



Evaluation of Antilipidperoxidation, A-Amylase and Lipase Inhibitory Activity of *Boswellia Ovalifoliolata* Bal. Henry

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Abstract

The objective of the present study was to determine the antilipidperoxidation and *in vitro* α -amylase and lipase inhibitory activity of the *Boswellia ovalifoliolata* Bal Henry (BOB) extract. The polyphenolic compounds, flavonoids, and tannins content of the extracts were estimated by spectrophotometry. Antioxidant activity on goat liver lipid peroxidation and linoleic acid was determined. Moreover, α -amylase and lipase inhibitory activities were evaluated. All the extracts showed antioxidant, α -amylase, and lipase inhibitory properties. Among the other extracts of BOB, macerated methanol extract could extract the highest concentration of polyphenols, flavonoids, and tannins. Even macerated methanol extract showed antioxidant activity on goat liver lipid peroxidation and linoleic acid were 62.83 ± 4.723 ($p < 0.01$) and 56.67 ± 1.43 ($p < 0.01$) respectively. Maximum α -amylase and lipase inhibitory activities were expressed as 56.34 ± 2.1 ($p < 0.01$) and $79.36 \pm 1.58\%$ ($p < 0.01$) respectively for macerated methanol and ethanol extracts. The results indicated that all the extract exhibited low inhibition activity as compared to standard.

Keywords: *Boswellia ovalifoliolata* Bal Henry, phenolic compounds, flavonoids, tannins, antioxidant, amylase, lipase

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1. Introduction

The α -amylase is one of the main enzymes in human that catalyses the hydrolysis of 1,4-glucosidic linkage of complex carbohydrates like starch into maltose and oligosaccharides in the small intestine. Inhibition of this enzyme reduces the rate of digestion of starch and result in a decrease in the post-prandial blood glucose levels in diabetic patients [1]. Reducing

postprandial hyperglycemia prevent glucose uptake in adipose tissue to inhibit synthesis and accumulation of triacyl glycerol. On the other hand, it is well known that dietary lipid is not directly absorbed from the intestine unless it has been subjected to the action of pancreatic lipase. Lipases are enzymes that digest fats, including triacylglycerol and phospholipids. It removes fatty acids from the alpha and α' position of dietary triglycerides and yields β -monoglycerides and long chain saturated and polyunsaturated fatty acids as the lipolytic products [2]. Lipase is responsible for the hydrolysis of 50–70% of total dietary fats. Lingual lipase secreted by serous gland, digests approximately one third of ingested fat. Gastric lipase secreted in response to mechanical stimulation, ingestion of food or sympathetic activation, accounts for the hydrolysis of 10–40% of dietary fat [3]. These two enzymes, thus, potentially limit the nutritional impact of the inhibition of lipid absorption that could result from the reduction in the activity of Pancreatic Lipase alone. Pancreatic Lipase inhibition is one of the most widely studied mechanisms for the determination of the potential efficacy of natural products as antiobesity agents. One of the most important strategies in the treatment of obesity includes development of inhibitors of nutrient digestion and absorption, in an attempt to reduce energy intake through gastrointestinal mechanisms, without altering any central mechanisms. Since dietary lipids represent the

major source of unwanted calories, specifically inhibiting triglyceride (TG) digestion forms a new approach for the reduction of fat absorption.

The major initial reaction products of lipid peroxidation are lipid peroxides and their quantitation serves as a direct and valuable index of the oxidative status of polyunsaturated fatty acid-containing tissues (membranes) or biosystems. Oxidatively modified human serum low density lipoprotein (LDL) has recently gained increasing interest in atherosclerosis research. It might represent a form of LDL by which monocyte~macrophages can be transformed to lipid-laden foam cells, a cell type that is often found in early atherosclerotic lesions [4]. Several methods currently exist for estimating the oxidation of lipids and some of them have been used to assess lipid oxidation in oxidized lipoproteins. Based on the reaction of malondialdehyde, a breakdown product of lipid peroxides, with thiobarbituric acid (TBA), the measurement of so-called thiobarbituric acid reactive substances (TBARS) has been used commonly to check lipoproteins for products of lipid peroxidation. One therapeutic approach to reducing post-prandial hyperglycaemia is to prevent glucose absorption via the inhibition of the carbohydrate-hydrolysing enzymes such as α -amylase [5]. Examples of such inhibitors in clinical use today are acarbose, miglitol and voglibose [6]. However, side effects of these compounds such as diarrhoea, vomiting, flatulence, abdominal cramp, fullness, and pain

have been reported [7,8]. Hence, there is a constant search for a better lipase and α -amylase inhibitors from natural sources. Some researchers reported that the different extracts of *Physalis minima* [9], *Crotalaria Juncea Linn* [10] and *acacia bark* [11] exhibited the inhibitory action on the amylase and lipase activity *in vitro individually*. In this study, *Boswellia ovalifoliolata* Bal. Henry (BOB) was selected and distributed in hot spots of India's Tirupati-Tirumala- Nallamalai hills, belonging to Burseraceae family. It is vernacularly known as konda guggilum in Telugu. The leaves are used in the mouth and throat ulcers. The stem is used in stomach ulcers, diabetes. Gum is used in dysentery, inflammations, joint pains, ulcers, arthritis, and amoebic dysentery [12]. Hence, the aims of this study are to investigate the *in vitro* effect of BOB extracts on α -amylase and lipase inhibition and antilipidperoxidation capacity.

2. Material and Methods

2.1. Collection and Authentication

The plant was collected from the surrounding areas of Talakona, A.P, India and was identified and authenticated by Mr. Madhavachetty, Botanist, S.V.University, Thirupati, Andhra Pradesh. The plant was identified as *Boswellia ovalifoliolata* bal.henry belonging to a family – Poaceae. The plant was dried in shade and ground to uniform powder using a milling machine.

2.2. Chemicals

Petroleum ether, chloroform, ethanol was purchased from SD Fine chemicals Ltd., (India). Chemicals used for determination of contents of total phenols, tannins and flavonoids were obtained from Sigma, Merck, and SD Fine chemicals Ltd. All the reagents used were of laboratory and analytical grade. Diagnostic kits were purchased from the Reckon diagnostic kit, India. Moreover, all the parameters were estimated using an automatic analyzer (Robonik Touch, version 2.622A) and Elico SL196 double beam spectrophotometer.

2.3. Preparation of Extracts

The extracts of *BOB* were prepared by soxhlet apparatus which was done with various solvents. The shade dried whole plant powder was packed in thimble kept in the soxhlet apparatus and extraction was allowed to run successively using the solvents, petroleum ether (60±80°C), chloroform and ethanol. Finally, the marc was dried and macerated with chloroform-water for 24 hours to obtain the aqueous extract. Petroleum ether and chloroform were used to defat the final extract. Only ethanol and aqueous extract was concentrated by evaporating the solvent on the Water-bath and the obtained extracts were weighed.

Even using maceration technique, the extract of *BOB* was prepared by the following. 50 g of shade dried whole plant powder was suspended and extracted with 10 volumes of methanol by

shaking at room temperature for 15 hours. The extracts were filtered through filter paper, and the supernatants were pooled. The residue was re-extracted under the same conditions. Pooled extracts were condensed (and methanol was removed) with a rotary evaporator at 50°C.

2.4. Polyphenol, Flavonoid and Tannin Estimation:

Total phenolic content [13], flavonoid content [14] and condensed tannin content [15,16] were analyzed spectrophotometrically. For phenolic content estimation, Gallic acid was used to prepare a standard curve (0.2 to 10µg/ml; $y=0.06218x+0.131$; $r^2 = 0.9850$; y is the absorbance; x is the solution concentration) and the results were expressed as milligrams of Gallic acid equivalents (GAE) per gram of the powdered crude drug. For flavonoid content estimation, Rutin was used to prepare a standard curve (0.2 to 10µg/ml; $y=1.4297x-0.3178$; $r^2=0.9854$; y is the absorbance; x is the solution concentration) and the results were expressed as milligrams of rutin equivalents (RE) per gram of the powdered crude drug. For condensed tannin content, Tannic acid was used to prepare a standard curve (0.2 to 10µg/ml; $y=0.0271x+0.157$; $r^2 = 0.9951$; y is the absorbance; x is the solution concentration) and the results were expressed as milligrams of tannic acid equivalents (TAE) per gram of the powdered crude drug.

2.5. Amylase Inhibition Activity

Activity measured by a test kit obtained from Reckon diagnostics Pvt. Ltd. Aliquots (50µl) of amylase standard and samples (using water as blank) added and mixed gently and incubated for 5min at 37°C. Working reagent (1000µl) added and mixed by gentle inversion, and the samples incubated again for 1min at 37°C. The recorded rate of decrease in absorbance at 405nm from 1min to 3min with the regular interval of 30sec. Atorvastatin used as a standard. Determine the alpha amylase activity in the samples prepared using for following kinetic formula, Alpha amylase activity (IU/L)= $\Delta A/\text{min} \times F$, whereas, F is a factor and is equal to $(1/12.9) \times (\text{Total volume}/\text{Sample volume}) \times 1000$ where, 12.9 is the milimolar absorbance of 2-Chloro-4-Nitrophenol and 1000 for conversion of activity per ml to per liter.

2.6. Lipase Inhibition Activity

Working reagent and lipase calibrator (450U/L) obtained from Reckon diagnostics Pvt. Ltd. Aliquots (50µl) of lipase standard and samples (using water as blank) added and mixed gently and incubated for 5min at 37°C. Working reagent (1000µl) added and mixed by gentle inversion, and the samples incubated again for 4min at 37°C. The recorded rate of decrease in absorbance at 340nm from 4min to 8min with the regular interval of 60sec. Atorvastatin used as a standard. Determine the pancreatic lipase activity in the samples prepared using for

following kinetic formula; Lipase activity (U/L) = $(\Delta A/\text{min of sample} / \Delta A/\text{min of calibrator}) \times \text{calibrator value}$.

2.7. Antilipid Peroxidation Activity in Goat Liver

Goat liver was collected from local market of Kakinada. Goat liver perfused with normal saline through the hepatic portal vein was harvested and its lobes were briefly dried between filter papers to remove excess blood and thin cut with a heavy-duty blade. The small pieces were transferred in a sterile vessel containing phosphate buffer (0.1 M, pH 7.4) solution. After draining the buffer solution as completely as possible, the liver was immediately grinded to make a tissue homogenate (1g/ml) using freshly prepared phosphate buffer (0.1 M, pH 7.4). The homogenate was centrifuged at (1000 rpm, 15 min) to remove debris. The supernatant was used to assay the lipid peroxidation activities. Extracts were used in various concentrations (500 and 250 $\mu\text{g/ml}$) individually. 3ml of liver homogenate was added with 100 μl of 15mM ferric chloride solution and was shaken for 30 min. From collected mixture, 100 μl was added with 1ml of different concentration of both plant extracts individually in different test tubes. The same procedure was followed for control and Standard (ascorbic acid). All the test tubes were incubated for 4hrs at 37°C. After incubation, 1.1 ml of 30% trichloroacetic acid (TCA) and 1.1 ml

of 0.65% thiobarbituric acid (TBA) were added to all tubes containing the mixture. The tubes were kept in a shaking water bath for 30 min at 80 °C. After 30 min of incubation the tubes were taken out and kept in ice-cold water for 10 min. These were then centrifuged at 800 g for 15 min. The amount of malondialdehyde[17] was assessed by measuring the absorbance of supernatant at 530 nm at room temperature against an appropriate blank. The percentage inhibition of Lipid peroxidation was calculated using the equation. The percentage of anti lipid peroxidation = $((\text{Control} - \text{Sample}) / (\text{Control})) \times 100$.

2.8. Antioxidant Activity on Linoleic Acid

Two dilutions of each extract (250 and 500 $\mu\text{g/ml}$) were prepared. For a typical assay an aliquot of 20 μl of each dilution was mixed with 20 μl of 2 mg/ml linoleic acid in ethanol and incubated at 80°C for 60 min. Incubated samples were cooled in an ice bath, followed by the addition of 200 μl of 20mM butylated hydroxytoluene (BHT, Sigma), 200 μL of 8% sodium dodecyl sulphate and 400 μl of distilled water. After mixing 3.2 ml of 12.5 mM 1,3-diethyl-2-thiobarbituric acid in sodium phosphate buffer (0.125 M, pH 3.0) was added. After mixing, the tubes were heated at 95°C for 15 min, and cooled in an ice bath. Then 4 ml of ethyl acetate was added to each tube, vortexed to extract the pink adduct from the aqueous phase, and centrifuged at 700 g for 10 min. A control

Table 1. *In vitro* Activity of *Boswellia Ovalifoliolata* Bal. Henry Extracts.

<i>Samples (mcg/ml)</i>	<i>amylase activity (IU/L)</i>	<i>Amylase inhibitory</i>	<i>Lipase activity (U/L)</i>	<i>Lipase inhibitory</i>	<i>%antilipid peroxidation</i>	<i>% antioxidant activity</i>
Standard (250)	1.62±0.235	90.47±1.37	78.57 ±7.14	82.53±1.58**	53.18±0.4107**	57.28±0.41**
BOI (250)	11.26±0.358**	34.12±2.1*	271.42 ±7.14	39.68±1.58	52.97±5.955**	44.14±5.33**
BOI (500)	7.46±0.358**	56.34±2.1*	242.85 ±7.14	46.03±1.58	62.83±4.723**	56.67±1.43**
BOE (250)	13.97±0.135**	18.25±0.79**	114.28 ±7.14**	74.60±1.58**	22.79±1.643*	18.68±1.64
BOE (500)	11.12±0.358**	34.92±2.1*	92.85 ±7.14**	79.36±1.58**	48.45±1.848**	44.55±1.64**
BOW (250)	14.65±0.235**	14.28±1.37**	378.57 ±14.28**	15.87±3.17**	26.69±1.437**	22.58±1.43*
BOW (500)	11.53±0.358**	32.53±2.1*	307.14 ±7.14**	31.74±1.58**	43.12±5.544**	39.01±5.54**

Results represents means±SEM, n =6. **P<0.01; *P<0.05; BOI= macerated methanol extract; BOE=ethanol extract; BOW= water extract; Standard for amylase & lipase is atorvastatin whereas ascorbic acid is for antioxidant activity

containing linoleic acid and other additives without antioxidants, representing 100% lipid peroxidation was also prepared [18]. The absorbance of an ethyl acetate layer of the sample and control solution was measured at an excitation wavelength of 515 nm. The antioxidant activity was calculated as the percent of peroxidation inhibition using the following equation:

$$\text{Percent of peroxidation inhibition} = ((\text{Control} - \text{Sample}) / (\text{Control})) \times 100.$$

3. Results and Discussion

BOB extracts were investigated for their total phenolic compounds, flavonoids and tannins using spectrophotometry. The results of phenolic content, flavonoids and tannin by spectrophotometry were expressed by

mean±standard deviation. In the phenolic content determination, the milligrams of Gallic acid equivalents (GAE) per gram of extracts were found to be 0.082±0.01, 0.243±0.24 and 1.099±0.31 for ethanol, aqueous and macerated methanol extracts respectively. The macerated methanol extract contained maximum total phenolic content (1.099mg GAE/g) than other extract. In flavonoid content estimation, the milligrams of rutin equivalents (RE) per gram of extracts were found to be 0.065±0.015, 0.047±0.005 and 0.524±0.159 for ethanol, aqueous and macerated methanol extracts respectively. The macerated methanol extract contained maximum total flavonoid content (0.524mg RE/g) than other extract. In tannin content estimation, the milligrams of tannic acid equivalents (TAE) per gram of extracts were

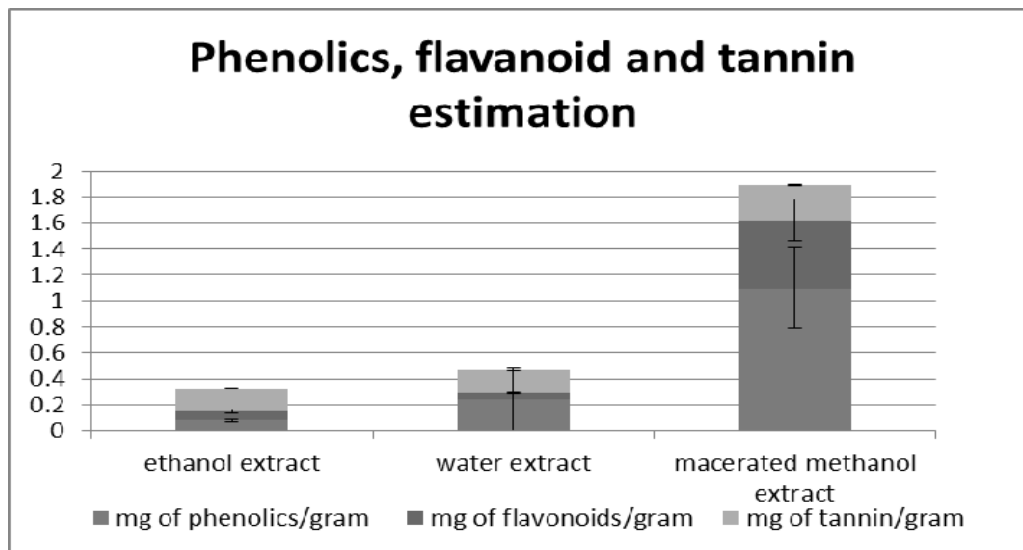


Figure 1. phenolic, flavonoid and tannin estimation on *Boswellia ovalifoliolata* Bal. Henry extracts.

found to be 0.172 ± 0.0007 , 0.174 ± 0.002 and 0.27 ± 0.008 for ethanol, aqueous and macerated methanol extracts respectively. The macerated methanol extract contained maximum total condensed tannin content (0.27 mg TAE/g) than other extract. Figure 1 expresses the results of phenolic, flavonoid and tannin content in *BOB* extracts. In our experiments, all the extracts with two different concentrations (250 mcg/ml and 500 mcg/ml) showed *in vitro* inhibitory activity on amylase and lipase and antioxidant activity in a dose dependent manner significantly (** $p < 0.01$) and also exerted a moderate inhibitory action on amylase and lipase and antioxidant activities (Table 1) when compared with standards. Standard (250 mcg/ml) was shown 90.47% and 82.53% inhibition of amylase and lipase activity and 53.18% and 57.28% on antilipid peroxidation and

antioxidant on linoleic acid emulsion. Among the all extracts, Macerated methanol extract (500 mg/kg) was shown greater inhibition on amylase (56.34%) but in lipase inhibition, ethanol extract was shown maximum (79.36%) inhibition. Among the all extracts, Macerated methanol extract (500 mg/kg) was shown high antilipid peroxidation (62.83%) and antioxidant on linoleic acid emulsion (56.67%). In all inhibitory activity, water extract was shown less than other extracts. The correlation between the constituents and the activity of herbal extracts explains the inhibition activity on amylase and lipase, antilipid peroxidation and antioxidant activity on the linoleic acid emulsion of the macerated methanol extract is due to the presence of high concentration of phenolic compounds, flavonoids and tannins.

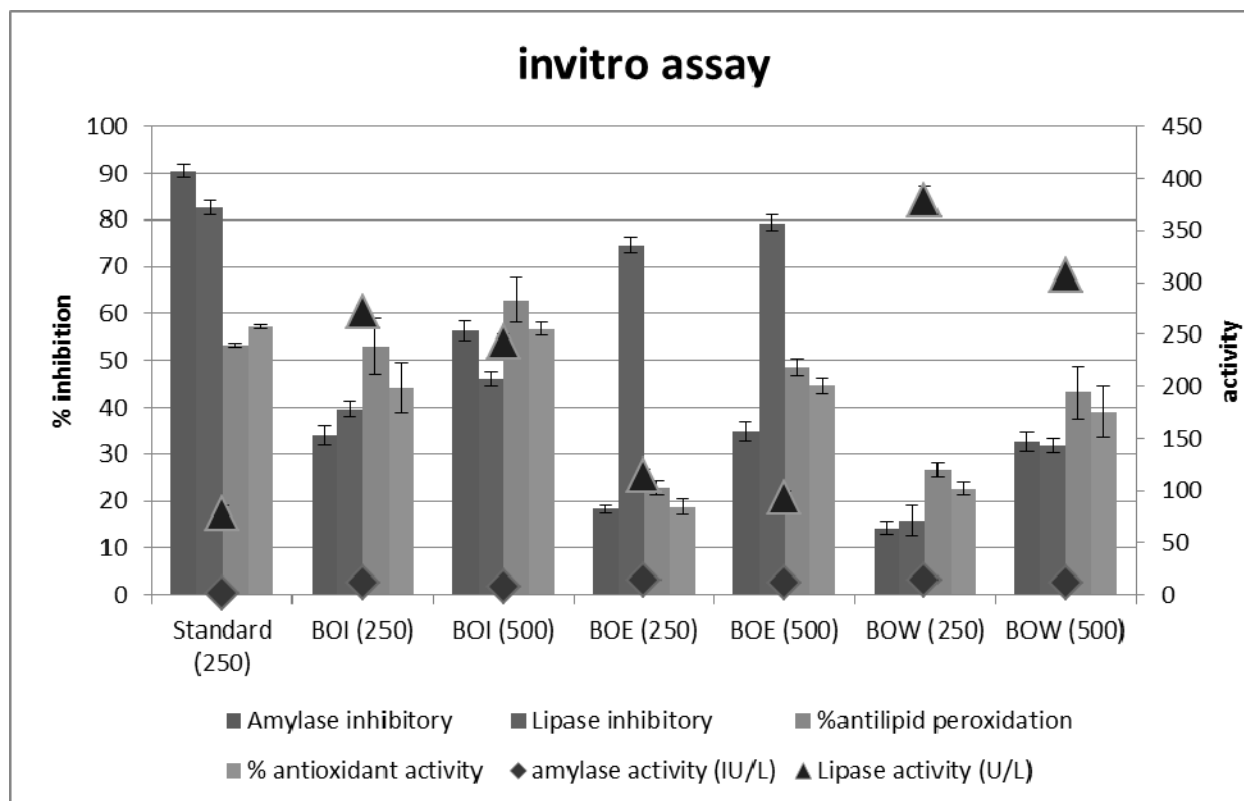


Figure 2. Amylase, lipase and antioxidant activities on *Boswellia ovalifoliolata* Bal. Henry extracts.

Alpha amylase uses a chromogenic substrate Gal-G3-CNP which, by the reaction of alpha-Amylase breaks down to release 2-Chloro-4-Nitrophenol. The release of 2-Chloro-4-Nitrophenol is measured at 405nm and is proportional to the alpha amylase activity. Lipases catalyse the hydrolysis of triolein in the presence of colipase to form glycerides and fatty acids. The rate of decrease in turbidity measured at 340nm is proportional to the lipase activity.

As lipid peroxides are increasingly thought to have a pathogenic role in many disorders a specific and reliable test for their concentrations in plasma is greatly needed. Oxidative stress has been implicated in the pathology of many

diseases and conditions including cardiovascular disease, diabetes, inflammatory conditions, cancer and ageing. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid per-oxidation and other mechanisms. Bioactivity of *BOB* extracts may be caused by the potentiating action of several compounds within the active fractions of the extracts.

4. Conclusion

In this investigation, we concluded that macerated methanol could extract the highest concentration of polyphenols, flavonoids and tannins from the *BOB* plant which are

responsible for the above mentioned *in vitro* activity. So further studies are required to confirm its pharmacological potency through *in vivo* studies, by that we can assure its potential for exploitation to promote human and animal health.

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