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SCREENING OF PHYTOCHEMICALS AND *IN VITRO* ANTIDIABETIC POTENTIAL OF *RAPHANUS SATIVUS* LEAVES EXTRACT

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ABSTRACT

Now a day, plants are gaining importance globally due to pharmacological and medicinal behavior. The current study aimed to evaluate the phytochemical screening and antidiabetic potential of *Raphanus sativus* leaves extract. The leaves extract of *Raphanus sativus* was prepared through maceration using different solvents such as n-hexane, chloroform, ethyl acetate, n-butanol, and methanol for one week. Qualitative analysis was carried out using standard protocols. Quantitative analysis was also performed for the determination of total alkaloids, saponin, total phenolic content, total flavonoid content, and antidiabetic activity. The total phenolic contents (TPC) and total flavonoid contents (TFC) were analyzed by spectroscopic technique. The alpha amylase inhibition was also done through a standard procedure cited in the literature. The preliminary phytochemical screening showed that the ethyl acetate extract extracted the maximum amount of flavonoids and alkaloids compared to other extracts. The quantitative analysis showed that the total phenolic and flavonoid contents ranged from (640.7 ± 6.4 to 154.9 ± 1.6) GAE mg/100 g and (103.6± 1.1 to 6.5 ± 0.1) per mg/100 g GAE, respectively. All of the plant extracts (*Raphanus sativus*) inhibited alpha amylase, but the ethyl acetate extract (36.2% inhibition) was the most potent. The findings of the studies reflect the importance of medicinal flora to counteract the adverse effects of drugs as well as the internally produced free radicals, responsible for metabolic disorders, including diabetes. So, the plant extract *Raphanus sativus* may be used as an herbal remedy alone as well as with drugs to cure metabolic disorders.

Keywords: Maceration, Antidiabetic, Medicinal flora, Alkaloids, Saponins.

INTRODUCTION

According to the health concern of the individuals as well as communities, medicinal plants gaining a great importance world widely (Naveen *et al.,* 2021). Chemical substances in medicinal plants have a physiological action on the human body. The important bioactive constituent of plants includes phytochemicals and phenolic compounds, usually called secondary metabolites (Roaa, 2020). The secondary metabolites act as herbal products which are used to cure several diseases in human. The substantial properties of natural/medicinal plants include anti-inflammatory activity, cytotoxicity, antidiabetic, and antioxidant ability has renewed the researches interest (Chekole, 2017). These properties are helpful to cure health diseases in mankind and keep strong the defensive mechanism of plants against disease (Hammerbacher *et al.,* 2019).

Raphanu ssativus belonging to Brassicaceae family, diuretic nature, is playing the role of medicinal plant. *Raphanus sativus* has a great role in the treatment of jaundice, gynaecological disorder, misfunctioning of the liver, antitumor, and poor digestion (Yang *et al.,* 2021). It also has a laxative effect and used for the

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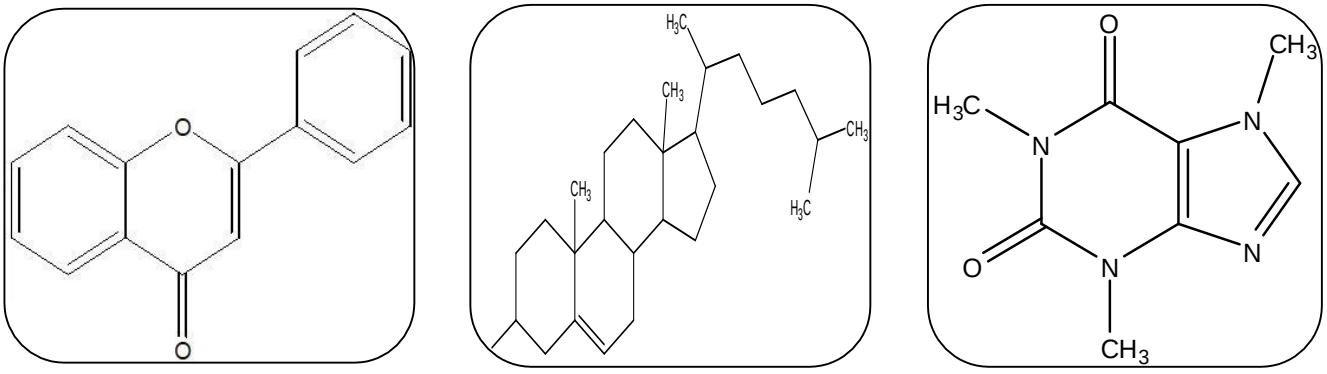
treatment of diarrhoea, bronchitis, constipation, and act as an appetizer (Jaafar *et al.,* 2020). The other name is said to be radish in English, Mooli/muli in Punjabi and Hindi language. Radish is a rich source of vitamin C, minerals which are helpful to build up tissues, maintain blood vessels and teeth (Dias, 2012). Some phytochemicals are structurally showed here which having medicinal importance (Figure 1).



Flavones Cholesterol Caffeine

To check the medicinal activity of *Raphanus sativus* leaves, various biological methods have been performed. Through these methods, assessment of phytochemicals and other properties of plants such as antioxidant, antimicrobial potential have been taken place (Zrouri *et al.,* 2021). It is revealed that the phytochemical of the said plant has a strong defensive mechanism against pesticides. Besides these phytochemicals, Diabetes mellitus, a globally concerned disease in which the quantity of glucose unable to maintain considered a major cause of death (reference). So, this systematic study is a connection between Radish and diabetes to meet death risk (Goyal *et al.,* 2017).

Radish is a potential to enhance defensive mechanism at the same time it reduces the oxidative stress. This defensive mechanism causes an imbalance between antioxidants and reactive oxygen species in case of the cellular system (Dumanović *et al.,* 2021). Diabetic conditions have been improved favorably by their basic components such as anthocyanin, flavonoids, sugar dietary fibers, and carbohydrates (Governa *et al.,* 2018). Radish can enhance the capacity of blood to carry oxygen in other parts of the body as well as the ability to purify the blood(Kota *et al.,* 2017). Overall radish considered to be a bioactive component has a beneficial effect on the human bodyfrom a medicinal perspective. The current research work was carried out to evaluate the medicinal activity such as antidiabetic, and phytochemicals of *Raphanus sativus* leaves extract.

MATERIALS AND METHODS

Collection and identification of plant material: *Raphanus sativus* leaves were gathered from district Faisalabad, Pakistan. At that point, the plant was identified and verified by Prof. Dr Muhammad Hanif, Department of Botany, University of Sargodha Women Campus Faisalabad. The voucher sample was deposited in the collection Department of Botany, University of Sargodha women Campus Faisalabad.

Chemicals used: All the chemicals used in the study trial are of analytical grade and were purchased from Sigma Aldrich and Merck. There are the following reagents or chemicals used: Ferric chloride ($FeCl_3$), olive oil, hydrochloric acid, concentrated sulphuric acid, dilute ammonia solution, acetic acid, chloroform, diethyl ether, concentrated ammonium hydroxide, NaCl, sodium carbonate (Na_2CO_3), folinciocalteau phenol reagent, sodium nitrite ($NaNO_2$), Aluminum chloride ($AlCl_3$), sodium hydroxide, Alpha-amylase, starch solution, DNSA, methanol, ethanol, n-butanol, n-hexane, ethyl acetate.

Preparation of plant extract: To prepare the extract, initially plant leaves were washed. Then washed leaves were shade dried by spreading on the floor. The shade dried leaves were ground to fine powder form by mechanical ways. At that point, 250 grams of dry powder plant material was extracted thrice by maceration with different polarity based solvents i.e. n-hexane, chloroform, n-butanol, ethyl acetate and 80 % methanol. Leaves powder was soaked for around 72 hours into solvents one by one with increasing polarity order i.e. n-hexane, chloroform, ethyl acetate, n-butanol and finally with 80% methanol having relatively highest polarity. At that point by using a rotatory evaporator, the material was concentrated to dryness under reduced

pressure condition. The extract was collected and weighed. Additionally, put away in the refrigerator at -4ºC temperature until used for analysis (Akhtar *et al.,* 2022).

Phytochemical Screening: The preliminary phytochemical analyses were performed using the standard protocol with some modifications with respect to sample utilization, reagents, and room conditions. The phytochemicals estimated through the protocol are tannins, saponins, phlobatanin, flavonoids, steroids, terpenoids and cardiac glycosides (Harborne, 1973).

Quantitative determination: The quantitative determination was carried out using the standard protocol (Edeoga *et al.,* 2005). Through the given procedure, the following phyto-constituents were evaluated: total alkaloids, saponin, total phenolic contents, and total flavonoid contents.

Defatted sample preparation: 2 g of the weighed amount of powder sample of (*Raphanussativus*) leaves was made defatted with 100 mL of diethyl ether by using a soxhlet apparatus for the time duration of about 2 hours.

Alkaloid determination: In a 250 ml beaker, about 5 g of sample was taken, and then 200 ml of 10% of acetic acid ethanol were added. Then covered and allowed to stand for 4 hours. After filtration dropwise concentrated ammonium hydroxide was added to the sample extract then no precipitates were formed which indicate the absence of alkaloid.

Saponin determination: Twenty grams of powder sample was mixed with 100 mL of 20 % aqueous ethanol in a conical flask. With continuous stirring at a temperature of about 55 ^{0}C the sample was heated over the water bath. Then filtration was done of the mixture and 20% ethanol about 200 mL was mixed to the residue for re-extraction. Then at 90 ^{o}C both extracts were combined and heated over the water bath. Then the residue was shifted to a separatory funnel with 20 ml of diethyl ether and shaken vigorously and then separated ether layer. Then the mixture of 60 mL of n-butanol extracts was mixed with 10 ml of 5% NaCl. The filtrate was heated over a water bath, after the departure of water dried and weighed the residue. This indicated the presence of saponin. Finally calculated the yield of saponin.

Determination of Total Phenolic content: Determination of total phenolic content was determined by the method explained by (Riaz *et al.,* 2012). 50 mg (0.05g) of sample extract was mixed with 0.5 mL of Folin ciocalteau phenol reagent was also added in it. Then 7.5 ml of distilled water added to it. At room temperature mixture was kept for 10 minutes, and then 1.5 ml of 20 % Na_2CO_3 was added to the mixture. The mixture was heated on the water bath for 20 min at 40°C and afterwards cooled promptly on an ice bath, and then absorbance was noted at 755 nm with the help of spectrophotometer. The results of plant samples were represented as Gallic acid equivalent (GAE).

Determination of Total Flavonoid content: Determination of total flavonoid content was measured by using the method adopted by Riaz *et al*. (2012). About 0.01 g of sample extracts dissolved in their respective solvents in the test tube and then added 5 ml of distilled water. At that point, 0.3 mL of 5 % $NaNO_2$ was additionally added. After 5 minutes, 0.3 ml of 10 % $AlCl_3$ was added, mixed well, followed by an expansion of 2 ml of 1 M NaOH. Then the whole mixture was shaken well gently. Noted the absorbance at 510 nm with a spectrophotometer. Total flavonoid contents were expressed as catechin equivalents (CE)/g of the dry matter.

***In vitro* alpha (α) amylase inhibition assay:** The alpha amylase inhibition assay was performed by using the method demonstrated by Kifle and Enyew, (2020) with some modifications. Extracts in respective solvents were mixed with 500 µL of alpha-amylase solution. This mixture was incubated for 10 min at 25 ^{o}C. Then 500 µL starch solution was added, again this mixture was incubated for 10 min at 25 ^{o}C. 1 mL of DNSA solution was added in it and heated this mixture for 5 min in the water bath. On cooling, 10 ml of distilled water was added and measured the absorbance at 540 nm by a spectrophotometer. The same procedure was followed by all extracts prepared in different solvents. Acarbose used as a standard drug.

The % inhibition was calculated by using the formula

$$\text{Inhibition (\%)} = \frac{(\text{A control} - \text{A sample})}{\text{A control}} \times 100$$

Where A control was the absorbance of the control (without sample); A $_{\text{sample}}$ was the absorbance in the presence of sample.

RESULTS AND DISCUSSIONS

Recently, the research on medicinal plants has increased, and the use of plants as medicines in various traditional systems has also increased (Forni *et al.,* 2019). The medicinal plants are preferred because they produce a variety of compounds with therapeutic properties

(Aboyewa *et al.,* 2021). All over the world, complex diseases are treated with plant-based medicines, also known as herbal medicines (Khan and Ahmad, 2019). Currently, approximately three to four quarters of the world's population relies on the medicinal plant for treatment (Nagulapalli Venkata *et al.,* 2017). Therefore, in the present research, phytochemicals and bioactive compounds are investigated in plants that have prominent biological and pharmacological properties. In the present research*, Raphanus sativus*, leaves were selected as a medicinal plant for the investigation of the phytochemical constituent and antidiabetic activity.

Percentage Yield: The percentage yield of plant extracts was obtained in the range of 1.3 to 3.2 g/100g of the dry plant. The highest estimation of the yield was found in the Methanol (3.2%) while the least value (1.3%) was measured for n-hexane. The percentage yields of extracts of different solvents such as ethyl acetate, n-butanol and chloroform is shown in Table (1). It was concluded that the yield of the extract depends on the nature of solvents. The highest yield of several extracts was reported with 80 % methanol revealing the greater efficacy of this solvent to extract the maximum amount of antioxidant components (Zhang *et al.,* 2016).

Table 1. The percentage yield of *Raphanus sativus* leaves extracts

Solvent used	Percentage yield (%)
n-Hexane	1.3 ±0.02
Chloroform	3.12 ±0.03
Ethyl acetate	2.83 ±0.09
n-Butanol	2.83 ±0.09
Methanol	3.2 ±0.04

Phytochemical Study: The phytochemical activity of the *Raphanus sativus* leaves is shown in Table 2. Alkaloids, tannins, flavonoids, saponin, steroids, terpenoids, and cardiac glycosides were found in plant extracts. But phlobatanins were absent in all extracts of *Raphanus sativus*.

Table 2. Qualitative estimation of phytochemicals in *Raphanus sativus* leaves extracts

Plant	Solvents	Phyto-constituents						
		Flavonoids	Tannins	Saponins	Terpenoids	Alkaloids	Cardiac glycosides	Pholabotannins
Raphanus sativus	n-Hexane	+	-	-	+	+	-	-
	Chloroform	+	+	+	+	++	+	-
	Ethyl acetate	+++	+	+	+	+++	+	-
	n-Butanol	+	+	+	+	++	+	-
	Methanol	+++	+	+	++	++	+	-

Table 2: Qualitative phytochemical analysis of a different plant extracts. '+++' shows strong presence, '++' shows moderately presence, '+'shows normal presence and "-" shows the absence of respective phytochemicals.

The preliminary analysis showed that all the extracts contained flavonoids, tannin, saponinsterpenoids, alkaloids, and cardiac glycosides, while phleobtanins were absent. The finding is somehow in agreement with the results reported by Bharthvaj and Kumar (2022), who demonstrated that an alcoholic extract of *Raphanus sativus* contained carbohydrates, glycosides, and flavonoids.

Yield of Saponin: Saponin having percentage yield was 26.8 % when the sample weight was 10 g and the extract weight was 2.86 g.

Total Phenolic content: The methanol extract had the highest TPC concentration, 640.69 mg/100 g GAE, while the n-hexane extract had the lowest TPC concentration, 154.98 mg/100 g GAE. The values of TPC for other extracts, for example, n-butanol, chloroform, and ethyl acetate, were found in the range of 246.15 mg/100g, 241.02 mg/100g, and 252.70 mg/100 g GAE respectively (Table 3).

Table 3. Determinationof Total phenolic content of *Raphanus sativus* leaves extracts

Name of Sample	Gallic acid equivalent mg/100 g
n-Hexane	154.98 ±1.574
Chloroform	241.02 ±2.51
Ethyl acetate	252.70 ±2.59
n-Butanol	246.15 ±2.473
Methanol	640.69 ±6.411

The plant leaves have been reported to be rich in phenolic compounds (Thangavelu *et al.,* 2022). Phenolic compounds are major constituents that have an important role in nutritional values, commercial properties and stabilization of lipid peroxidation due to the scavenging abilities of their hydroxyl group (Vijayakumari *et al.,* 2022).

Total flavonoid content: The finding have shown that the maximum results of TFC 103.9 mg/100 g were found in methanol extract while the least value of the TFC was presented in n-hexane 6.49 mg/100 g (Table 4). The value of TFC for other extracts, for example, chloroform, n-butanol, ethyl acetate, were found in the range of 11.59 mg/100 g, 22.45 mg/100 g and 16.85 mg/100 g, respectively.

Table 4. Determination of TFC in *Raphanus sativus* leaves extracts

Name of Sample	Catechin equivalent (CE) mg/100 g
n-Hexane	6.49 ±0.071
Chloroform	11.59 ±0.121
Ethyl acetate	16.85 ±0.169
n-Butanol	22.45 ±0.232
Methanol	103.56 ±1.041

The percentage of higher total flavonoid content in leaves extract suggest a higher nutritional value of leaves, as flavonoids have strong antioxidant activity and inhibit oxidative stress (Forni *et al.,* 2021). From previous reports the occurrence of total flavonoid content in leaves extract suggested that it can be used as an alternative source of total flavonoids; flavonoids play the advantageous role for different beneficial health effects (Walia *et al.,* 2019).

Antidiabetic activities of *Raphanus sativus* extracts: The inhibitory studies accomplished, explained that all the extracts have shown significant inhibitory potential. The maximum inhibitions (Table 5) showed by ethyl acetate extract (36.29%) which is closest to the inhibition showed by standard acarbose (62.5%). Alpha-amylase is the enzyme that catalyzed the carbohydrates into simple sugar, and this sugar increased the blood glucose level high. There is insufficient insulin in diabetic patients that controlled blood sugar level. So this can be controlled by inhibition of Alpha-amylase (Ballesteros-Álvarez and Andersen, 2021).

Table 5. Percentage inhibitions of *Raphanus sativus* leaves extract

Sample	Percentage inhibitions (%)
n-Hexane	20.13 ±0.24
Chloroform	23.88 ±0.29
Ethyl acetate	36.24 ±0.31
n-Butanol	35.36 ±0.41
Methanol	35.36 ±0.44
Acarbose	62.47 ±0.69

The findings showed that the plant extract possessed inhibitory potential, which might be due to the presence of phytochemicals such as flavonoids, alkaloids, terpenoids, saponins, and tannins. The finding is in agreement to the results reported by Sai *et al.,* (2019), who demonstrated that the phyto-constituents found in plants extract may be responsible for inhibitory potential. Similarly, various researchers also performed this assay to evaluate the alpha amylase inhibitory potential of plant extracts (Sachan *et al.,* 2019; Kifle and Enyew, 2020).

CONCLUSIONS

The findings of the current studies showed that plant extracts of different solvents possessed various phytoelements responsible for their antidiabetic potential. *Raphanus sativus* contains flavonoids, alkaloids, terpenoids, tannins, and saponins. The quantitative analyses have also shown that the plant extracts contained flavonoids and phenolic acids, which were confirmed through standard protocols. So, the maximum amounts of flavonoids and phenolic acid were found in the methanol extract. The medicinal plants have vast applications in different fields of pharmacology to cure various metabolic disorders like diabetes, may be taken as herbal remedies or foods.

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