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Antidiabetic, Thrombolytic, Antimicrobial, Antioxidant and Cytotoxicity Studies of Brinjal (*Solanum Melongena*) Leaves Extracts

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ABSTRACT: *Phytochemical, antioxidant, cytotoxicity, antimicrobial, and antidiabetic of brinjal (Solanum melongena) leave extracts in the present study. The selected plant material was dipped in different polarity-based solvents for one weak such as chloroform, n-hexane, methanol, ethyl acetate, and n-butanol. By using spectroscopic techniques total phenolic contents (TPC) and total flavonoid contents (TFC) were analyzed. The range of total phenolic contents was 159.48 ± 1.49 to 23.19 ± 0.40 GAE mg/100 g. The range of total flavonoid contents was 4.87± 0.28 to 29.6 ± 0.24 CE mg/100 g. Extracts had an antioxidant activity which was evaluated by using free radical scavenging DPPH assay. Total phenolic contents and the ratio inhibition in linoleic acid oxidation were also calculated. The present study result revealed that the leaves of the Solanum melongena plant may be the main source of natural antioxidants and it is also used as an antidiabetic activity. In the present study, the potential of extracts against the selected bacterias (Bacillus subtilis and Escherichia coli) and fungi (Fusarium solani and Aspergillus nigar) was evaluated. The plant extracts were examined against human red blood cells (RBCs) and the percentage lysis was also found in this research work. Statistical data analysis was done by Analysis of variance (ANOVA) using SPSS version 21.0.*

Key words: *Solanum melongena, Antioxidant, Antidiabetic, Plant extract, Thrombolytic*

INTRODUCTION

The plant biodiversity included the chief source of herbal medication in the world population, the medicines which comprise of about 60 – 80 % in their average depend on plants are being used as the health of traditional care

system since the ancient ages. Consequently, these plants have medicinal values which leave the physiological effect on the bodies of human. These plants also have bioactive phytochemical constituents. Today we use modern drugs which are formed from these natural compounds (Edeoga et al., 2005).

The naturally occurring compounds in plants are phytochemical. Phytochemical involves the flavor, color, and smell of plants. Collectively, they convert plant parts into natural defenders against diseases. It has been reported that phytochemicals can be used for therapeutic purposes like human health and disease prevention (Okwu, 2004). The naturally occurring bioactive compounds called phytochemicals are found in plants like, fruits, vegetables, medicinal plants, leaves, roots, and flowers which show work with nutrients and fiber employed in the treatment of disease condition or defend against the action of disease (Jimenez-Garcia et al., 2013; Rajasree et al., 2016; Alamgir, 2017; Chhikara et al., 2018; Chhikara et al., 2020). Based on their function in plant metabolism, the phytochemicals have two groups, which include primary constituents and secondary constituents. In this study, Primary constituents consist of amino acids, common sugars, chlorophyll, and proteins, on the other hand, secondary constituents involve alkaloids phenolic compounds, and terpenoids (Krishnaiah et al., 2007) and a lot of others like tannins, flavonoids, etc.

MATERIALS AND METHODS

The work of research was conducted at the Department of Chemistry University of Sargodha, Women Campus, city Faisalabad Pakistan.

Collection of sample and its identification

The brinjal plant (*solanum melongena*) leaves were collected from Faisalabad city Pakistan. The sample of the plant was dried by air and converted into uniform powdered by a grinding machine.

Sample extraction

Leave samples were washed with tap water followed by distilled water to get rid of all dust particles then air dried and crushed into uniform powdered by grinding machine (AB-03, Absons, Karachi Pakistan. Dipped in different polarity order solvents for one week then filtered the solutions, evaporated, dried, and took its extract then dried and preserved it by following the methods of (Riaz et al., 2012a).

Qualitative tests

The analysis for quality of phytochemicals like tannins, saponins, flavonoids, phlobatannins, terpenoids, steroids, cardiac glycosides (Keller-Kelani test) was performed on *Solanum melongena* leaves extracts and technique defined by (Edeoga et al., 2005).

Quantitative tests

The procedure used to determine the quantitative analysis of saponins was described by (Obadoni and Ochuko, 2002). The method which were used for

the determination of alkaloid, total phenolic, total flavonoid contents, DPPH free radical scavenging activity were followed as described by (Molyneux, 2004; Edeoga et al., 2005; Negi et al., 2005; Sarker et al., 2007). The antioxidant activity process for the percentage inhibition of linoleic acid and reducing power activity of the *Solanum melongena* leaves was given by (Yen et al., 2000). The procedure used for the antimicrobial activity of *solanum melongena* roots extract was given by (Sarker et al., 2007). The method used in the thrombolytic activity of sample extracts is determined by (Prasad et al., 2007) and haemolytic activity of sample extracts was as described by (Powell et al., 2000). The method used for the antidiabetic activity of *Solanum melongena* leaves was described by (Conforti et al., 2005).

RESULTS AND DISCUSSION

Yield of extracts

The plant extracts yield was found in the range of 0.724±0.006 to 5.892 ±0.05g/100 g of the dry plant and shown in table 1. In the present study a higher yield was noticed with methanol and according to the earlier reports, methanol gives maximum percentage yield due to its polarity. It is also used for the free radical scavenging compounds extraction from the plant parts due to its polar nature and solubility with many antioxidant compounds (Sultana et al., 2007). Statistical analysis of percentage yield of *Solanum melongena* leaves extract of various solvents was performed by use of one way ANOVA which revealed significant results ($p< 0.05$).

Table 1: Percentage yield of Solanum melongena leaves extracts

Sample	Percentage yield
Methanol	5.892±0.05
n-Hexane	3.346±0.02
Chloroform	5.78±0.05
Ethyl acetate	1.506±0.01
n-Butanol	0.724±0.006

Phytochemical study

The components of Phytochemical such as flavonoids, alkaloids, saponins were analyzed and the other phytochemical components like Phlobatannins, steroids, terpenoids, cardiac glycosides, and tannins were absent in the leaves of the *Solanum melongena* plant. These secondary metabolites results were given in Table 2. As per composed works assessment, the present studies carried out on the leaves sample of *Solanum melongena* plant which calculates the common and organized secondary metabolites percentages such as flavonoids, alkaloids, and saponins inside the *Solanum melongena* as reported by Edeoga et al. (2005) and the plant used in this work almost show same development.

Table 2: Qualitative Phytochemical results of *Solanum melongena* leaves extracts

Phytochemical Names	Results
Saponins	Present
Steroids	Absent
Phlobatannins	Absent
Terpenoids	Absent
Cardiac glycosides	Absent
Tannins	Absent
Flavonoids	Present
Alkaloids	Present

Total phenolic contents

In this study, the total phenolic contents were determined through the spectroscopic technique. The quantity of TPC of *Solanum melongena* leaves extracts was investigated by the means of folin ciocalteau. Due to their high sensitivity, the folin ciocalteau method preferred to quantify the phenolic contents rather than other competitive tests. The maximum value of TPC (159.48± 1.49 mg/100 g GAE) was found in methanol solvent while the lower amounts of TPC (23.19 ± 0.40mg/100 g GAE) was observed with n-hexane solvent but the value of extracts with other solvents such as chloroform, ethyl acetate, n-butanol was 48.35± 0.49, 62.56 ± 0.64and 98.72± 1.10 mg/100 g GAE respectively. The amounts of TPC represent in Table 3 (mg /100 mg of dry weight as GAE).

The research explains that *Solanum melongena* contains high phenolic contents which can explain its high scavenging activity of free radicals (Sarker et al., 2007). Analysis of total phenolic contents of *Solanum melongena* leaves extracts were statistically performed using one-way ANOVA which showed significant results ($p< 0.05$).

Table 3: Total phenolic contents in brinjal (*Solanum melongena)* leaves extracts

Name of the Sample	TPC GAE mg/100g
n-Hexane	23.19 ± 0.40
Chloroform	48.35 ± 0.49
Ethyl acetate	62.56 ± 0.64
n-Butanol	98.72 ± 1.10
Methanol	159.48 ± 1.49

Total flavonoid contents

The maximum value of TFC (29.6± 0.24 mg/100g CE) was found in methanol solvent while the lower amounts of TFC (4.87 ± 0.28mg/100 g CE) was observed with n-hexane solvent but the yield of extracts with other solvents such as chloroform, ethyl acetate, n-butanol was 8.36 ± 0.06, 9.22 ± 0.11and 21.26 ± 0.30mg/100 g catechin equivalent (CE) respectively and also summarized in table 4. Free radical scavengers include flavonoids that strong water-soluble antioxidants, which prevent the cell from oxidative damage and hold the strong activities of anticancer and findings were same as reported by Okwu and Josiah (2006).

Total flavonoid contents values in different solvents were decreases in following order: methanol > ethyl acetate > n-butanol > chloroform > n-hexane. Statistical analysis of total flavonoid

contents of *Solanum melongena* leaves extracts was performed by using one-way ANOVA which showed significant results ($p < 0.05$).

Table 4: Total flavonoid contents in brinjal (*Solanum melongena*) leaves extracts

Name of the sample	TFC CE mg/100 g
n-Hexane	4.87± 0.28
Chloroform	8.36 ± 0.06
Ethyl acetate	9.22 ± 0.11
n-Butanol	21.26 ± 0.30
Methanol	29.6 ± 0.24

Antioxidant study

Percentage inhibition of linoleic acid oxidation

extract represented percentage inhibition ranging from 21.12 ± 0.19 to 84.5 ± 0.81%. The percentage inhibition in maximum value was represented in the extract of methanol and minimum inhibition showed by the n-hexane whereas Butylated hydroxytoluene (BHT) used as positive control showed 90.64 ± 0.90% inhibition. So these results suggested that the *Solanum melongena* leaves are used for the slower oxidation processes of lipid. The methanol extract was showed the maximum percentage inhibition value in linoleic acid oxidation as antioxidant activity (Table 5). The methanol extract showed the highest level of percentage inhibition of oxidation may be due to the occurrence of TFC and TPC in greater concentration, due to this condition methanol could appear higher activity of antioxidant. In the present study, the n-hexane showed the minimum value of percentage inhibition. Due to the lower concentration of phenolic, the lower % inhibition level is explained in n-hexane extract. This study was the same as the investigation of Iqbal et al. (2005) who reported the percentage inhibition of linoleic acid.

Table 5: Percentage inhibition in linoleic acid peroxidation and free radical scavenging by DPPH assay of *Solanum melongena* leaves extracts

Extracts	Percentage inhibition of oxidation in linoleic acid	DPPH percentage scavenging
n-Hexane	21.12 ± 0.19	60.58 ± 0.50
Chloroform	46.42 ± 0.45	64.95 ± 0.64
Ethyl acetate	60.13 ± 0.57	69.54 ± 0.68
n-Butanol	73.5 ± 0.73	79.64 ± 0.77
Methanol	84.5 ± 0.81	87.71 ± 0.80
BHT	90.64 ± 0.90	89.64 ± 0.88

DPPH free radical percentage scavenging assay

The value of DPPH free radical percentage scavenging for the different solvents extracts like chloroform, n-hexane, ethyl acetate, methanol, and n-butanol was determined and summarized in Table 5. The n-hexane solvent exhibited the lowest value 60.58 ± 0.50 µg/ml and methanol showed the highest value 87.71 ± 0.80 µg/mL. The antioxidants which showed the least value are the best antioxidants and those which value is greater are bad antioxidant. In this assay, for the positive control, the butylated hydroxyl toluene (BHT) was used which is a synthetic antioxidant. For the evaluation of the antioxidant potential, the DPPH radical is generally used. The variation in the free radical scavenging ability of plants produced by the nature and amount of the secondary metabolites of a plant (Sudjaroen et al., 2005).

DPPH free radical percentage scavenging value for the leaves extracts of *solanum melongena* by using the following solvents; chloroform, n-hexane, ethyl acetate, methanol, and n-butanol was 60.58 ± 0.50, 64.95 ± 0.64, 69.54 ± 0.68, 79.64 ± 0.77 and 87.71 ± 0.80 µg/mL respectively showed in table 4.10. Statistical analysis of DPPH free radical scavenging activity of *Solanum melongena* leaves extracts was performed by using one-way ANOVA which showed significant results ($p< 0.05$).

Reducing power

The Reducing power is the ability to determine the reductive potential of antioxidants and it is measured through the conversion of ferricyanide complex to ferrous form in the presence of sample extracts (Gulcin et al., 2003). The reducing power of the *Solanum melongena* leaves extracts was analyzed and summarized in Fig. 1. According to figure 1, when the concentration of extracts increased then their reducing power also increased. The data showed that the reducing ability of the entire sample increased when extracts concentration was increased, it was estimated through spectrophotometer by noting the absorbance. These results were in agreement with (Gulcin et al., 2003, Noriham et al., 2004). Statistical analysis of reducing the power of *Solanum melongena* leaves extracts was performed by the use of one-way ANOVA which showed significant results ($p< 0.05$). The reducing power ability to reduce Ferricyanide is due to the donation of hydrogen from phenolic compounds (Shimada et al., 1992).

According to this assay the test solution color change from yellow to different shades like blue and green which is associated with reducing the power of each compound. The presence of radicals which are also called antioxidants causes to convert Fe^{3+} (Ferricyanide) complex to the Fe^{2+}ferrous form. The complex of Ferricyanide (reducing power) was used in this method.

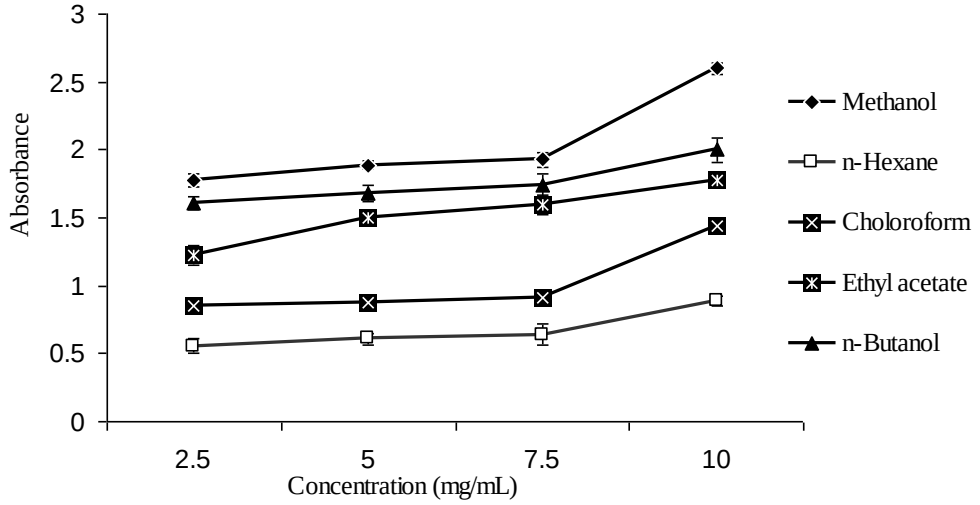

Fig. 1: Reducing potential of *Solanum melongena* leaves extracts

Antimicrobial activity

The *Solanum melongena* leaves extracts showed the antimicrobial activity against the selected fungus and bacteria with the methods of disc diffusion and (MIC) minimum inhibitory concentration, which were summarized in tables 6 and 7.

According to the antimicrobial activity results, the extracts of different solvents showed different values like n-hexane and chloroform showed no activity against the fungal strain and bacterial strain. Methanol showed the result against bacterial strain included *B. subtilis* and *E.coli* which was 16±0.14 and 15±0.16 mm respectively. Methanol showed the result against fungal strain included *A. nigar* and *F. solani* which was 12± 0.11 and 10 ±0.10 mm respectively. The ethyl acetate also showed positive results against bacterial and fungal strains. Ethyl acetate showed value against the bacterial strain which included *B. subtilis* and *E. coli,* the value was 16±0.16 and 20±0.23 mm. Other solvent extracts values were summarized in table 7.

Table 6: Antimicrobial and antifungal activity in millimeter of *Solanum melongena* leaves extracts

Extracts/Standard	Bacterial strains		Fungal strains	
	B. subtilis	*E. coli*	*A. nigar*	*F. solani*
n-Hexane	*N.A	N.A	N.A	N.A
Chloroform	N.A	N.A	N.A	N.A
Ethyl acetate	16±0.16	20±0.23	14±0.14	28±0.25
n-Butanol	8±0.06	6±0.05	N.A	12.5±0.11
Methanol	16±0.14	15±0.16	12±0.11	10±0.10
Rifampicin	28±0.24	32±0.30	N.A	N.A
Terbinafin	N.A	N.A	26±0.26	31±0.33

*N.A. = No activity

Rifampicin and Terbinafin were used as reference standards for bacterial and fungal strains, respectively.

Table 7: The minimum inhibitory concentration (MICs) in µg/mL by *Solanum melongena* leaves extracts

Extracts/Standard	Bacterial strains		Fungal strains	
	B. subtilis	*E. coli*	*A. nigar*	*F. solani*
n-Hexane	N.A	*N.A	N.A	N.A
Chloroform	N.A	N.A	N.A	N.A
Ethyl acetate	202	N.A	189	165
n-Butanol	276	304	229	270
Methanol	320	356	310	285
Rifampicin	78	29	N.A	N.A
Terbinafin	N.A	N.A	99	31

*N.A = no activity

Rifampicin and Terbinafin were used as reference standards for bacterial and fungal strains, respectively.

According to the MICs results, the methanol showed the highest value in bacterial strains which include *B. subtilis* and *E. coli*, the values were 320 and 356 µg/mL respectively. Methanol also showed the highest value of fungal strains which includes *A. nigar* and *F. solani* values were 310 and 285 µg/mL respectively. The N-butanol showed the values of fungal strains in which *A. nigar* and *F. solani* include, had the values 229 and 270 µg/mL respectively. Bacterial strain values also showed by the n-butanol which includes *B. subtilis* and *E. coli* had 276 and 304 µg/mL respectively. The ethyl acetate gives a result against bacterial strain which includes *B. subtilis*, had a value of 202 µg/mL, and showed no activity against the *E. coli*. Ethyl acetate represented activity against fungal strain in which values were 189 µg/mL and 165 µg/mL of *A. nigar* and *F. solani*

respectively. The Rifampicin and Terbinafin which were used as reference standards in this MICs activity, also showed their results against the bacterial and fungal strains are showed in table 8. The overall results showed that both extracts n-hexane and chloroform showed no antimicrobial activity against the fungal strainsandbacterial strains. The other three extracts which include ethyl acetate, n-butanol and methanol showed antimicrobial activity against bacterial strains *B. subtilis* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria).

Cytotoxicity activity

Haemolytic activity

The Haemolytic action of *Solanum melongena* leaves extracts was evaluated against the red blood cells (RBCs) of humans, for positive control used Triton X-100 and is summarized in table 8. The breakdown of heam protein is called hemolysis. Red blood cells contain this protein. The sample of *solanum melongena* leaves extracts were compared with Triton X-100 which was used as positive control and recorded percentage lysis of RBCs. Triton X-100 gave higher percentage lysis of red blood cells (99.640 ± 1.03%). The higher percentage lysis was observed in the n-butanol extract (6.82 ± 0.06%) and the minimum lysis was observed in methanol extract (2.58 ± 0.02%). The yield of extract in various solvents like n-hexane, chloroform, and ethyl acetate was 5.38 ± 0.05, 5.38 ± 0.040, 3.71 ± 0.03 % respectively. Statistical analysis of cytotoxicity assay by the haemolytic activity of *Solanum melongena* leaves extracts was accomplished with the help of one-way ANOVA that showed significant results ($p< 0.05$).

Table 8: Cytoxicity studies by haemolytic activity, thrombolytic activity and antidiabetic studies by *Solanum melongena* leaves extracts

Extracts	Percentage (%) RBCs lysis	Percentage (%) thrombolysis	Percentage (%) antidiabetic
n-Hexane	5.38 ± 0.05	6.32 ± 0.05	14.91±0.14
Chloroform	5.38 ± 0.04	2.67 ± 0.02	13.84±0.15
Ethyl acetate	3.71 ± 0.03	3.38 ± 0.03	20.58±0.21
n-Butanol	6.82 ± 0.06	1.31 ± 0.01	23.33±0.23
Methanol	2.58 ± 0.02	4.66 ± 0.03	25.02±0.25
Acarbose	-	-	62.47±0.60
Triton X-100	99.64 ± 1.03	-	-
Streptokinase	-	87.01±0.78	-

Thrombolytic activity

The thrombolytic activity of *Solanum melongena* leaves extracts was evaluated against the human red blood cells and is summarized in table 8. Thrombolysis is a treatment in which the dangerous clots dissolve in blood vessels and improve the flow of blood. It is also known as thrombolytic therapy. The minimum yield of thrombolytic activity was observed in methanol 4.66 ± 0.03% extract and the maximum value was observed in the n-hexane extract 6.32 ± 0.05 %. The values of the other solvents like chloroform, ethyl acetate, n-butanol were 2.67 ± 0.02, 3.38 ± 0.03, 1.31 ± 0.01 respectively. Streptokinase used as positive control showed 87.01% clot lysis. Statistical analysis of cytotoxicity assay by the thrombolytic activity of *Solanum melongena* leaves extracts was performed by the use of one-way ANOVA which showed significant results ($p< 0.05$).

Antidiabetic activity

The *Solanum melongena* leaves extracts showed the antidiabetic activity within different solvents according to their polarity order like chloroform, n-hexane, ethyl acetate, methanol, and butanol. These solvents showed different results against antidiabetic activity. The highest value was shown by the methanol extract which was 25.02 ± 0.25% and the chloroform extract was showed the lowest value which was 13.84 ± 0.15%. The other solvent extracts like n-hexane, ethyl acetate, n-butanol showed 14.91 ± 0.14, 20.58 ± 0.21, 23.33 ± 0.23% values respectively. These extracts values showed in table 8. The acarbose which was a reference standard showed the highest value than other solvent extracts. The value of acarbose is also summarized in table 8

Statistical analysis of the antidiabetic activity of *Solanum melongena* leaves extracts was performed by using one-way ANOVA which showed significant results ($p< 0.05$).

CONCLUSION

It was concluded that *Solanum melongena* leaves extracts especially methanol has more potential as an antioxidant as compared to other solvents due to presence of flavonoids, saponins, and other phytochemical constituents included alkaloids, TPC, and TFC. A minor effect of cytotoxicity on RBCs was also noticed. Moreover, the extract and fraction of this plant also showed the antimicrobial activity against the selected bacterial and fungal strains. Furthermore, the antioxidant potential of the plant *Solanum melongena* leaves extracts showed by the oxidation activities of DPPH scavenging and linoleic acid. Due to the slight cytotoxicity, this plant may be used as herbal medicines.

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