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IMPACT OF OPTIMAL EXTRACTION ON PHARMACOLOGICAL ATTRIBUTES OF *ARTEMISIA ABSINTHIUM LINN* FROM PAKISTAN

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Abstract
The oxidative stress is the precursor of many diseases including diabetes and obesity. The side effects of synthetic drugs and commercial antioxidants urge to explore plant based sources for novel pharmacological agents and natural antioxidants for stress and disease management. In the current work, various compositions of methanol (20%, 40%, 60%, 80% and pure methanol) were used to optimized the extraction process and consequent analysis for extract yields, DPPH scavenging, total phenolic contents, total flavonoid contents, α-glucosidase and pancreatic lipase inhibitions for *Artemisia absinthium Linn*. The extraction was assisted with freeze drying and ultrasonication. The findings revealed that 80% methanol yielded comparatively higher extract yields (15.80±0.55gm), total phenolic contents (84.20±1.09 mg GAE/g DE) and total flavonoid contents (49.01±1.20 mg RE/g DE) for *Artemisia absinthium Lin*. The DPPH scavenging % value of 85.80±1.20 was also observed for 80% methanolic extract.The 80% methanolic extract also exhibited higher α-glucosidase inhibition value of 54.20±1.45% and pancreatic lipase inhibition value of 73.58±1.24%. The high phenolic and flavonoid contents were responsible for better antioxidant activity and dietary enzyme inhibitions. The *Artemisia absinthium Linn* was observed as a potential source for obesity and diabetes mellitus type-2 management which may be further investigated for novel antioxidant system and therapeutic agents.

Keywords:*Artemisia absinthium*, extraction optimization, antioxidant, antiobesity, antidiabetic, phytochemistry.

Introduction
Reactive oxygen species (ROS) are produced in bodyas a result of metabolic functions. The human body also gets exposure to ROS through various environmental factors. The ROS and other free radicals produced in body are neutralized by natural antioxidant defense of body or antioxidant

system added through food intake. However, sometimes the ROS production is triggered due to some endogenous or exogenous factor and the antioxidant system of body fails to control the overproduction of ROS [1]. The imbalance between ROS and antioxidant defense results in establishing the state of oxidative stress. The oxidative stress may be temporary or long term depending upon circumstances. The ROS especially in state of oxidative stress are highly damaging to body tissues, organs, enzymes and other biochemical entities [2]. The detrimental impacts of ROS are not simple but of complex nature causing many ailments like diabetes, obesity, neural disorders, kidney dysfunctioning, retinal and many more health issues [3].
The synthetic medicines used to treat these diseases are not only costly but also associated with serious health complications and side effects and their continuous use is creating socio-economic burden. The synthetic antioxidants are very efficient in controlling the process of oxidation but their toxicity issues make them unfit for continuously high dose oral intake [4]. Ultimately, the only possible and effective choice is the plant based antioxidants and phytotherapeutic agents which have the ability to control or mitigate oxidative stress but also to treat chronic ailments like diabetes mellitus type 2 (DM-T2), obesity, neural disorders, digestive problems and to stabilize the food systems [5].

Many studies on plants have been reported under various aspects to assess therapeutic role of plants and their products. An important aspect is the role of extraction strategy, especially the solvent. The conventional local medicinal practitioners are not using modern technologies to get maximum therapeutic and pharmacological output from medicinal plants. Methanol is considered as optimum choice for extraction from plant material to assess its pharmacological attributes [6].

Artemisia absinthium Linn (*A. absinthium*) has been reported for the treatment of constipation, splenomegaly, jaundice, obesity, diabetes, anemia, bladder diseases, and insomnia [7].*A. absinthium Linn* is being used to treat diabetes and obesity in local medicinal system of Pakistan but no scientifically proven evidence exists in this context. The current work was designed to select the optimum methanol composition for better extract yields and consequent testing for antioxidant, phenolics, flavonoids and in vitro antidiabetic and antiobesity attributes.

Material and methods

Collection of plants:

A. absinthium Linn. is an evergreen and widely spread woody shrub that is found everywhere, especially in the Mediterranean and Pacific areas. This herb is abundantly found everywhere near pathways, roads, and fields. Due to this availability, it is also called woodworm[8]. The plant material was collected from Pattoki area of District Kasur, Pakistan.

Chemicals

Methanol, DPPH, distilled water, Follin-Cicoalteau reagent, gum Arabic, Tris-HCl Buffer, pancreatic lipase, α-amylase, Na_2CO_3, Gallic acid, $AlCl_3$, and α-glucosidase. The chemicals and reagents utilized were of analytical grade.

Extract Preparation:

Plants were freshly taken from the source andthe fresh plant was quenched in liquid nitrogen to preserve all possible metabolites then grinded and powdered [9] The solution was made in different compositions of methanol and distilled water at different ratios. Simply, 100gm of dried plant powder obtained after lyophilization was dissolved in 1000mL distilled water, and 5 compositions of 20%, 40%, 60%,80% methanol, and pure methanol. Stored the mixture for 48 hours in a dark cool place. Then, the mixture was sonicated for 30 minutes @150 reps at 10°c below temperature. The sonicated sample was evaporated in a rotary evaporator under a vacuum to remove the remaining water or moisture. The sample was filtered to remove plant matrix and debris and

obtained a clear solvent of extract. The filtrate was then taken to lyophilization where is completely dried to powder form and extract was obtained.

DPPH activity

DPPH scavenging Assay for the antioxidant was carried out for the plant extraction and evaluated by using spectrophotometric measurements [10]. The polyphenolic contents in the sample resulted in the changing of color from violet to the yellow color of the DPPH reagent. The % inhibition value was measured as the antioxidant potential. The reference standard was used as (BHA) Butylated hydroxy anisole.

$$Scavanging\ Activity\ \% = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance}$$

Total Phenolic Contents TPC determination

TPCs of extract from the plant were determined by a modified well-known method [11]. Beirfly, a solution was made with the methanol and 200uL of the extract of plants and mixed with FC reagent, 20% Na_2CO_3 solution with 4mL addition to the solution. The prepared solution was kept in a stand for 90 minutes. After that, the samples were subjected to absorbance measurement at a wavelength of 750nm. The TPC result expresses gallic acid in milligrams equivalent per gram of dry weight.

Determination of Total Flavonoid Contents

A method used for the determination of flavonoid contents present in plant extract is carried out by a well-known method based on aluminum [12]. For this process, rutin has been used as a standard sample. The plant extract was dissolved in methanol with a sample solution of 200 µl. 0.3 M of $AlCl_3$.$6H_2O$ (o.15ml), 0.5M of $NaNO_2$, and 30% methanol was added in solution. Put in a stay at ambient temperature for 5 minutes and add 1ml of NaOH in the mixture of the reaction solution. The absorbance was measured at a wavelength of 510nm. The TFC result was noted and expressed in milligrams of rutin in dry weight per gram.

Pancreatic Lipase Inhibition Activity

A reported method used famously was followed up with some changes and modifications for in vitro and in-vivo assays for the anti-obesity activity of the extracted plant compounds. A freshly prepared solution of pancreatic lipase (PLP) was added with 0.01 M Tris-HCL in porcine pancreatic lipase [13]. This solution was freshly prepared and a plant extraction solution was added to this solution, then Gum Arabic was added with a mixture of olive oil. 1:1 ethanol and acetone were added to stop the reaction. The mixture was then titrated by the titrant as 0.02 M NaOH to determine the contents of free fatty acids. The inhibition %age of the enzyme was calculated by using the following formula relation.

$$\%\ Inhibition = 100\% - \left[\left(\frac{Vs}{Vc}\right) X100\right]$$

Vs = volume of Sample
Vc = Volume of Control

α-Glucosidase Inhibition Assay

By following a well-known reported method, the activity of α-glucosidase inhibition of plant extract was determined with a slightly changed and modified method [14]. Here, a reaction mixture is prepared that consists of 80µl of phosphate buffer (pH=6.8), α-glucosidase 91 unit/ml) from yeast, and 10µg of plant extract was prepared. The mixture put in stays for 10 minutes at a temperature of 37°c. After that, p-nitrophenyl glucopyranoside of 10µl was added. Then the mixture was placed for 30 minutes in incubation and measurement of absorbance was done at 405nm wavelength for p-nitrophenol released.

The %age inhibition of activity was calculated by following formula:

$$Enzyme\ Inhibition\ \% = \left[\frac{Ab - As}{Ab}\right] X100$$

Where, Ab= Absorbance at blank
As = Absorbance of sample
The standard acarbose was used as standard antidiabetic agent.

Statistical Analysis

The SD standard deviation (±) for multiple results values was applied. ANOVA 17.0 software using Minitab was considered for one-way analysis variance testing computed the difference significantly.

Results and Discussion

Extract yields, TFC, and TPC values.

The % yield extracts result of TFC and TPC are given in table 1 below. With the variety of solvent compositions, the extract yields also varied.The results indicated that 80% methanol yielded higher extract yield, TPC and TFC contents and statistically significant as compared to other methanol compositions ($p<0.05$).

Table 1: Solvent Effect on Extract Yield, TFC, TPC

Extracting Solvent	Extract yields %	TFC (mg GAE/g DE)	TPC [mg RE/g DE)
20% Methanol	9.92 ± 0.54^{e}	14.93 ± 0.55^{e}	35.10 ± 1.04^{d}
40% Methanol	8.97 ± 0.14^{de}	18.92 ± 0.45^{d}	31.56 ± 1.23^{e}
60% Methanol	11.08 ± 0.42^{c}	33.45 ± 0.32^{c}	56.70 ± 1.25^{c}
80% Methanol	15.80 ± 0.55^{a}	49.01 ± 1.20^{a}	84.20 ± 1.09^{a}
Pure Methanol	12.45 ± 0.98^{b}	38.02 ± 1.03^{b}	78.20 ± 1.98^{b}

The values not having common letter are statistically significant($p<0.05$).

The high extract yield obtained by 80% methanol was most probably due to polarity of solvent due to its composition. The high extract yield also generated high TPC and TFC. The 80% methanol was the most optimal choice for improved extract yield, TPC and TFC from *A. absinthium Linn*. The polarity of solvent was the most probable cause behind the linkage between higher extract yield, TPC and TFC [15].

The results of DPPH scavenging, α-glucosidase and pancreatic lipase inhibition were presented in table 2.

Table 2: DPPH scavenging % and Enzymatic inhibitions

Extracting Solvent	DPPH scavenging %	α-Glucosidase inhibition %	Pancreatic lipase inhibition %
20% Methanol	44.50 ± 1.5^{e}	24.46 ± 1.23^{e}	25.75 ± 1.25^{e}
40% Methanol	58.33 ± 1.11^{d}	28.15 ± 1.05^{d}	41.88 ± 1.44^{d}
60% Methanol	65.57 ± 0.91^{c}	30.33 ± 1.11^{c}	62.10 ± 1.05^{c}
80% Methanol	85.80 ± 1.20^{a}	54.20 ± 1.45^{a}	73.58 ± 1.24^{a}
Pure Methanol	70.05 ± 1.05^{b}	37.55 ± 2.05^{b}	67.23 ± 1.11^{b}
StandardsCompounds	BHA = 96.66 ± 0.33	Acarbose = 86.02 ± 0.11	Orlistat = 84.15 ± 0.15

The values not having common letter are statistically significant($p<0.05$).

The results indicated that solvent composition substantially effected the antioxidant and enzyme inhibitions. The 80% methanolic extract exhibited highest values of DPPH scavenging, α-glucosidase and pancreatic lipase inhibition. The high phenolic and flavonoid contents present in 80% methanolic extract were responsible for comparatively higher antioxidant activity. The phenolic compounds have the ability to stabilize the DPPH radical by proton donation changing the color from violet to yellowish. The reasonable antioxidant activity by 80% methanolic extract of *A. absinthium*was observed.Similarly, 80% methanolic extract of *A. absinthium Linn* also resulted in higher α-glucosidase and pancreatic lipase inhibition values which owed to the existence of high TPC and TFC values [16]. However, the high inhibition % value for pancreatic lipase enzyme

indicated that the 80% methanolic extract of *A. absinthium Linn* was more effective to control obesity as compared to diabetes. The phenolic compounds have ability to bind the active sites of enzymes to restrict their activities. The binding of metabolites with glucosidase enzyme results in slow digestion of carbohydrates which consequently reduces post prandial blood glucose level [17].
The binding of plant metabolites with pancreatic lipase enzyme inhibits or reduces the enzyme efficiency resulting in less digestion of fats which resultantly reduces the absorbable fats in body intestine. A previous study on *Conocarpus lancifolius* reported that the plant secondary metabolites bind with the active sites of enzymes to reduce their activity for diabetes and obesity management. The plant based compounds like gallic acid, ellagic acid, rutin, quercetin and their derivatives were reported to induce antidiabetic and antiobesity effects by inhibiting the dietary enzyme activities [18].The plant metabolites were reported to exhibit the antioxidant and glucose lowering effect to manage the oxidative stress related diseases [19]. The presence of secondary metabolites in plant extracts was also reported to be responsible for immune system improvement and disease management.
The findings of current work emphasized that 80% methanolic extract of *A. absinthium Linn* was quite effective source of natural antioxidants to manage diabetes and obesity confirming its ethnophramacological use for blood sugar control and obesity.

Conclusion

The 80% methanolic extract of *A. absinthium Linn* was the most effective fraction having high antioxidant, TPC and TFC values due to suitable polarity of solvent system. The high TPC and TFC values were also responsible for better α-glucosidase and pancreatic lipase inhibition. The findings confirmed that *A. absinthium Linn* is a potential candidate for naturopathic approach to treat oxidative stress oriented diseases like diabetes mellitus type 2 and obesity. Further characterization of extract may be performed to identify the secondary metabolites present in the plant extract.

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