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Antioxidant and pancreatic lipase inhibitory attributes of *Conocarpus lancifolius* leaf extracts

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ABSTRACT

The present study describes the antioxidant and pancreatic lipase inhibitory properties of different hydroethanolic leaf extracts of *Conocarpus lancifolius*. Freeze drying assisted ultra-sonication was adopted for extract preparation. The results showed that highest total antioxidant power of 291 ± 0.50 mg ASE/g DE was exhibited by 60% ethanolic extract which was slightly lower than antioxidant power of BHA (309.16 ± 3.90 mg ASE/g DE). The β-carotene bleaching inhibition of 87.44 ± 1.20% was computed for 60% ethanolic leaf extract being statistically non-significant from BHA (89.41 ± 2.45%). The in-vitro anti-obesity attribute of extracts was assessed by measuring the inhibition of enzymatic activity of pancreatic lipase. The 60% ethanolic leaf extract exhibited maximum pancreatic lipase inhibition of 54.55 ± 0.72% but lower than standard drug orlistat (66.65 ± 0.68%). Findings explored the hidden antioxidant and pancreatic lipase inhibitory potential of *Conocarpus lancifolius* that may be exploited for naturopathic treatment of obesity and functional food development with anti-obesity attributes.

Keywords: Antioxidant, Lipase inhibition, Ultrasonication, Antiobesity, *Conocarpus lancifolius*

INTRODUCTION

The term obesity describes a state of metabolic dysfunction characterized by disproportionate fat deposition in adipose tissues of body (Cohen-Cole & Fletcher, 2008). The reasons behind obesity development may be dietary, environmental or genetic. It is widely spread health disorder with about 500 million obese adults on planet (Finucane *et al.,* 2011). It also initiates and propagates many other health disorders including hyperlipidemia, diabetes, arteriosclerosis, hypertension and arthritis (Ogunbode *et al.,* 2009). The obesity has become a socio-economic burden and its management is a matter of keen concern on the globe. Therefore, prevention and management of obesity is a key concern both in developed and developing countries. Usually, optimized nutritional modification, physical exercise and medication are adopted to reduce or mitigate the obesity. These measures cover the domain leading from low fat intake to allopathic treatment. However synthetic anti-obesity drugs like orlistat and sibutramine have potential health risks like high blood pressure, cardiac failure, insomnia, headache and permanent constipation (Bray and Popkin, 1998; Azman *et al.,* 2012).

Dietary lipids are important component of energy homeostasis to maintain proper metabolic functions. Lipids are degraded in intestine by pancreatic lipase to hydrolyze fats into simpler forms for subsequent assimilation in body. Inhibition of enzymatic activity of pancreatic lipase is a workable choice to delay the digestion of lipids which results in low uptake of fatty acids and monoglycerides from intestine (Aabideen *et al.,* 2020). This phenomenon prevents the fat accumulation in adipose tissues of body to eliminate the obesity induction or propagation (Derksen *et al.,* 2012). Anti-lipase agents may be employed for effective control of dietary lipid uptake to manage its further expansion. The fat deposition also produces oxidative stress which is responsible for the initiation and intensification of obesity

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oriented side effects. The cytokines from adipose tissues produce reactive oxygen species (ROS) for alteration in molecular structures to limit the antioxidant defense (Fernández-Sánchez *et al.,* 2011). The imbalance between ROS production in body and antioxidants introduces state of oxidative stress. The oxidative stress is responsible for obesity related disorders and health complications (Marseglia *et al.,* 2015). The phytotherapeutic approach for obesity control and related disorder management is widely adopted due to safe nature and low cost. The phytochemicals from plants are well known for their antioxidant and enzymatic inhibitory properties. Studies have revealed that plant bioactives also tend to be promising antioxidants and anti-lipase agents providing a potential approach towards obesity management (Hanefeld and Sachse, 2002). The simultaneous antioxidant and lipase inhibitory action of plants can be predicted as efficient way to mitigate the obesity development and propagation (Junior and Almeida, 2017). The side complications of synthetic drugs for obesity management are of serious nature. The plants provide a great deal of space to explore them for hidden anti-obesity potential generally governed by lipase inhibitors and antioxidants.

Conocarpus lancifolus (*C. lancifolius*) is a member of *Combretaceae* family and usually grows in tropical to subtropical areas of world. The *C. lancifolius* has emerged as potent medicinal plant due to recently reported biological activities line antioxidant, anti-urease, phytotoxic, α-glucosidase inhibition, antibacterial, anti-inflammatory and cytotoxic (Saadullah *et al.,* 2014; Saadullah *et al.,* 2016; Al. Taweel *et al.,* 2016). Keeping in view such promising medicinal attributes of *C. lancifolius*, the current work was designed to assess anti-oxidant and lipase inhibitory characteristics of its leaves as complement to obesity mitigation, prevention and management.

MATERIALS AND METHODS

Collection of plant material and treatment

The fresh leaves were harvested from Lahore city, submitted for authentication from Department of Botany, GC University Lahore and confirmed videGC.Herb.33.Bot. 3378 voucher specimen. The fresh leaves were quenched in liquid nitrogen, continuous ground to form powder. The powder was lyophilized under reduced pressure at -68°C for 48 hours on Christ Alpha 1-4 LD (Germany) lyophilizer. The powder was extracted with binary solvent system comprising of ethanol and water in six combinations (pure water, ethanol 20% to pure ethanol with an interval of 20%) at ambient conditions in 1:10 by mass and sonicated on Soniprep 150 ultrasound disintegrator (UK). The fractions were vortexed for 2 hours and filtered. The rotary evaporator was used to remove excess solvent. The obtained samples were freeze dried at -68°C for 48 hours on Christ Alpha 1-4 LD (Germany) and stored for further use.

Total antioxidant power (TAP) assay

The TAP assay of all extracts was based on synthesis of phosphomolybdenum complex with little modification as previously reported (Prieto *et al.,* 1999). Initially, 250 µg/mL of extract was dissolved in reagent solution consisting of sulphuric acid (0.6 M), ammonium molybdate (4 mM) and sodium phosphate (28 mM) in capped plastic vials. The total volume of reagent mixture used was 4mL. The obtained mixtures along with blank (without extract) were subjected to water bath for 90 min heating at recommended temperature of 95°C. Absorbance was noted at 695 nm after cooling of solution. A standard curve was drawn using ascorbic acid. The butylated hyroxyanisole (BHA) was utilized as reference compound/positive control to compare antioxidant potential of extracts. The findings were computed in ascorbic acid equivalents per gram of dried extract (ASE/g PE).

The β-carotene bleaching assay (BCB)

To add further conformity to antioxidant potential, plant extracts were also evaluated by assessing the bleaching of β-carotene (yellow color) in the presence of linoleic acid. Simply, 10 mL of chloroform were taken and 2 mg β-carotene was added to it followed by subsequent addition of linoleic acid (0.02 mL) and Tween 40 (0.2 mL). Plant extracts (0.2 mL) were added in the prepared mixture and a control was also run. The resultant mixtures were incubated for 15 min at 20°C. Chloroform was evaporated at 40°C and added by distilled water. The obtained sample was vortexed for 2 min and an emulsion was obtained. The absorbance for all samples was measured immediately (0 time) and after incubation of samples for 120 min at 50°C.

$$Inhibition\% = [1-(Ao-At)/(Co-Ct)]\times 100$$

The terms Ao and Co are zero time the absorbance of sample and control, respectively. Where, At and Ct are the values of absorbance for sample and blank after 120 min of incubation.

Positive control contained BHA under same set of conditions (Shon *et al.,* 2003).

Pancreatic lipase inhibition (PLI)

The anti-obesity activity (*In-vitro*) of plant extracts was performed by measuring the inhibition of pancreatic lipase enzyme. The extracts were added to Tris-HCl buffer (0.01M) along with pancreatic lipase. Olive oil was mixed with gum Arabic (dissolved in Tris-HCl buffer of 0.1 M strength, pH 8.0, 20 mM $CaCl_2$ and 0.5 M NaCl). The lipase inhibition of plant extracts was calculated by previously reported scheme with slight changes (Fukumoto *et al.,* 1963). To carry out procedure, the 0.5 mL of plant extracts were added to 0.2 mL of lipase solution followed by 30 minutes stay at 4°C to allow reaction to occur completely, following addition of 2 mL of substrate. A further incubation of 30 min was accomplished at 37°C. The reaction was discontinued by adding ethanol and acetone in 1:1 composition. The free fatty acids (FFA) released were titrated against 0.02 M NaOH till the pH became 9.4. All the measurements were performed in triplicates and percent inhibition was calculated by the following equation:

$$\%\text{Inhibition} = 100\ \%-[(\text{Vs/Vc}) \times 100]$$

Where, 100% is the enzymatic activity of control. The Vs and Vc represents the volume of base used in titration for sample and control, respectively.

Statistical analysis

The findings were exported to analysis of variance with Tukeys test to evaluate the level of significance difference. Minitab 17.0 statistical software was used. Triplicate values were typically shown with standard deviation (±).

RESULTS AND DISCUSSION

Total antioxidant power (TAP)

The conversion of Mo (VI) form to Mo (V) provided a base to judge antioxidant potential of extracts. This reduction resulted in green complex which absorbed at 695 nm. The results showed that TAP of hydroethanolic leaf extracts of *C. lancifolius* ranged from 65.66 ± 0.28 mg ASE/g DE to 291.50 ± 0.50 mg ASE/g DE with varied solvent composition (Fig. 1). The antioxidant power exhibited by 60% ethanolic extract was near to value associated with BHA but statistical analysis indicated that 60% extract was less potent than BHA but most active fraction among all under study extracts ($\rho<0.05$).

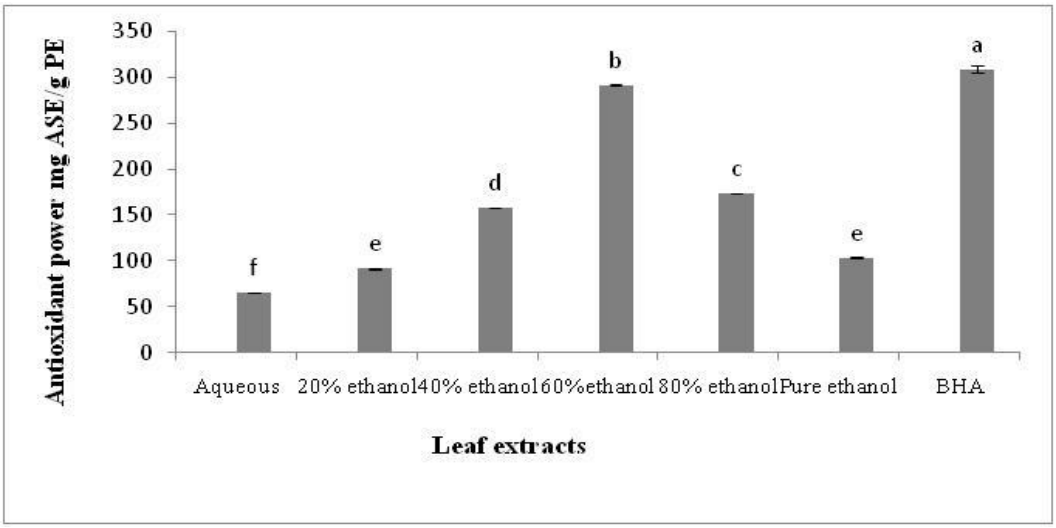

Fig. 1: Total antioxidant power exhibited by hydroethanolic extracts of *C. lancifolius*

β-carotene bleaching assay (BCB)

The antioxidant activity of plant extracts in this method was calculated by noting the inhibition of conjugated dienes production by oxidation of linoleic acid. Greater the inhibition, greater would be the antioxidant potential (Barriere *et al.,* 2001). This method is believed to be a quick and reliableantioxidant screening test especially in context of foods containing fats and oils as major

component. The finding of this assay for *C. lancifolius*, indicated that 60% ethanolic leaf extract was most effective fraction with inhibition of 87.44 ± 2.11% (Fig., 2). This value was statistically non-significant with the value of inhibition 89.41 ± 2.45% showed by BHA ($\rho < 0.05$).

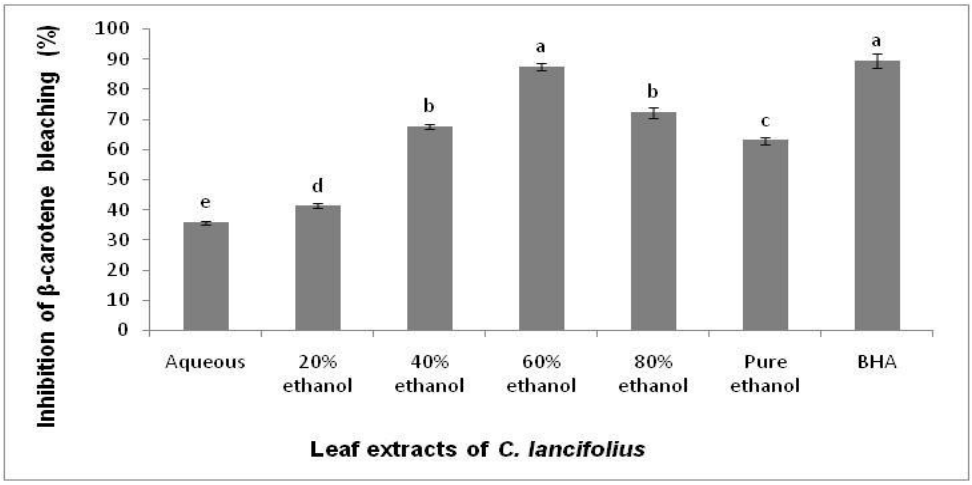

Fig. 2: The inhibition% of color beaching of β-carotene by leaf extracts of *C. lancifolius*

The β-carotene assay has special importance in systems where the lipids and lipid containing foods are involved. The restriction in bleaching of β-carotene color by plant extract might be a function of phytochemicals which scavenged free radicals to protect the substrate from harmful impacts of ROS (Alam *et al.,* 2013).The antiradical/antioxidant properties of 60% ethanolic leaf extract of *C. lancifolius* was much higher than recently reported inhibition percentage of 17.88 ± 3.13% for ethanolic extract of *Bromelia laciniosa* (Selamoglu *et al.,* 2017).

Pancreatic lipase inhibition (PLI)

Inhibition of pancreatic lipase retards degradation and absorption of fats in intestine which lead to low fat accumulation in tissues. Plants being rich source of natural lipase inhibitors so can be a resource for management of obesity along with other associated disorders like diabetes (Sellami *et al.,* 2017). The lipid metabolism is essential component of energy homeostasis. Dietary uptake of fatty and high caloric foods is the major reason for weight gain and fat deposition. Lipase enzyme hydrolyzes triglycerides in intestine and facilitates their absorption to become part of blood stream. Human dietary habits and life style are responsible for high fat intake and deposition. It is very difficult to amend the existing life style and prevailing dietary habits to a great extent for obesity management (Thurairajah *et al.,* 2003; Sukhdev and Singh, 2013). The complementary use of synthetic drugs to control obesity is an issue of keen concern due to questionable safety. Under such circumstances, the use of natural lipase inhibitors are the most feasible and effective strategy for obesity management. The plants can serve this purpose smoothly and effectively. The current investigation regarding PLI presented 60% ethanolic extract as most efficient anti-obesity fraction followed by 80% ethanolic extract (Fig. 3). Statistical analysis indicated the significant level of difference among the percentage inhibition values of extracts. However, statistically speaking the PLI for 40% and 80% ethanolic extracts was non-significant ($\rho < 0.05$). The maximum PLI was shown by orlistat, a synthetic drug with value of 66.65 ± 0.68 and no extract could match the standard drug.

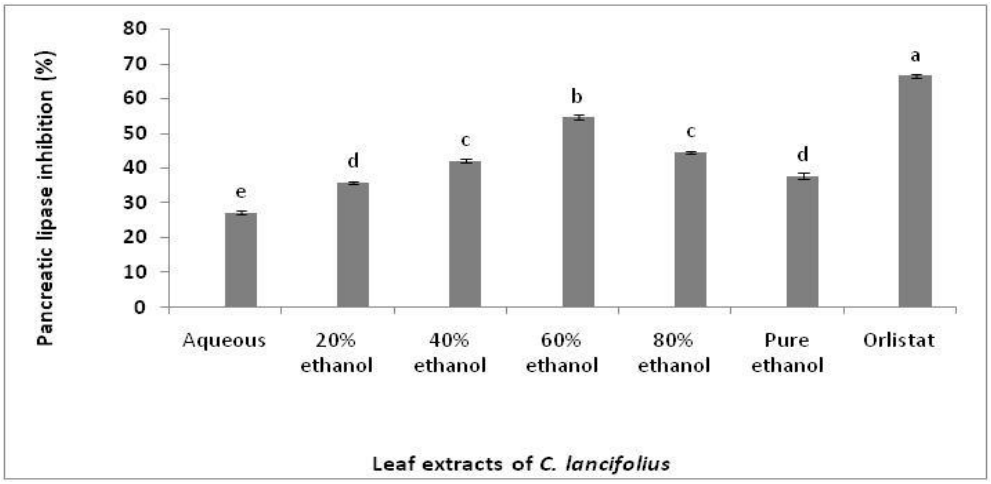

Fig. 3: Inhibition of pancreatic lipase by hydroethanolic leaf extracts of *C. lancifolius*

CONCLUSION

The antioxidant and lipase inhibitory properties of *C. lancifolius* leaf extract suggested the new addition to naturopathic approach for obesity management. The findings revealed that the leaves of *C. lancifolius* might have substantial phytochemicals of medicinal importance. The antioxidant and pancreatic lipase inhibitory potentials of ultra-sonicated leaf extracts of *C. lancifolius* computed in this work were of great significance. The synergistic outcomes of antioxidant and lipase inhibition by plant extracts may be employed to improvise the dietary intake of natural antioxidants and lipase inhibitors for obesity management and functional food development after careful *in vivo* trials.

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