

# Diapin<sup>®</sup>: A food supplement with diverse therapeutic potentials

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## ABSTRACT

**Background and Aims:** For many centuries herbs and spices have traditionally been used to treat or manage a variety of diseases. The formulation of food supplements containing single or multiple herbs or spices is now popular. These formulations provide biochemical, pharmacological and medicinal benefits due to their diverse phytochemical constituents.

**Methods:** In the present study, Diapin<sup>®</sup> – a food supplement containing *Olea europaea* L. leaves extract, *Cinnamomum cassia* (L.) J. Presl stem extract, *Nigella sativa* L. seed oil, *Cocos nucifera* L. oil and vitamin D3 – dissolved in absolute ethanol was evaluated for  $\alpha$ -amylase,  $\alpha$ -glucosidase, acetylcholinesterase, elastase, neuraminidase, adenosine deaminase and arginase inhibitory activity.

**Results:** The supplement strongly inhibited neuraminidase ( $IC_{50} = 0.272 \pm 0.007$  mg/mL), while adenosine deaminase, acetylcholinesterase, elastase and arginase were moderately inhibited (with an  $IC_{50}$  of  $4.562 \pm 0.052$ ,  $5.396 \pm 0.563$ ,  $5.783 \pm 0.058$  and  $6.800 \pm 0.067$  mg/mL respectively). The less inhibition activity was on  $\alpha$ -amylase and  $\alpha$ -glucosidase ( $IC_{50} = 9.593 \pm 0.582$  and  $14.010 \pm 2.280$  mg/mL respectively).

**Conclusion:** The pharmacological activities of Diapin<sup>®</sup> can be attributed to its opulent phytochemical composition. The present findings support the folkloric claim of Diapin<sup>®</sup> supplement having antidiabetic, anticancer, anti-inflammatory, antimicrobial, anti-ageing, and immune bolstering properties, in addition to the mitigation of Alzheimer's disease and the alleviation of neurological dysfunction.

**Keywords:** Diapin<sup>®</sup>, *Olea europaea*, *Cinnamomum cassia*, *Nigella sativa*, *Cocos nucifera*, Enzyme inhibition, Extracts

## INTRODUCTION

Food supplements, also known as dietary supplements, are diet based formulations (from a singular or combined source) containing vitamins, minerals, essential fatty acids, amino acids or even fibre. Beside these, plant based food supplements contain bioactive compounds that include phenols, terpenoids, thiols, saponins, glycosides, amines, essential oils etc. Thus, they exert pharmacological and therapeutic effects above and beyond their nutritional functions (Garcia-Alvarez *et al.*, 2014). Supplements are usually sold as tablets, capsules or in liquid forms. They are easily administered or consumed orally, either before, during or after regular meals. Since they are food based, it is claimed that they have minimal or no adverse effects. In general terms, no clear cut difference exist between food supplements and nutraceuticals. Nevertheless, nutraceuticals (also bioceutical, or sometimes

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functional foods) are more or less food based pharmaceutical alternatives, believed to have biochemical or physiological significance (Hardy, 2000; Kalra, 2003). They are categorised as food supplements and/or food additives by the Food and Drug Administration (FDA) of the United States. Despite the wide use/acceptability of food supplements and nutraceuticals, doubt about the declared benefits and pharmacological effects of these products remains a serious concern. More so, the safety and quality of such products are significant issues (Hasler, 2005).

Olive (*Olea europaea* L.) is a predominant plant in the Mediterranean region (Waterman & Lockwood 2007). The plant fruits are rich sources of unsaturated fatty acid, vitamin E, minerals and carbohydrate. In addition, its fruits and leaves are composed of biologically active phytochemicals, with diverse therapeutic benefits (Jilani, Cilla, Barberá, & Hamdi, 2016; Guo *et al.*, 2018). Hitherto, the therapeutic and/or medicinal benefit of the Mediterranean diet has been attributed to the olive rich component of the diet (Obied *et al.*, 2012; Roman, Jackson, Gadhia, Roman, & Reis, 2019). The health benefits are said to include higher life expectancy and decreased occurrence of degenerative diseases (Vogel *et al.*, 2014; Morris *et al.*, 2015; Guo *et al.*, 2018; Roman *et al.*, 2019). Therefore, the therapeutic benefits of olives cannot be over emphasized.

Cinnamon (also cassia) is a spice obtained from the inner bark of a plant species of the genus *Cinnamomum*. The spice is chiefly composed of cinnamaldehyde and many essential oils (including eugenol), the compounds responsible for aromatic and flavouring properties (Jayaprakasha & Rao, 2011). This spice has a history from ancient time, and it is both sacred and highly priced (Gray & Miller, 1970). Cinnamon is reported to have a lowering effect on total cholesterol and triacylglycerols (Maieran *et al.*, 2017), and it is said to aid digestion as well as to have a controversial effect on both diabetes (Leach & Kumar, 2012) and glycated haemoglobin levels (Akilen, Tsiami, Devendra, & Robinson, 2012; Leach & Kumar, 2012; Costello *et al.*, 2016).

Black seed (*Nigella sativa* L.) has been historically used as an essential herb. In accordance with prophetic sayings, Muslims believe it can cure all diseases except death (Al-Bukhari, 1976). In addition to protein, carbohydrate and unsaturated/essential oils, the plants is rich in active phytochemical such as thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, 4-terpineol, t-anethol, sesquiterpene longifolene,  $\alpha$ -pinene and thymol. Moreover, nigellicimine, nigellicimine-N-oxide, nigellidine, nigellicine and alpha-hederin are found in trace amount. Vitamins, provitamins (carotene, stigmasterol among other sterols) and minerals (copper, zinc, phosphorus and iron) are found in the plant seed (Al-Jassir, 1992; Nickavar, Mojab, Javidnia, & Amoli, 2003). A review by Ahmad *et al.* (2013) indicates that black seeds have tremendous pharmacological benefits such as antimicrobial, anti-schistosomiasis, antioxidants, anti-inflammatory and anticancer effect. The plant was also reported to have antidiabetic effect, to act as a vasodilator in the lung, and to protect gastrointestinal, hepatic and renal tissues.

Coconut (*Cocos nucifera* L.) is a member of the palm family Arecaceae, whose edible fruit is widely consumed due to its fat composition and milk (Lebrun, Grivet, & Baudouin, 2013; Nayar, 2017). The fresh fruits contains high amount of saturated fats, and lower amounts of carbohydrates and proteins. It is reported to contain significant amount of micronutrients that includes selenium, zinc, copper, iron, manganese and phosphorus (Naik, Raghavendra, & Raghavarao, 2012). Despite containing appreciable levels of antioxidant elements, excessive and chronic consumption of coconut is associated with high risk of cardiovascular diseases due to its high levels of saturated fats. This is manifested as high levels of LDL cholesterol and lauric acid in the blood (Neelakantan, Seah, & van Dam, 2020).

Diapin® (NatiVital) is a Turkish food supplement made from *O. europaea* L. leaves extract, *Cinnamomum cassia* (L.) J. Presl stem extract, *N. sativa* L. seed oil, *Cocos nucifera* L. (coconut) oil and vitamin D3 (cholecalciferol). It is believed to have ample therapeutic benefits, thus prompting the present research. The present study is aimed at investigating the inhibitory effects of Diapin® on the activity of some important metabolic enzymes (viz;  $\alpha$ -amylase,  $\alpha$ -glucosidase, acetyl cholinesterase, elastase, neuraminidase, angiotensin-converting enzyme and arginase).

## MATERIALS AND METHODS

### Sample preparation

Diapin® capsule was pierced, and the content emptied into a weighed empty beaker. A stock solution of 50 mg/ml was prepared by dissolving the obtained contents in absolute ethanol via sonication. Thereafter, serial dilution was prepared from the stock and used for enzyme inhibition study.

### Enzyme inhibition assay

The inhibitory effect of Diapin® on activities of  $\alpha$ -amylase,  $\alpha$ -glucosidase, acetylcholinesterase, elastase, neuraminidase, arginase and adenosine deaminase were determined according to the method of Bhutkar & Bhise (2012); Tao, Zhang, Cheng, & Wang, 2013; Ingkaninan, Temkitthawon Chuenchon, Yuyaem, & Thongnoi, 2003; Moon, Yim, Song, Lee, & Hyun, 2010; Myers *et al.*, (1980); Corraliza, Campo, Soler, & Modolell, 1994, and Blum & Schwedt (1998) respectively. The findings of this research are expressed as mean  $\pm$  standard deviation of three replicate values. Percentage enzyme inhibition activities of the inhibitors were used to calculate half maximum inhibitions ( $IC_{50}$ ) for individual enzymes, via regression analysis data. The lower the  $IC_{50}$  values, the higher the inhibition activity.

## RESULTS

The effect of Diapin® on the activities of  $\alpha$ -amylase,  $\alpha$ -glucosidase and acetylcholinesterase are presented in Table 1. Diapin® inhibited  $\alpha$ -amylase,  $\alpha$ -glucosidase and acetylcholinesterase with an  $IC_{50}$  of  $9.593 \pm 0.582$ ,  $14.010 \pm 2.280$  and  $5.396 \pm 0.563$  mg/mL respectively. These inhibition activities were below that of the corresponding standard inhibitors of the enzymes. Acarbose inhibited  $\alpha$ -amylase and  $\alpha$ -glucosidase with an  $IC_{50}$  of  $0.059 \pm 0.002$  and  $0.177 \pm 0.010$  mg/mL, while 1,2,3,4-tetrahydroacridin-9-amine hydrochloride (tacrine) inhibited acetylcholinesterase with a very low  $IC_{50}$  of  $8.833 \times 10^{-4} \pm 2.943 \times 10^{-5}$  mg/mL.

**Table 1. Inhibitory effect of Diapin® on  $\alpha$ -amylase,  $\alpha$ -glucosidase and acetylcholinesterase activities.**

| Enzyme                | Extract/ Standard | Concentration (mg/mL) | Inhibition (%)* | IC <sub>50</sub> (mg/mL)*                      |
|-----------------------|-------------------|-----------------------|-----------------|--|
| $\alpha$ -Amylase     | Diapin®           | 30.000                | 91.614±1.929    | 9.593±0.582                                    |
|                       |                   | 20.000                | 88.476±0.943    |  |
|                       |                   | 10.000                | 61.892±2.729    |  |
|                       |                   | 1.000                 | 16.356±1.061    |  |
|                       | Acarbose          | 0.050                 | 43.042±1.994    | 0.059±0.002                                    |
|                       |                   | 0.030                 | 28.620±0.875    |  |
|                       |                   | 0.010                 | 12.458±1.179    |  |
|                       |                   | 0.005                 | 8.642±0.927     |  |
| $\alpha$ -Glucosidase | Diapin®           | 50.000                | 88.001±1.412    | 14.010±2.280                                   |
|                       |                   | 40.000                | 69.464±1.082    |  |
|                       |                   | 30.000                | 64.168±1.880    |  |
|                       |                   | 20.000                | 58.044±2.032    |  |
|                       | Acarbose          | 0.050                 | 16.092±1.249    | 0.177±0.010                                    |
|                       |                   | 0.030                 | 13.291±0.300    |  |
|                       |                   | 0.010                 | 6.791±0.755     |  |
|                       |                   | 0.005                 | 4.390±0.458     |  |
| Acetylcholinesterase  | Diapin®           | 5.000                 | 45.284± 3.070   | 5.396±0.563                                    |
|                       |                   | 2.500                 | 35.814±2.812    |  |
|                       |                   | 1.000                 | 27.659±2.086    |  |
|                       |                   | 0.500                 | 12.101±0.396    |  |
|                       | Tacrine           | 0.003                 | 90.721±0.152    | 8.833×10 <sup>-4</sup> ±2.943×10 <sup>-5</sup> |
|                       |                   | 0.001                 | 79.376±1.122    |  |
|                       |                   | 0.0001                | 33.597±0.623    |  |
|                       |                   | 0.00001               | 9.807±0.858     |  |

\*Mean ± SD of triplicate values

As shown in Table 2, Diapin® had a high inhibitory effect on neuraminidase that corresponds to a low IC<sub>50</sub> of 0.272 ± 0.007 mg/mL, while quercetin inhibited neuraminidase with an IC<sub>50</sub> of 0.013 ± 0.001 mg/mL. On the other hand, Diapin® had a lower inhibitory effect of adenosine deaminase (IC<sub>50</sub> = 4.562 ± 0.052 mg/mL), elastase (IC<sub>50</sub> = 5.783 ± 0.058 mg/mL) and arginase (IC<sub>50</sub> = 6.800 ± 0.067 mg/mL). The standard inhibitors, erythro-9-(2-Hydroxy-3-nonyl)adenine hydrochloride (EHNA), ursolic acid and quercetin inhibited these enzymes with an IC<sub>50</sub> of 0.053×10<sup>-3</sup> ± 0.002×10<sup>-3</sup>, 2.907 ± 0.146 and 7.619×10<sup>-3</sup> ± 1.243×10<sup>-5</sup> mg/mL respectively (Table 2).

## DISCUSSION

For centuries, herbs and spices have been used as food additives or food supplements due to their immense therapeutic/health benefits. They are widely used in folk medicinal practices, and are proven to contain pharmacologically active compounds. The inhibition of enzymes is among the major techniques employed by modern medicine for the treatment of disease and infection, as well as for the management of metabolic diseases. In the present study, a Turkish food supplement, Diapin® was investigated for its inhibitory effect on  $\alpha$ -amylase,  $\alpha$ -glucosidase, acetyl cholinesterase, elastase, neuraminidase, arginase and adenosine deaminase activity.

$\alpha$ -Amylase and  $\alpha$ -glucosidase are involved in the degradation of food based carbohydrate in the intestine. They break carbohydrates down into molecular components, which are readily absorbed into the blood stream. The normal activity of these

enzymes, accompanied by deranged insulin action (i.e. insulin deficiency or insulin resistance) can be detrimental to normal metabolic processes (Ramasubbu, Paloth, Luo, Brayer, & Levine, 1996). For instance, the activity of these enzymes leads to high postprandial blood glucose in diabetic patients. The excess unutilised blood glucose is then channelled to alternative pathways such as aldose reductase and sorbitol dehydrogenase due to inefficient insulin action. These fallouts are accompanied by increased osmotic pressure, non-enzymatic glycation of macromolecules, and the accumulation of oxidants and advance glycation products. Hence, precipitating diabetic complications such as cataract, kidney failure, heart diseases, autoimmune reactions, increased oxidative stress, neural complications etc. (Villarreal, Reyes, Angelo, Reines, & Ramo, 2011). Therefore, inhibiting the activity of these enzymes plays a vital role in controlling postprandial blood glucose level and attenuating the progression of diabetic complication (Kim, Kwon, & Son, 2000; Zhen *et al.*, 2017).

Previous studies have shown that several herbs and species, including components of the food supplement (Diapin®) used in the present study, exhibit antidiabetic properties. Temiz & Temur (2019) demonstrated that the extract of olive leaves significantly inhibited intestinal  $\alpha$ -amylase and  $\alpha$ -glucosidase of streptozotocin-induced diabetic rats. In addition, the levels of insulin increased, while those of blood glucose and glycated haemoglobin decreased. Reports by Komaki *et al.* (2003) and Nickavar & Yousefian (2011) revealed that olive extracts directly have inhibitory effects on the activities of  $\alpha$ -amylase. In

**Table 2. Inhibitory effect of Diapin® on neuraminidase, adenosine deaminase, elastase and arginase activities.**

| Enzyme              | Extract/ Standard | Concentration (mg/mL) | Inhibition (%)* | IC <sub>50</sub> (mg/mL)*                      |             |
|---------------------|-------------------|-----------------------|-----------------|--|-------------|
| Neuraminidase       | Diapin®           | 0.500                 | 82.669±0.488    | 0.272±0.007                                    |             |
|                     |                   | 0.250                 | 49.678±2.511    |  |             |
|                     |                   | 0.100                 | 25.956±0.614    |  |             |
|                     |                   | 0.050                 | 13.751±1.101    |  |             |
|                     | Quercetin         | 0.040                 | 93.720±1.272    | 0.013±0.001                                    |             |
|                     |                   | 0.020                 | 60.959±3.091    |  |             |
|                     |                   | 0.010                 | 45.433±2.174    |  |             |
|                     |                   | 0.005                 | 37.440±1.824    |  |             |
| Adenosine deaminase | Diapin®           | 10.000                | 91.599±0.162    | 4.562±0.052                                    |             |
|                     |                   | 5.000                 | 63.005±0.162    |  |             |
|                     |                   | 2.000                 | 27.464±1.131    |  |             |
|                     |                   | 0.500                 | 11.632±0.323    |  |             |
|                     | EHNA              | 0.06×10 <sup>-3</sup> | 52.784±0.798    | 0.053×10 <sup>-3</sup> ±0.002×10 <sup>-3</sup> |             |
|                     |                   | 0.04×10 <sup>-3</sup> | 44.631±0.204    |  |             |
|                     |                   | 0.02×10 <sup>-3</sup> | 39.475±1.135    |  |             |
|                     |                   | 0.01×10 <sup>-3</sup> | 34.940±0.488    |  |             |
|                     | Elastase          | Diapin®               | 5.000           | 45.260±0.306                                   | 5.783±0.058 |
|                     |                   |                       | 4.000           | 35.474±0.917                                   |             |
| 3.000               |                   |                       | 33.028±0.306    |  |             |
| 1.000               |                   |                       | 16.616±2.212    |  |             |
| Ursolic Acid        |                   | 1.000                 | 28.030±0.309    | 2.907±0.146                                    |             |
|                     |                   | 0.100                 | 22.854±0.945    |  |             |
|                     |                   | 0.010                 | 17.424±1.856    |  |             |
|                     |                   | 0.001                 | 12.879±0.619    |  |             |
| Arginase            | Diapin®           | 10.000                | 87.451±0.432    | 6.800±0.067                                    |             |
|                     |                   | 8.000                 | 58.538±0.654    |  |             |
|                     |                   | 5.000                 | 45.169±1.732    |  |             |
|                     |                   | 4.000                 | 6.631±0.283     |  |             |
|                     | Quercetin         | 0.010                 | 64.975±1.547    | 7.619×10 <sup>-3</sup> ±1.243×10 <sup>-5</sup> |             |
|                     |                   | 0.008                 | 56.419±0.066    |  |             |
|                     |                   | 0.006                 | 28.154±0.458    |  |             |
|                     |                   | 0.004                 | 14.404±0.458    |  |             |

\*Mean ± SD of triplicate values

another study, aqueous extract of olive leaves were shown to inhibit maltase and sucrose, as well as intestinal glucose uptake and transport (Kerimi *et al.*, 2019). In addition to preventing the digestion of carbohydrate, standardised olive leaf extract (20% oleuropin) has been shown to effectively increase the activity of pancreatic beta cells in obese Australia men (de Bock *et al.*, 2013). Furthermore, the strong antioxidant action of the standardised olive extract protects pancreatic cells from diabetic induced oxidative damage, which might occur due to increasing levels of hydrogen peroxide and reactive oxygen (Cumaoglu *et al.*, 2011). Moreover, this standardised extract has been demonstrated to attenuate the formation of advanced glycation products- a secondary complication in diabetes that distort the structure and function of biomolecules, and damage tissues as well (Kontogianni *et al.*, 2013). Cinnamons are also reported to have antidiabetic effects. However, the specific antidiabetic mechanism and the efficacy of *C. cassia* is still doubted (Vanschoonbeek, Thomassen, Senden, Wodzig, & van Loon, 2006; Costello *et al.*, 2016). Several studies have expatiated the antidiabetic potentials of black seeds. In experimental animals, the seed extract is observed to induce a reduction in intestinal glucose absorption and blood glucose level, while

increasing insulin level and glucose tolerance (Kanter, Meral, Yener, Ozbek, & Demir, 2003; Meddah *et al.*, 2009). Studies by Adekola *et al.*, 2017 reveal that extract of coconut testa is capable of inhibiting both pancreatic  $\alpha$ -amylase and  $\alpha$ -glucosidase. Mohammed *et al.* (2017) found that blood glucose levels were markedly reduced upon administration of aqueous coconut oil extract to alloxan-induced diabetic rats. Vitamin D3 is also reported to strongly inhibit  $\alpha$ -glucosidase (Peng, Zhang, & Zen, 2016). The aforementioned reports support the findings of the present study as well as the folkloric claim that Diapin® has antidiabetic effect. However, in the present study, the antidiabetic effect of the aforementioned plants mixture could not be proven through the enzyme inhibition mechanisms of  $\alpha$ -glucosidase and  $\alpha$ -amylase.

In addition to the deposition of  $\beta$ -amyloid in brain and nervous tissue, the excessive activities of cholinesterases (acetylcholinesterase and/or butyrylcholinesterase) plays an important role in the development of Alzheimer's disease (Rao, Sridhar, & Das, 2007). The inhibition of these enzymes alter their catabolic activities, consequently retaining high systemic levels of their substrates. These are some of the basic strategies used for

the management of dementia and other related neurological diseases (Heinrich & Teoh, 2004). Studies have shown that olive leaves and cinnamon bark extract are capable of subsiding the intensity of Alzheimer's disease via promoting autophagy (Cordero, Garcia-Escudero, Avila, Gargini, & Garcia-Escudero, 2018), mitigating the formation and deposition of  $\beta$ -amyloid (Frydman-Marom *et al.*, 2011) or by inhibiting cholinesterase activity (Omar, Scott, Hamlin, & Obied, 2018; Park *et al.*, 2018), thereby aiding cognitive function. Similarly, acetylcholinesterase is reported to be inhibited by both black seed oil extract (Kannan, Ittiyavirah, & Harindran, 2019) and coconut extract (Nafar & Mearow, 2014; Mirzaei, Khazaei, Komaki, Amiri, & Jalili, 2019) or to improve cognitive functions by decreasing plaque formation and neuron death. Conversely, altered levels of vitamin D are reported in people with Alzheimer's disease (Shah *et al.*, 2012; Johansson *et al.*, 2013), and the administration of this vitamin tends to downplay the progression and symptoms of the disease as well as the activity of acetylcholinesterase (Anweiler, Karras, Anagnostis, & Beauchet, 2014). The findings of the present study, in addition to the aforementioned reports, suggest that Diapin<sup>®</sup> supplementation may play a vital role in the attenuation of plaque formation, progression of dementia as well as retaining adequate systemic levels of acetylcholine.

Neuraminidases have a substantial effect on the pathogenesis and virulence microorganisms (Rothe, Rothe, Roggentin, & Schauer, 1991). These enzymes hydrolyse neuraminic acid and its derivative, thus aiding pathogens-host cell interaction, virion progeny elution, aggregation and motility (von Itzstein, 2007; McAuley *et al.*, 2017). The outcome of the present study indicated that Diapin<sup>®</sup> is a good inhibitor of neuraminidase ( $IC_{50} = 0.262 \pm 0.012$  mg/mL). This finding is in line with previous reports that demonstrated the antimicrobial effect of the various components of Diapin<sup>®</sup> which are: *O. europaea* L. leaves extract (Pereira *et al.*, 2007; Korukluoglu, Sahan, Yigit, Ozer, & Gucer, 2010; Liu, McKeever & Malik, 2017). *C. cassia* stem extract (Munazza, Najam-us-Sahar, Deeba, & Farhan, 2016), *N. sativa* seed oil (Bakathir & Abbas, 2011) and *C. nucifera*oil (Silva *et al.*, 2013; Hovorková, Laloučková & Skřivanová, 2018). Moreover, the hypothesised effect of the Mediterranean diet against COVID-19 and other respiratory syndromes (Angelidi, Kokkinos, Katechaki, Ros, & Mantzoros, 2021; Baeta, Bagina, & Canilhas, 2020; Tamer, Fayed, Ayman, & Ibrahim, 2020) may not be unconnected to the anti-neuraminidase activity of the diet - as displayed by Diapin<sup>®</sup> (a supplement composed of herbs commonly used in the Mediterranean diet).

Adenosine deaminase is an important enzyme of purine metabolism. Its primary function is the irreversible deamination adenosine to inosine (Losey, Ruthenburg, & Verdine, 2006). This enzyme is also believed to be associated with normal immune function, neurotransmission, epithelial cell differentiation and gestation (Moriwaki, Yamamoto, & Higashino, 1999). Deficient level/activity of adenosine deaminase is linked to pulmonary fibrosis, while its over expression or hyperactivity is observed in some disease conditions such as autoimmune dysfunction (e.g. arthritis, psoriasis and sarcoidosis), cancer, ischemia, haemolytic anaemia and AIDS (Blackburn & Kellems, 2005). Thus, inhibiting this enzyme may help in the management of these

diseases, and/or alleviate their symptoms. Cubukcu, Durak, Kocaoglu, & Durak, (2018) demonstrated that the adenosine deaminase activity of cancerous gastric tissue was strongly inhibited by aqueous olive leaves extract. Likewise, nanoparticles of the extract were shown by Farhan *et al.*(2016) to inhibit this enzyme in sera of arthrosclerosis patients. Another study reveals the cytotoxic effect of the extract on cancer cells (Korkmaz, Sarimahmut, Ozel, & Ulukaya, 2016). Similarly, *N. sativa* (Shafiq, Ahmad, Masud & Kaleem, 2014; Gholamnezhad, Rafatpanah, Sadeghnia, & Boskabady, 2015) and *C. cassia* extract are proven to exhibit cytotoxic effects or prevent mutations (Ngoc *et al.*, 2014). These reports support the claim of Diapin<sup>®</sup> having both anti-inflammatory, anticancer and autoimmune stabilizing effects.

Elastase are protein proteases responsible for the degradation of elastic- a connective tissue protein critical for elasticity in association with collagen (Bieth, 2001). These enzymes are associated with the degradation and recycling of extracellular tissue matrixes, which in turn aid tissue repair, wound healing and re-epithelialization processes. Moreover, they play an important immunological role through degradation of the outer membrane protein A Gram-negative bacteria (e.g. *E. coli*) and the Shigella virulence factors. However, elastase may instigate the progression of inflammatory anomalies, heart diseases, cancer, fibrosis, as well as virulence factor of some microbes (Girish, Kemparaju, Nagaraju, & Vishwanath, 2009; Alam, Newby, & Henriksen, 2012). Findings suggest that elastase inhibitors could mitigate inflammatory responses and decrease the release of inflammatory cytokine (Alam, Newby, & Henriksen, 2012), as well as lung cancer metastasis (Moroy, Alix, Sapi, Hornebeck, & Bourguet, 2012). The outcome of the present study indicates that Diapin<sup>®</sup>inhibits elastase activity. This finding is in agreement with previous reports that demonstrate elastase inhibitory activity of *O. europaea* (Battinelli *et al.*, 2006; Angelis *et al.*, 2020) and *N. sativa* (Kacem & Meraihi, 2006), as well as the wound healing and anti-inflammatory activities of *C. nucifera* (Zakaria *et al.*, 2006).

Arginase is an enzyme of ureagenesis that catalysis the conversion of L-arginine into L-ornithine and urea (Wu & Morris, 1998). This ureohydrolase is abundant in liver, kidney and prostate, and to a lesser extent in the brain, macrophages and lactating mammary glands (Morris, 2002). The arginase II isozyme is believed to be co-expressed with its substrate competitor - nitric oxide synthase - in the genitals and other smooth muscle tissue. The activity of nitric oxide synthase on the other hand is correlated with the bioavailability of nitric oxide, a molecule that induces nitric oxide-dependent smooth muscle relaxation and is capable of acting as an oxidant as well. Over expression/activity of arginase and inhibition of nitric oxide synthase is accompanied by competitive depletion of arginine pool, and the ultimate depletion of nitric oxide respectively. This is believed to precipitate erectile dysfunction and decreased smooth muscle relaxation (Cama *et al.*, 2003; Christianson, 2005; Kim *et al.*, 2009). Moreover, elevated activity of arginase is reported in asthma, oxidative stress induced diabetes (Kiss *et al.*, 2014), chronic obstructive pulmonary disease (van den Berg, Meurs, & Gosens, 2018) and in cystic fibrosis (Maarsingh, Khazaei, Koma-

ki, Amiri, & Jalili, 2008). Therefore, inhibitors of arginase such as Diapin® may have positive implications on the aforementioned maladies.

## CONCLUSION

The present study demonstrates that Diapin® inhibits the activities of  $\alpha$ -amylase,  $\alpha$ -glucosidase, acetyl cholinesterase, elastase, neuraminidase, adenosine deaminase and arginase. The previously reported antioxidant, anti-inflammatory and beta-cell enhancing activity of Diapin® is an added advantage to its inhibitory potentials. Its broad pharmacological and biochemical activities are attributed to the rich phytochemical composition of the herbs, spices, vitamins/provitamins (i.e. *O. europaea* L. leaves extract, *C. cassia* (L.) J. Presl stem extract, *N. sativa* L. seed oil, *Cocos nucifera* L. oil and vitamin D3) contained in the supplement. Moreover, the present findings support the folkloric claim of Diapin® supplement having antidiabetic, anticancer, anti-inflammatory, antimicrobial, anti-aging, and immune bolstering properties, and that it alleviates neurological dysfunction and mitigates Alzheimer's disease.

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