *Ns*AqE and *Pg*AqE+*Fv*AqE+*Ns*AqE**

| Conc. (µg/mL) | Ascorbic Acid | *Psidium* Guajava | *FoeniculumV ulgare* | *Niigella Sativa* | *PFN*.aqueous |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| 20 | 45.15 ± 0.38 | 08.15 ± 0.07 | 11.78 ± 0.17 | 28.03 ± 0.31 | 25.28 ± 0.43 |
| 40 | 67.97 ± 0.33 | 14.84 ± 0.10 | 13.24 ± 0.25 | 34.32 ± 0.35 | 33.98 ± 0.45 |
| 60 | 77.99 ± 0.79 | 19.98 ± 0.13 | 16.53 ± 0.18 | 39.97 ± 0.28 | 47.64 ± 0.38 |
| 80 | 88.98 ± 0.33 | 25.52 ± 0.19 | 21.98 ± 0.14 | 45.63 ± 0.39 | 59.70 ± 0.26 |
| 100 | 99.21 ± 0.98 | 31.34 ± 0.20 | 26.74 ± 0.26 | 52.93 ± 0.27 | 63.11 ± 0.33 |
| 120 | 99.36 ± 0.04 | 37.00 ± 0.24 | 33.34 ± 0.23 | 67.68 ± 0.43 | 72.31 ± 0.31 |

## 4. Conclusion

In the present study, we have investigated the antioxidant potential of *Psidiumguajava, Foeniculumvulgare*and *Nigella sativa.* Therefore, it would seem likely that both solvents were able to extract those compounds which are responsible for the antioxidant activity of the plant. Hence, our study concludes that *Psidiumguajava, Foeniculumvulgare* and *Nigella sativa* can be considered as potential antioxidant natural drugs for the treatment of various diseases. The present finding would be useful for future research directionson the application of traditional medicinal plants in the development of nutraceuticals and pharmaceuticals.

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