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In vitro antioxidant and lipoprotein lipase inhibitory properties of freeze drying assisted ultrasonicated extracts of *Solanum nigrum*

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ABSTRACT

Solanum nigrum is used in local medicinal system to cure many metabolic disorders and oxidative stress-oriented health problems. Current work was performed to optimize the extraction process using freeze drying assisted ultrasonication for better antioxidant activities and lipoprotein lipase inhibitory properties. The 80% ethanolic extract exhibited considerable Fe-chelating activity (65.82 ± 1.26%), β-carotene bleaching inhibition (75.10 ± 0.90%) and lipoprotein lipase inhibitory properties (IC_{50} = 45.99 ± 2.12 µg/mL). The 20% ethanolic extract exhibited least antioxidant and enzyme inhibition activities. The variations in the activities of different extracts were probably due to solvent polarity set by the solvent components. The antioxidant and enzyme inhibition properties were most probably due to variety of phytochemicals present in extract. The *Solanum nigrum* was proved as a good source of natural antioxidants and anti-obesity agents which might be explored for novel pharmacological leads to treat obesity in safe and effective way.

Keywords: Antioxidant, Lipoprotein lipase, Freeze drying, Ultrasonication, *Solanum nigrum*.

INTRODUCTION

Obesity is a metabolic disorder characterized by abnormal or excessive fat deposition in adipose tissues (Qureshi & Abrams, 2007). Obesity is a widespread disease caused by interlinked factors of genetic, dietary, and environmental nature. It is spreading rapidly, and an estimate stipulates 600 million obese adults worldwide (Sweeting et al., 2015). It also initiates and intensifies other health disorders including hyper lipidemia, arteriosclerosis, diabetes, hypertension, and arthritis (Azman et al., 2012; Poulos et al., 2010). Obesity has become a social problem characterized by multiple economic and social aspects. Therefore, prevention and management of obesity is a key concern both in developed and developing countries. To control obesity, nutritional regime, physical exercise, and medication are the main adaptations. However synthetic anti-obesity drugs like orlistat and sibutramine might be associated with potential health risks like high blood pressure, cardiac failure, insomnia, headache, and permanent constipation (Derksen et al., 2012; Thurairajah et al., 2005).

Dietary lipids are important component of energy homeostasis to maintain proper metabolic functions. Lipoprotein lipase enzyme is responsible for the hydrolysis of triglyceride rich lipids into free fatty acids and monoglycerides. Restriction in lipid digestion and absorption lowers the amount of fats available for deposition in adipose tissues. Inhibition of enzymatic activity of lipoprotein lipase is one of the workable choices to reduce the fat deposition in adipose tissues, hence preventing the elevated levels of fats accumulation (Kusunoki et al., 2013). Anti-lipase agents may be used as an effective tool to control obesity development and to manage its further expansion. Plants are well known for their medicinal value due to the presence of phenolic compounds to reduce the reactive oxygen species (ROS) and oxidative stress (Nadeem et al., 2020; Nita & Grzybowski, 2016). Reactive oxygen species are produced during metabolism and their production may be enhanced under the influence of internal or environmental factors. Antioxidants encounter ROS to maintain the homeostatic functioning of system but imbalance in ROS and

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antioxidants results in state of oxidative stress. Oxidative stress has been scientifically proved to be a major factor in chronic disease development and prolongation. Role of ROS and oxidative stress is obvious in obesity development and its inevitable extension (McMurray et al., 2016). Studies have revealed that plant bioactives also tend to be promising antioxidants and anti-lipase agents providing a potential approach towards obesity management (Abdul Rahman et al., 2017; Sellami et al., 2017). The scavenging of ROS eliminates the oxidative stress and may furnish therapeutic benefits to control obesity. Keeping in view the side complication of synthetic drugs used for obesity management, plants provide a great deal of space to explore them for hidden anti-obesity potential generally governed by lipase inhibitory mode. Moreover, antioxidants also scavenge metals like iron to reduce its load for systematic reduction in free radical production. Plants are well known for their metal chelating potential (Raza et al., 2020).

The *Solanum nigrum* (*S. nigrum*) of Solanaceae family is well known for its medicinal potential and many studies have revealed its use to treat chronic ailments (Gbadamosi & Afolayan, 2016; Peng et al., 2020).

Current investigation was performed to assess the antioxidant and lipoprotein lipase inhibitory potential of wild grown *S. nigrum* as possible treatment of obesity.

MATERIALS AND METHODS

Extract Preparation

Fresh leaves of *S. nigrum* were immediately treated with liquid nitrogen and freeze dried. The freeze-dried powdery material was extracted with 20%, 40%, 60% 80% hydroethanolic and pure ethanol using ultrasonication. The ultrasonicated fractions were filtered to remove the suspended plant material and debris. The filtrate was subjected to rotary evaporation for complete removal of extra solvent in the extract (Aabideen et al., 2020). The extracts obtained at the end were used for computing different antioxidant and enzyme inhibitory properties.

Fe-Chelating Activity

Fe-chelating activity is an important parameter to assess the medicinal potential of plants. The *in vitro* chelating activity was performed by considering an already reported protocol with minute changes (Dinis et al., 1994). The plant extracts were mixed with mixture of $FeSO_4$ and ferrozine. The absorbance of resultant mixture was noted at 562 nm for every extract. The following formula was used for calculation of % Fe-chelating activity.

% Fe-chelating = (Absorbance of control – Absorbance of Sample/Absorbance of control) x 100

β-carotene bleaching assay

The extracts were subjected to β-carotene bleaching assay for antioxidant activity evaluation. A previously reported method was followed for the purpose (Lelono et al., 2009). The β-carotene solution was prepared in chloroform and this mixture was added to linoleic acid in Tween 40. The extract was added to the reaction mixture and stayed for 15 min at 20 ^oC. Then water was added to form emulsion and absorbance was noted at 470 nm.

Inhibition % = [1-(Ai - Af) / (Ci - Cf)] x 100

The Ai and Ci were the absorbance for sample and control, respectively at start of protocol. Whereas Af and Cf were absorbance of sample and control after 120 min incubation period. Butylated hydroxylanisole (BHA) was used as reference standard.

Lipoprotein lipase inhibition

An already established method with slight modifications was used for lipoprotein lipase assay substrate preparation (Chung & Scanu, 1974). The substrate was prepared by diluting activator from human plasma with TrisHCl and 1% triton X-100. The sonication process was carried out for 3 min in ice cold conditions. Lipoprotein lipase was dissolved in 0.02M TrisHCl to formulate the concentration of about 25 units/mL. The enzyme inhibition activity was monitored using a previously published method (Schotz et al., 1970). The plant extract was mixed with the lipase solution in substrate. The epicatechin was used as standard compound. The reaction mixtures were stored at 4°C for a period 30 min. The hydrolysis was started by maintaining the temperature at 37°C and later, the reaction was retarded by adding aqueous NaCl. The free fatty acids released from reaction were titrated against 0.01 M NaOH until complete neutralization of fatty acids. The percentage inhibition was calculated by taking triplicate readings.

$$\text{Inhibition (\%)} = \left[100\% - \frac{V_S}{V_C}\right] \times 100$$

V is the volume of acid used to neutralize free fatty acid.

RESULTS

Fe-chelating activity

The findings of *S. nigrum* extracts for Fe-chelating potential are given in fig. 1. The results clearly demonstrated the 80% ethanolic extract as most effective to chelate Fe. The statistical analysis confirmed that Fe-chelating activity of 80% ethanolic extract was significantly higher among all extracts ($\rho<0.05$).

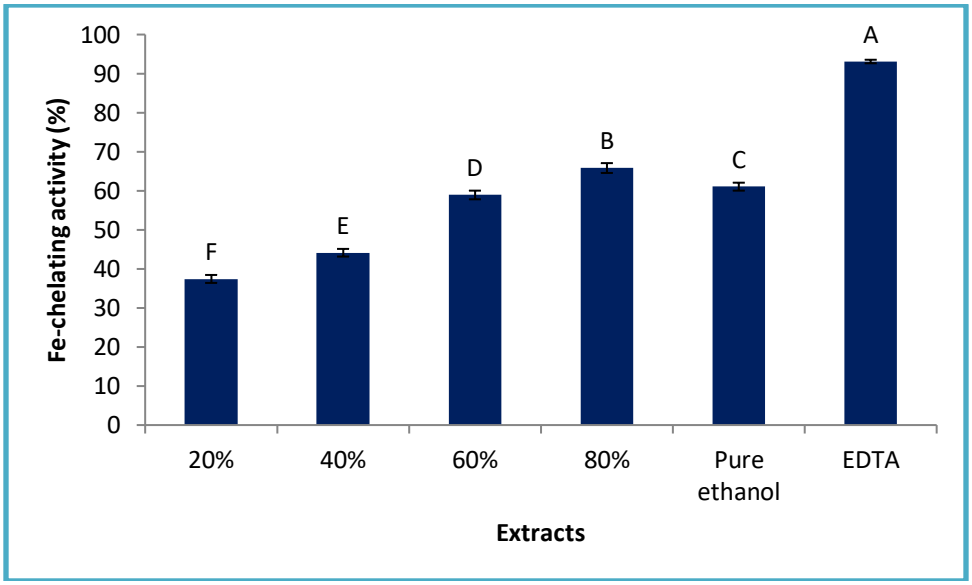

Fig. 1: Fe-chelating activity of hydroethanolic extracts of *S. nigrum.* Values did not share a letter were significantly different

The iron is known to generate free radicals by gain or loss of electron. The free radical production by iron overload is a contributing factor towards oxidative stress. The oxidative stress is associated with many metabolic discrepancies including obesity. Plant extracts having the ability to chelate metals like iron may improve the antioxidant system of body. Therefore, metal chelating/Fe-chelating activities of plants are of great therapeutic significance (Sudan et al., 2014).

β-carotene bleaching assay

The values of β-carotene bleaching assay are given in fig. 2. The results showed that 80% ethanolic extract was the most effectual extract to prevent the color bleaching by inhibiting the β-carotene oxidation followed by pure ethanolic extract.

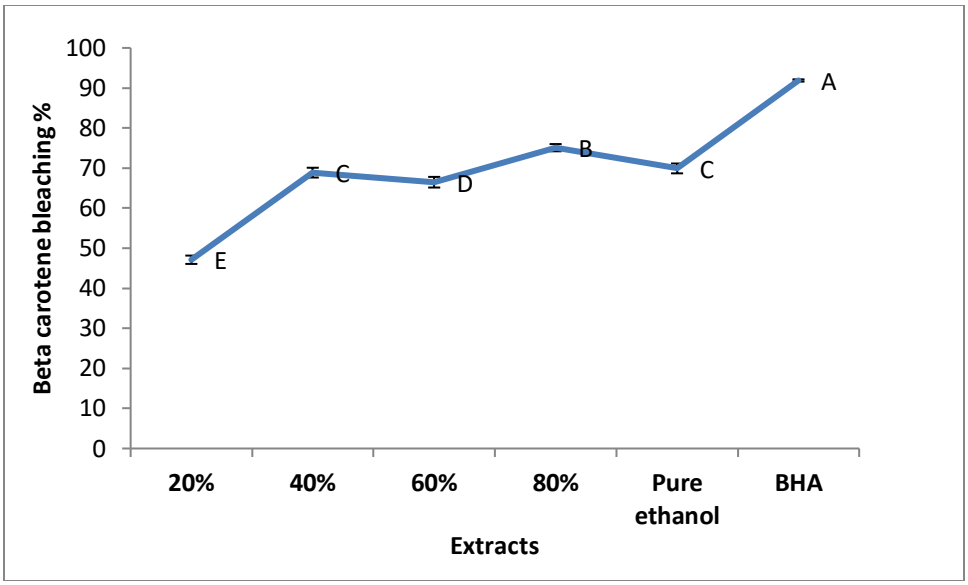

Fig. 2: Beta-carotene bleaching assay results. Values did not share a letter were significantly different

The statistical comparison of means reflected that 80% ethanolic extract was the most effective extract with ρ value <0.05.

The high inhibition activity of 75.10 ± 0.90% by 80% ethanolic extract showed that the *S. nigrum* extract might be a good source of antioxidants. Plant based antioxidants are generally polyphenolic entities including phenolic acids and flavonoids. The polyphenols contribute to antioxidant and metal chelating activity of plant extract and are associated with vital therapeutic role (Raza et al., 2020; Sudan et al., 2014). The variation in antioxidant activities observed for *S. nigrum* extracts was most probably due to difference in solvent polarities. The solvent polarity is an important feature which improves the extraction process for maximum recovery of phytochemicals. The high phenolic and flavonoid contents are believed to be an important factor behind the antioxidant activity of plant extracts. Many studies have supported the solvent efficacy to improve the phytochemical yields for better antioxidant and pharmacological properties (Raza et al., 2020; Raza et al., 2018).

Lipoprotein lipase inhibition

The fig. 3 indicates IC_{50} values (µg/mL) for plant extracts against lipoprotein lipase enzyme.

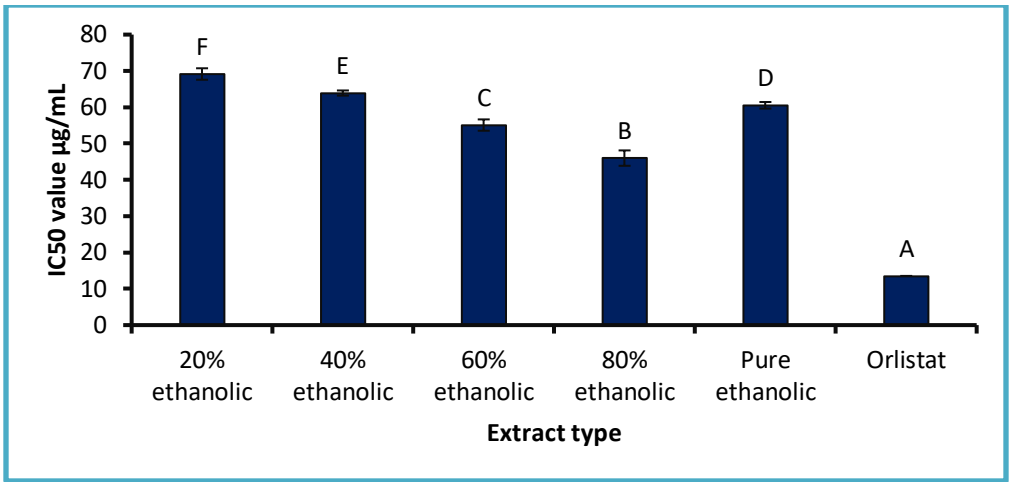

Fig. 3: Lipoprotein lipase inhibitory properties of extracts. Values not sharing a letter were categorized as statistically different

Different extracts of *S. nigrum* (20-80% HE and pure ethanolic) showed different lipoprotein lipase inhibitory activities ranging from 45.99 ± 2.12 to 69.15 ± 1.58 µg/mL in terms of IC_{50} values. Results revealed that 20% *S. nigrum* HE extract displayed the lowest inhibition activity (69.15 ± 1.58 µg/mL), while 80% HE extract demonstrated the highest inhibition of enzyme (45.99 ± 2.12 µg/mL). All the *S. nigrum* HE and pure ethanolic leaf extracts indicated statistically different ($\rho < 0.05$) response in their inhibitory effects towards enzyme. The pure Orlistat showed IC_{50} value of 13.46 ± 0.09 µg/mL.

Lipoprotein lipase inhibition is an important therapeutic target for obesity management. The inhibition of this enzyme leads to low fat accumulations which in turn maintain the regular metabolic activities. Plants give a good deal of natural and safe substances to inhibit lipoprotein lipase enzyme and this option are considered better than use of synthetic drugs. The lipoprotein lipase inhibition was defined as a leading pathway behind anti-obesity properties of plants (Baek et al., 2013; Wei et al., 2011).

The *S. nigrum* extract was found very effective source of antioxidants and lipoprotein lipase inhibitory agents. The *S. nigrum* may be further explored for efficient and natural candidates to treat and manage obesity with low or negligible toxicity at low cost. The study may be helpful to reduce the socio-economic burden caused by obesity.

CONCLUSION

The 80% ethanolic extract exhibited good antioxidant activities and lipoprotein lipase inhibitory property. The same extract can be a good source of natural antioxidants of therapeutic significance. The study also confirmed the ethnopahrmacological use of *S. nigrum* to cure metabolic disorders. Further studies may be carried out on the phytochemical availability and characterization to search for novel anti-obesity agents.

REFERENCES

Aabideen, Z.U., Mumtaz, M.W., Akhtar, M.T., Mukhtar, H., Raza, S.A., Touqeer, T., Sarri, N., 2017. Anti-obesity attributes; UHPLC-QTOF-MS/MS-Based metabolite profiling and molecular docking insights of *Taraxacum officinale. Molecule.,* 25: 1-20.

Azman, K.F., Amom, Z., Azlan, A., Esa, N.M., Ali, R.M., Shah, Z.M., & Kadir, K.K.A., 2012. Antiobesity effect of *Tamarindus indica* L. pulp aqueous extract in high-fat diet-induced obese rats. *J. Nat. Med.,* 66: 333-342.

Baek, J., Lee, J., Kim, K., Kim, T., Kim, D., Kim, C., Tsutomu, K., Ochir, S., Lee, K., & Park, C.H., 2013. Inhibitory effects of *Capsicum annuum* L. water extracts on lipoprotein lipase activity in 3T3-L1 cells. *Nutr. Res. Pract.,* 7: 96-102.

Chung, J., & Scanu, A.M., 1974. Continuous pH-stat titration method for the assay of lipoprotein lipase activity *in vitro*. *Anal. Biochem.,* 62: 134-148.

Derksen, R., Brink-Melis, W.J., Westerman, M., Dam, J.T., Seidell, J., & Visscher, T., 2012. A local consensus process making use of

focus groups to enhance the implementation of a national integrated health care standard on obesity care. *Fam. Pract.,* 29: i177-i184.

Dinis, T.C., Madeira, V.M., & Almeida, L.M., 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.,* 315: 161-169.

Gbadamosi, I., & Afolayan, A., 2016. *In vitro* anti-radical activities of extracts of *Solanum nigrum* (L.) from South Africa. *J. Appl. Biosci.,* 98: 9240-9251.

Kusunoki, M., Tsutsumi, K., Tana, C., Sato, D., & Nakamura, T., 2013. Lipoprotein lipase activation improves the cachexia and obesity. *J. Obes. Weight-Loss Ther.,* 3: 1-6.

Lelono, R., Tachibana, S., & Itoh, K., 2009. *In vitro* antioxidative activities and polyphenol content of *Eugenia polyantha* Wight grown in Indonesia. *Pak. J. Biol. Sci.,* 12*:* 1564-1570.

McMurray, F., Patten, D.A., & Harper, M.E., 2016. Reactive oxygen species and oxidative stress in obesity-recent findings and empirical approaches. *Obesity,* 24:, 2301-2310.

Nadeem, M., Mumtaz, M.W., Danish, M., Rashid, U., Mukhtar, H., & Irfan, A., 2020. Antidiabetic functionality of *Vitex negundo* L. leaves based on UHPLC-QTOF-MS/MS based bioactives profiling and molecular docking insights. *Ind. Crops Prod.,* 152, 1-14.

Nita, M., & Grzybowski, A., 2016. The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. *Oxid. Med. Cell. Longev.,* 2016: 1-23.

Peng, C.H., Cheng, J.J., Yu, M.H., Chung, D.J., Huang, C.N., & Wang, C.J., 2020. *Solanum nigrum* polyphenols reduce body weight and body fat by affecting adipocyte and lipid metabolism. *Food Funct.,* 11: 483-492.

Poulos, S.P., Dodson, M.V., & Hausman, G.J., 2010. Cell line models for differentiation: preadipocytes and adipocytes. *Exp. Biol. Med.,* 235: 1185-1193.

Qureshi, K., & Abrams, G.A., 2007. Metabolic liver disease of obesity and role of adipose tissue in the pathogenesis of nonalcoholic fatty liver disease. *World J. Gastroenterol.,* 13: 3540-3553.

Raza, S.A., Chaudhary, A.R., Mumtaz, M.W., Adnan, A., Mukhtar, H., & Akhtar, M.T., 2020. Metabolite profiling and antidiabetic attributes of ultrasonicated leaf extracts of *Conocarpus lancifolius*. *Asian Pac. J. Trop. Biomed.,* 10: 353-360.

Raza, S.A., Chaudhary, A.R., Mumtaz, M.W., Bashir, S., Ahmad, M., William, J., & Arshad, M., 2020. Fe-chelating activity of *Conocarpus lancifolius* Engl. leaf extracts and 1H-NMR based metabolite's classification. *Acta Sci. Natura.,* 7: 1-7.

Raza, S.A., Chaudhary, A.R., Mumtaz, M.W., Ghaffar, A., Adnan, A., & Waheed, A., 2018. Antihyperglycemic effect of *Conocarpus erectus* leaf extract in alloxan-induced diabetic mice. *Pak. J. Pharm. Sci.,* 31: 637-642.

Schotz, M.C., Garfinkel, A.S., Huebotter, R., & Stewart, J., 1970. A rapid assay for lipoprotein lipase. *J. Lipid Res.,* 11: 68-69.

Sellami, M., Louati, H., Kamoun, J., Kchaou, A., Damak, M., & Gargouri, Y., 2017. Inhibition of pancreatic lipase and amylase by extracts of different spices and plants. *Int. J. Food Sci. Nutr.,* 68: 313-320.

Sudan, R., Bhagat, M., Gupta, S., Singh, J., & Koul, A., 2014. Iron (FeII) chelation, ferric reducing antioxidant power, and immune modulating potential of *Arisaema jacquemontii* (Himalayan Cobra Lily). *Biomed. Res. Int.,* 2014: 1-7.

Sweeting, A.N., Hocking, S.L., & Markovic, T.P., 2015. Pharmacotherapy for the treatment of obesity. *Mol. Cell Endocrinol.,* 418: 173-183.

Thurairajah, P.H., Syn, W.K., Neil, D.A., Stell, D., & Haydon, G., 2005. Orlistat (Xenical)-induced subacute liver failure. *Eur. J. Gastroenterol. Hepatol.,* 17: 1437-1438.

Wei, X., Wang, D., Yang, Y., Xia, M., Li, D., Li, G., Zhu, Y., Xiao, Y., & Ling, W., 2011. Cyanidin-3-O-β-glucoside improves obesity and triglyceride metabolism in KK-Ay mice by regulating lipoprotein lipase activity. *J. Sci. Food Agric.,* 91: 1006-1013.