# SYNERGISTIC ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF *ALLIUM SATIVUM* AND *SWERTIA CHIRATA* IN COMBINATION

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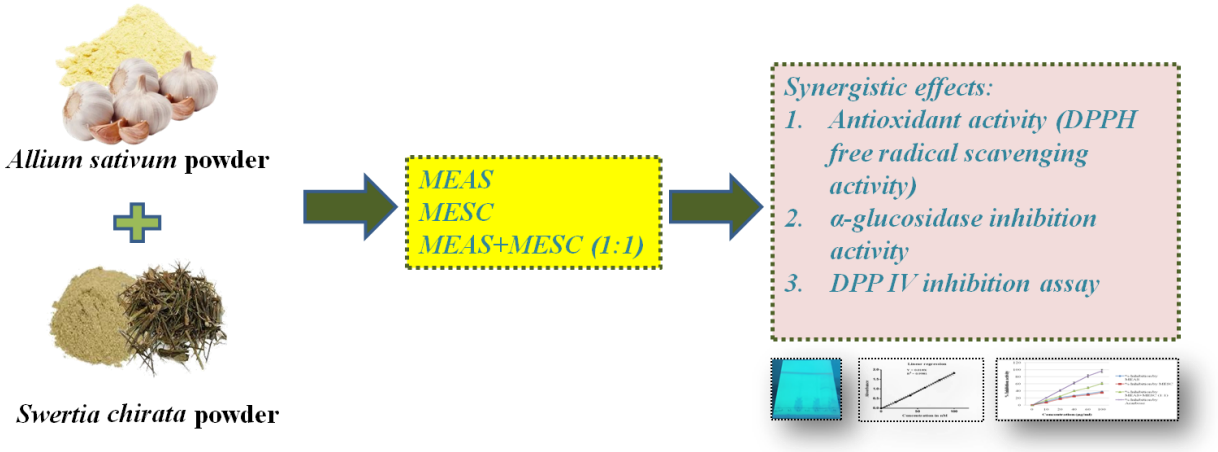
## Graphical abstract




## Abstract

The synergistic antioxidant and anti-diabetic properties of powdered methanolic extracts of *Swertia chirata* (Gentianaceae) and *Allium sativum* (Liliaceae) plants have been investigated utilizing in vitro methods. Using methanol as a solvent, the extraction was carried out using the Soxhlet apparatus. To carry out the experiments, methanolic extracts of each plant were used. The methanolic extracts of both plant materials were subjected to preliminary phytochemical screening. The enzymatic assays for DPPH, α-glucosidase activity, and DPP IV were performed in vitro. In α-glucosidase assay, acarbose was utilized as a standard, while sitagliptin was employed in the DPP IV assay. Preliminary phytochemical analysis of the MEAS found the presence of alkaloids, flavonoids, phenolic compounds, tannins, proteins, amino acids, resin, glycosides, and saponins. Phytochemical analysis of the MESC found the presence of alkaloids, glycosides, glycosides, glycosides, phenolic compounds, and tannins. This study showed that swertiamarin was not present in MEAS and gallic acid was not present in MESC while ferulic acid was present in both drugs. MEAS-MESC (1:1) included swertiamarin, gallic acid, and ferulic acid. MEAS, MESC, MEAS+MESC (1:1), and Ascorbic acid at 100 µg/mL inhibited DPPH free radicals by 84.23%, 81.51%, 88.34%, and 99.88%, respectively. Percentage of inhibition for α-Glucosidase inhibitory activity for MEAS (10.2, 21.3, 27, 32, 38)%; for MESC (7.5, 17.6, 24.8, 29, 35.2)%, for MESC+MEAS (1:1) it was (14.2, 25.4, 40, 49, 61.5)% while for Acarbose (20.6, 41.3, 62.4, 82.4, 96.5)%. MEAS (0.88, 12.54, 38.32, 65.24)%; MESC (0.69. 7.56, 25.21, 49.80)%; MEAS+MESC (1:1) (0.92, 16.44, 43.26, 68.24)%; and sitagliptin (positive control) (92, 93, 95, 98)% inhibited DPP-IV at 25, 50, 75, and 100 µg/ml,

respectively. The combination (1:1) of methanolic extract of *Swertia chirata* (MESC) and *Allium sativum* (MEAS) showed synergistic efficacy compared to the alone. The combination of these two plants may be utilized to create medicine for the treatment of diabetes since the 1:1 mixture of the methanolic extract is shown to be more effective.

**Keyword:** *Allium sativum, Swertia chirata*, DPPH, α- glucosidase activity and Dipeptidyl peptidase IV (DPP IV) inhibition assay

## 1. Introduction

Diabetes, commonly known as diabetes mellitus (DM), is a dangerous chronic illness caused by a collection of metabolic disorders that impair protein, lipid, and carbohydrate metabolism. The combination of several environmental and genetic factors leads to diabetes (Association, 2009; Galicia-Garcia et al., 2020). Health, lifespan, and quality of life are all negatively impacted by DM in patients. Costs associated with the health care system are also affected (Traish et al., 2009). The International Diabetes Federation (IDF) reported 4.2 million deaths from diabetes and 463 million persons aged 20–79 with diabetes, a figure that would likely climb to 700 million by 2045. T2DM (type 2 DM) prevalence and incidence vary by location, with more than 80% of patients in low-to-middle-income countries, making treatment difficult (Galicia-Garcia et al., 2020).

Seven plant extracts used in the treatment of DM, such as ethanolic extracts of *Rosmarinus officinalis* L. and *Cassia angustifolia,* enhanced glucose uptake via GLUT-4, and water extracts of *Helminthostachys zeylanica* and *Dendropanax morbifera* restored normal lipid genesis, as well as water extracts of *Dendropanax morbifera* regulated PARP-1 and FAS to reduce inflammation in DM. These results validated plant extracts role in anti-diabetic effects (De et al., 2021). Drug formulations in Ayurveda are based on two principles: single-drug usage and multiple-drug use. Any preparation including two or more plants is termed polyherbal. Since polyherbal formulations have higher therapeutic potential than individual plant extracts, researchers have developed several polyherbal formulations to treat DM (Perumal et al., 2022). In order to help develop this polyherbal combination into a pharmaceutical medicine to treat Type 2 DM, this research intends to investigate the antidiabetic properties of the mixture of extracts from *Swertia chirata* (Family: Gentianaceae) and *Allium sativum* Linn (Family: Liliaceae). Both *Swertia chirata* and *Allium sativum* are excellent choices of plants to study their combined impact on antidiabetic properties in order to fulfil the demand for a complementary treatment for DM since they have both been shown to have various essential antidiabetic components as well (Perumal et al., 2022).

## 2. Materials and Methods

### 2.1 Materials/ Equipments

UV-cabinet (CAMAG), Soxhlet apparatus, silica gel (230-400#) (Merck, Germany), twin trough TLC chamber (10x10), Rotavap (Buchi-R-210), methanol, DPP IV (Sigma Aldrich), were used for and DPP-IV enzyme (EC 3.4.14.5) was purchased from (Sigma-Aldrich-D4943), DPPH, ascorbic acid, disodium carbonate, (MerckLts,India), gallic acid (MoleChem, Mumbai, Inida), quercitin and α-glucosidase (*Saccharomyces cerevisiae*) (Sigma-Aldrich), incubator chamber; microplate reader; milliQ water; 70% isopropyl alcohol; plastic trays; waste beakers; micropipettes(1 ml, 200µl, 2 to 20µl); Micro tips(1 mL,200µl, 2 to 20µl) were used for an enzyme inhibition experimentation process (Kumar et al., 2020).

### 2.2 Procurement and authentication of powdered drug

*Allium sativum* powder was purchased from M/S Hindustan Herbs House in Kachiguda, Hyderabad, India and *Swertia chirata* powder (whole plant) purchased from Iconic Herbs, Ahmedabad, Gujarat, India. Authentication of powdered drug was done by FTIR.

### 2.3 Preparation of extract

Soxhlet extraction method was used to prepare methanolic extracts of *Allium sativum* (AS) powder and *Swertia chirata* (SC) whole plant powder. Methanolic extracts of the AS was prepared by adding 250g of AS powder and methanol (60-80°C) 2.5 liter, respectively, for 72 h according to the prescribed method (Bhanot and Shri, 2010; Raju et al., 2008). Extraction continued till completion. Colourless soxhlet apparatus siphoning arm indicated extraction completion. The crude methanol extract of SC was extracted from 250g of SC crude powder in 2.5 litres of methanol (60-80°C) and concentrated. The extracts were concentrated under reduced pressure using rotavap (Buchi, R-210) until all the solvent have been evaporated to obtain methanolic extract. The %yield of methanolic extract of AS (MEAS) and methanolic extract of SC (MESC) were obtained 7.23 and 5.29 (%w/w), respectively.

### 2.4 Phytochemical screening of MEAS and MESC

The preliminary phytochemical screening was carried out in accordance with the stated procedures to identify the phytochemical classes present in MEAS and MESC (Kumar et al., 2010, 2017).

### 2.5 Thin layer chromatography (TLC) of MEAS and MESC

For chromatographic investigations, precoated TLC plates (silica gel 60# F254(E.Merck)) had been used. The samples of MEAS and MESC were applied on the chromatographic plates. Chloroform and methanol were used for the mobile phase in gradient solvent system (7.5:2.5 v/v) and methanol, ethyl acetate, water, formic acid (10:3:5:2) for the comparison of MEAS, MESC, and mixture of MEAS and MESC (1:1), with standard compounds Swertiamarin (SWMN), Gallic acid (GA), Quercetin (QC), Ferulic acid (FA). TLC was observed under short UV 254 nm.$R_f$ values of each samples were determined by using formula (Kumar and Jairaj, 2018) in equation 1.

$R_f$=(Distance travelled by solute/Distance travelled by solvent)……..(eq.1)

### 2.6 Antioxidant activity

#### *2.6.1 DPPH free radical scavenging assay*

The radical scavenging impact of stable 1,1-diphenyl-2 picryhydrazyl (DPPH) free radical activity was used to evaluate the total antioxidant activity of crude methanol extracts of MEAS, MESC, and their 1:1 combination. In methanol, ascorbic acid and samples (10-100 µg/ml) were created. Each ascorbic acid and plant extract concentration was achieved in 0.5 ml sterile test

tubes. This sample was combined with 0.5 ml of 0.2 mM DPPH solution and incubated for 30 minutes in the dark. In place of the ascorbic acid or plant extract, methanol was used to create the control, which was then made as described above. Thermo Fisher Scientific's Genesystem-10-5) spectrophotometer was used to measure the absorbance at 517 nm wavelength (Khanal et al., 2015). Formula for DPPH free radical scavenging: (%) inhibition (DPPH free radical scavenging activity)=$[(A_0-A_I)/A_0]*100$…….(eq.2), where, $A_0$=absorbance of control, and $A_I$= absorbance of inhibitor and ascorbic acid served as a standard for this assay.

**2.7 Antidiabetic activity**

***2.7.1 α-Glucosidase inhibitory activity***

In a 96-well reaction combination, 50 µl phosphate buffer (100 mM, pH = 6.8), 10 µl α-glucosidase (1 U/ml), and 20 µl of samples (10, 20, 40, 60, 80, 100)µg/ml were preincubated at 37°C for 15 min. Following that, 20 µl p-NPG (5 mM) was added as a substrate and incubated at 37°C for 20 min. 50 µl Na2CO3 (0.1 M) halted the reaction. Multiplate Reader measured released p-nitrophenol absorbance at 405 nm. (10, 20, 40, 60, 80, 100)µg/ml acarbose was positive. A parallel control experiment without test material was run in triplicates (Telagari and Hullatti, 2015). The results were expressed as percentage inhibition, which was calculated using the formula:

Inhibitory activity (%) = $(1-A_S/A_C)*100$, where, Ac = Absorbance of the control and As = Absorbance of the sample. Further, % inhibition activity was calculated for MEAS, MESC and mixture of MEAS and MESC (1:1).

***2.7.2 DPP IV inhibition assay***

***2.7.2.1 Principle of DPP IV enzymatic inhibition assay***

DPP-IV enzyme inhibition assay was performed using the chromogenic substrate Gly-Pro-p-nitroanilde. In 100 mM Tris-HCl at pH 8.0 and 37 °C, one unit (1 U) produced 1.0 mole of p-nitroaniline from Gly-L-Pro p-nitroanilide every minute. Spectrophotometric analysis was performed using a Microplate Reader at absorbance 405nm (Aldrich, 1999; Kumar et al., 2020) Schematic representation of the basic principle of the conversion of Gly-pro and p-nitroaniline from Gly-pro-nitroanilide by DPP IV enzyme shown in Figure 1.

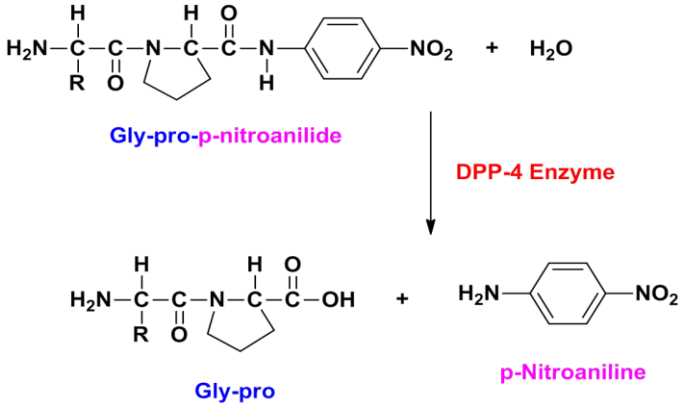



Figure 1: Schematic representation of the basic principle of the conversion of Gly-pro and p-nitroaniline (pNA) from Gly-pro-nitroanilide.

***2.7.2.2 Linear regression curve for pNA***

The linear regression curve was plotted as per reported method by Kumar et al., 2020 (Kumar et al., 2020) The experiment was performed in triplicate (n = 3).

***2.7.2.3 DPP IV enzyme inhibition activity***

Sitagliptin, MEAS, MESC, and MEAS/MESC (1:1) were tested for anti-diabetic potential by

inhibiting DPP-IV. DPP IV was 2000 µU/l mM. Concentrations of (25–100) µg/ml samples were tested. The (%) inhibition of the DPP-IV by sitagliptin, and test samples have determined as per reported method by Kumar et al., 2020 (Kumar et al., 2020). All experiment was performed in triplicates. The following formula (eq. 3) was used for the calculation of % inhibition:

% DPP IV inhibition=$(1-A_0/A_I)*100$, where $A_0$=Absorbance of control and $A_I$= Absorbance of test samples…..eq.(3)

## 3 Results and Discussion

### 3.1 Authentication of plant material

The FT-IR spectra of the *S. chirata* methanolic extract (MESC) displayed in Figure 2(A) indicated the presence of functional groups at several groups, including (O-H), 2908.97 cm-1 (C-H)stretching, 1617.71-1388.88 cm-1 (benzene ring), and 1045 cm-1 (C-O-C). The authenticity of crude *S. chirata* powdered medication is confirmed by these spectrum studies, which also provide microscopic diagnostic criteria for powdered medicines. The FT-IR spectra of *A. sativum* powder is shown in Figure 2(B). In addition to the O-H stretches of polysaccharides and water, proteins also contribute to the broad band at 3307 cm-1. The methyl group in lipids undergoes symmetric and antisymmetric strains that result in the bands at 2,844 and 2,979 cm-1 (C-H) stretching, respectively. Chemical components of plant-based medicine, including lipids, proteins, polysaccharides, and polyphenols, were detected in the spectrum values between 1800 and 800 cm-1. The strong peak between 1636 and 1635 cm-1 may be attributed to the C=C stretching vibration of the allyl group. In an FT-IR spectrum of garlic oil, the conjugated double bond at C=C may be seen at 1636 cm-1. *A. sativum* medication in powder shows CH3 bending in the protein methyl group at 1401 cm-1. The double signal at 1192 cm-1 is brought on by diallyl sulphide molecules vibrating in their skeletal structure. Polysaccharides and cellulose are responsible for the stretching value of 1120 cm-1 (C-C). Sulphides and other compounds with C-S and disulfide linkages have stretching bands that are between 700 and 600 cm-1 and close to 500 cm-1 in size. By using Fourier transform infrared spectroscopy, it was possible to determine the presence of sulphides groups at 655 and 602 cm-1 in powdered *A. sativum*.

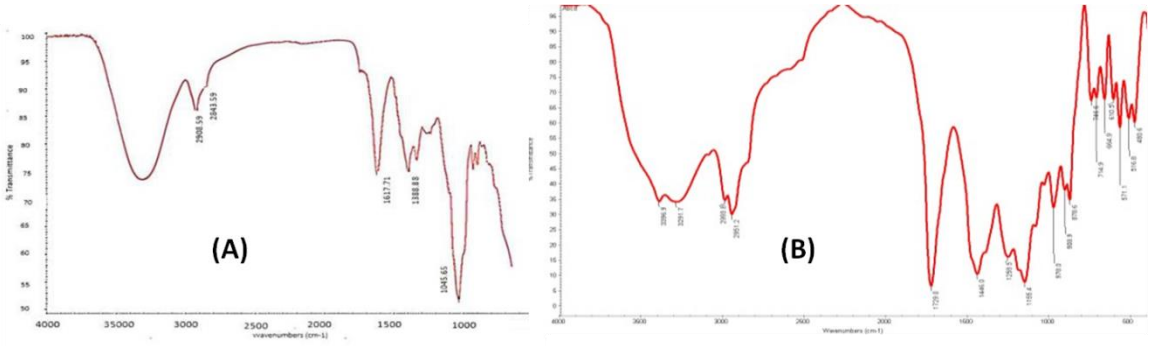



Figure 2: FTIR spectrum of the powdered drug of *Swertia chirata* and *Allium sativum.*

### 3.2 Preliminary phytochemical screening

Preliminary phytochemical studies of the MEAS revealed the presence of carbohydrates, resins, glycosides, phenolic compounds, tannins, proteins, amino acids, falvonoids and MESC revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, resin,

glycoside, and saponins as mentioned in the **Table 1.** The important lead compounds such as swertiamarin (Jalwal et al., 2022; Kumar and Jairaj, 2018; Seema et al., 2023), gallic acid, quercitin, ferulic acid, other compounds related to phenolic (Mateus et al., 2019), flavonoids, (Kumar et al., 2017) and tannins (Kumar et al., 2010) are very important for the various pharmacological activities in different plants including *Swertia chirata* and *Allium sativum*.

Table 1: Preliminary photochemical screening of methanolic extract of *Allium sativum (MEAS)* and *Swertia chirata (MESC).*

| **Chemical class** | ***MEAS*** | ***MESC*** |
|---|---|---|
| Alkaloid | - | + |
| Carbohydrates | + | - |
| Resin | + | + |
| Glycoside | + | + |
| Saponins Glycoside | + | + |
| Phenolics compound and tannins | + | + |
| Protein, and amino acids | + | - |
| Gum, and mucilage | - | - |
| Flavonoid | + | + |

**3.3 Chromatographic studies**

Thin layer chromatographic (TLC) studies were carried out in different solvent gradients. The mobile phase was used as solvent gradient (Methanol:Ethyl acetate:water:formic acid) in the ratio (10:3:5:2), respectively. Iodine vapor were used as derivatizing agent. Rf values in TLC plate shows four spots of various colours, each with a distinct Rf value in the iodine chamber. MEAS and MESC have shown four distinct TLC spots with varying Rf values as mentioned in Figure 3 and Table 2.

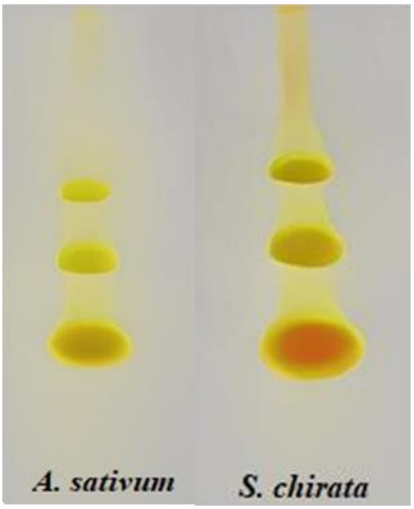



Figure 3: TLC of MEAS and MESC in mobile phase Methanol:Ethyl acetate:water:formic acid (10:3:5:2).

Table 2: $R_f$ values of the compounds present in MEAS and MESC.

| | **MEAS** | | **MESC** | |
|---|---|---|---|---|
| **Mobile Phase** | **Rf values** | **Compounds** | **Rf values** | **Compounds** |
| Methanol:Ethyl acetate:water:formic | 0.87 | Flavonoids | 0.85 | Flavonoid |
| | 0.31 | Terpenes | 0.34 | Terpenes |

| | | | | |
|---|---|---|---|---|
| acid (10:3:5:2) | 0.25 | Phenolic compounds | 0.29 | Phenol |
| | 0.19 | Tannins | 0.17 | Tannin |

Further TLC was done using mobile phase using solvent gradient of methanol and chloroform in ratio (7.5:2.5 v/v). Swertiamarin (SWMN) in yellow colour square as a glycoside, Gallic acid in light blue colour circle and Ferulic acid as phenolic and Quercetin as a flavonoid category were used to presence of compounds in MEAS and MESC. TLC denotes that swertiamarin, gallic acid, ferulic acid were present in the mixture of MEAS and MESC (1:1) in the first lane of TLC. This study showed that swertiamarin was not present in MEAS and gallic acid was not present in MESC while ferulic acid was present in both drugs. Swertiamarin, gallic acid and ferulic acid were present in the mixture of MEAS and MESC (1:1). TLC of the mixture of MEAS and MESC (1:1) in lane 1, MESC (lane 2), MEAS (lane 3), swertiamarin (lane 4), gallic acid (lane 5), ferulic acid (lane 6), and quercetin (lane 7) in mobile phase Methanol:chloform (7.5:2.5 v/v) as shown in Figure 4.

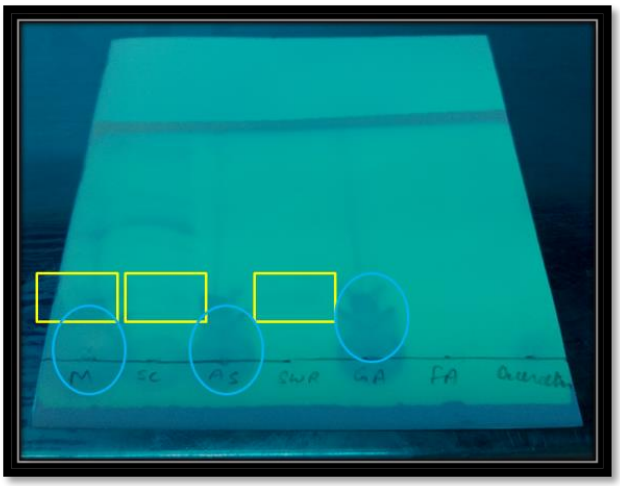


Figure 4 TLC of the mixture of MEAS and MESC (1:1) in lane 1, MESC (lane 2), MEAS (lane 3), swertiamarin (lane 4), gallic acid (lane 5), ferulic acid (lane 6), and quercetin (lane 7) in mobile phase Methanol:chloroform (7.5:2.5 v/v).

**3.4 Antioxidant activity**

Ascorbic acid was used as a standard for comparison. The experiment was carried out in triplicate and %inhibition (DPPH free radical scavenging) activity of was calculated.

MEAS, MESC, MEAS+MESC (1:1) exhibited a comparable antioxidant activity with that of standard ascorbic acid at varying concentrations tested (10, 20, 40, 60, 100 µg/mL). There was a dose–dependent increase in the percentage antioxidant activity for all concentrations tested. MEAS, MESC, MEAS+MESC (1:1) and Ascorbic acid at a concentration of 100 µg/mL exhibited a percentage inhibition of 84.23%, 81.51%, 88.34% and 99.88%, respectively (Table 3 and Figure 5). It is observed that combination of both extracts (MEAS+MESC) (1:1) shown better DPPH radical scavenging property than alone.

Table 3:DPPH free radical scavenging (% inhibition) activity of MEAS, MESC, MEAS+MESC (1:1) and Ascorbic acid.

| Concentrations (µg/mL) | %Inhibition by MEAS | %Inhibition by MESC | %Inhibition by MEAS+MESC (1:1) | %Inhibition by Ascorbic acid |
|---|---|---|---|---|
| 10 | 32.22 | 30.21 | 36.31 | 51.24 |

| | | | | |
|---|---|---|---|---|
| 20 | 41.23 | 40.21 | 45.26 | 64.51 |
| 40 | 50.21 | 48.23 | 55.31 | 79.26 |
| 60 | 73.21 | 74.21 | 76.23 | 96.34 |
| 100 | 84.23 | 81.51 | 88.34 | 99.88 |

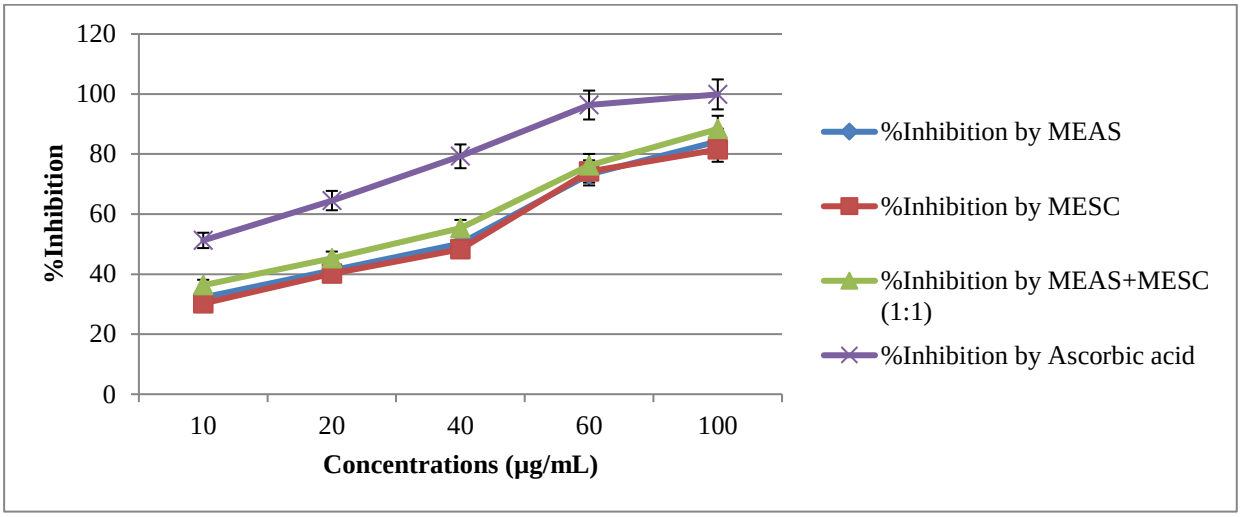



Figure 5: DPPH free radical scavenging (% inhibition) activity of MEAS, MESC, MEAS+MESC (1:1) and Ascorbic acid.

### 3.5 Antidiabetic activity

#### *3.5.1 α-Glucosidase inhibitory activity*

Percentage of inhibition for α-Glucosidase inhibitory activity for MEAS (10.2, 21.3, 27, 32, 38); for MESC (7.5, 17.6, 24.8, 29, 35.2), for MESC+MEAS (1:1) it was (14.2, 25.4, 40, 49, 61.5) while for Acarbose (20.6, 41.3, 62.4, 82.4, 96.5) was observed as shown in the Table 4 and Figure 6.

Table 4:Percentage inhibition activity of MEAS, MESC, MEAS+MESC (1:1) and Acarbose for α-glucosidase enzyme.

| **Concentration (µg/ml)** | **% Inhibition by MEAS** | **% Inhibition by MESC** | **% Inhibition by MEAS+MESC (1:1)** | **% Inhibition by Acarbose** |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 10 | 10.2 | 7.5 | 14.2 | 20.6 |
| 20 | 21.3 | 17.6 | 25.4 | 41.3 |
| 40 | 27 | 24.8 | 40 | 62.4 |
| 60 | 32 | 29 | 49 | 82.4 |
| 100 | 38 | 35.2 | 61.5 | 96.5 |

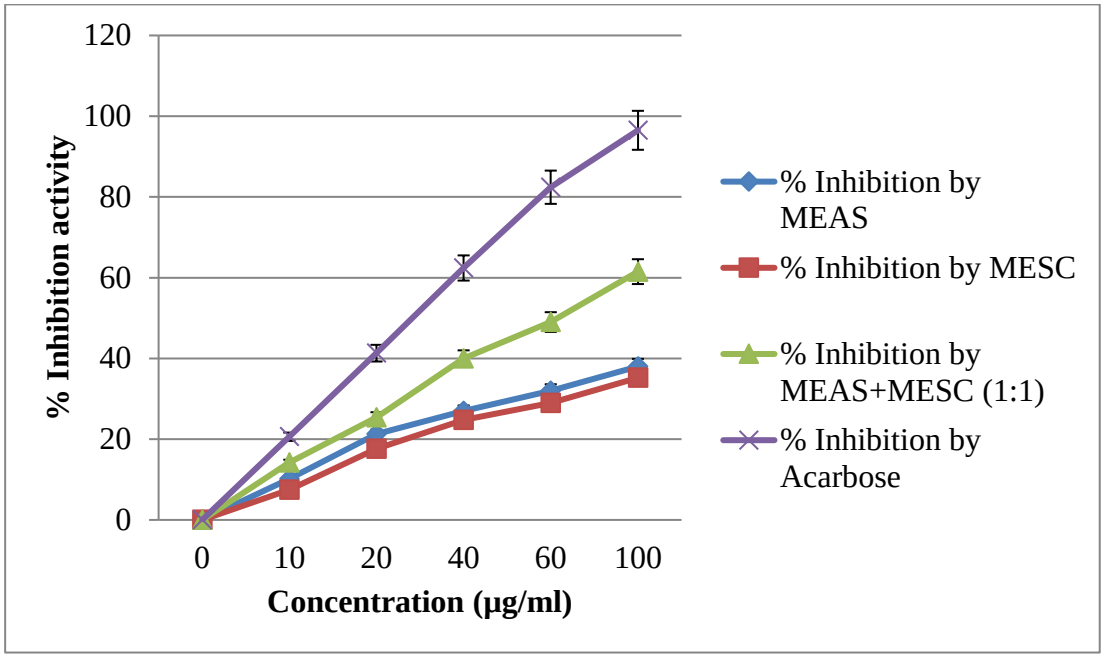



Figure 6: Percentage inhibition activity of MEAS, MESC, MEAS+MESC (1:1) and Acarbose for α-glucosidase enzyme.

**3.6 DPP IV inhibition assay**

Standard regression curve was plotted for pNA (p-nitroaniline) before the determination of the inhibitory activity of different test samples against DPP IV enzyme. Value for m=0.018and r2 value (0.09981) was determined after a plot of regression line (Figure 7) at different concentrations (20-100nM) of pNA. DPP IV inhibition activity for MEAS, MESC, and MEAS+MESC (1:1) was determined at different concentrations (25, 50, 75 and 100) µg/ml in comparison to sitagliptin (SG) (standard).

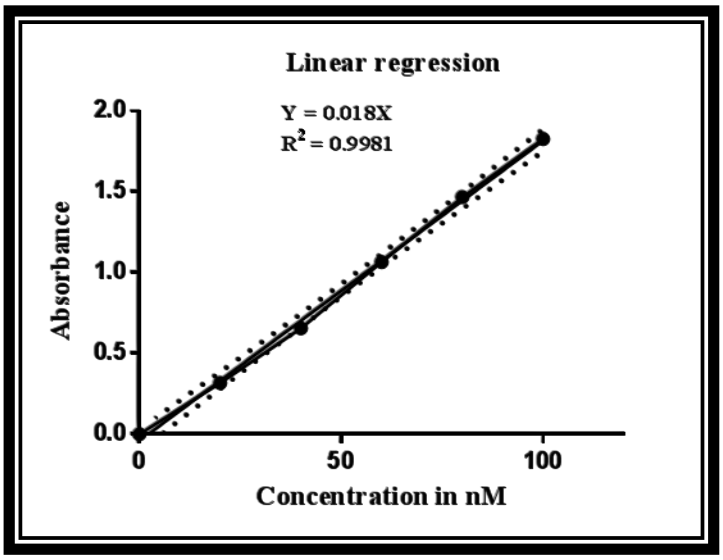



Figure 7: Standard regression curve plot for pNA.

The (%) inhibition of DPP-IV at different concentrations i.e. 25, 50, 75 and 100 µg/ml were obtained for MEAS (0.88, 12.54, 38.32, 65.24); MESC (0.69. 7.56, 25.21, 49.80); MEAS+MESC (1:1) (0.92, 16.44, 43.26, 68.24); and sitagliptin (positive control) (92, 93, 95, 98) respectively. The enzymatic assay revealed that only the combination of MEAS and MESC (1:1) had shown better inhibitory activity (~ 68.24%) in comparison to MESC (49.80%), and MEAS (65.24%). The absorbance values exhibited in Table 5 and % inhibition activity of all

tests samples for DPP IV enzyme has shown in Table 6 and Figure 8.

Table 5: Absorbance of MEAS, MESC, MEAS+MESC (1:1) and sitagliptin (SG) at 25, 50, 75 and 100 µg/ml concentration.

| Test Sample | Concentration | Mean absorbance (n=3)±std. dev. |
|---|---|---|
| **Enzyme+substrate** | --- | 0.50± 0.02 |
| ***MEAS*** | 25µg/ml | 0.49± 0.20 |
| | 50µg/ml | 0.43± 0.10 |
| | 75µg/ml | 0.31± 0.10 |
| | 100µg/ml | 0.17± 0.12 |
| ***MESC*** | 25µg/ml | 0.49± 0.20 |
| | 50µg/ml | 0.46± 0.10 |
| | 75µg/ml | 0.37± 0.10 |
| | 100µg/ml | 0.25± 0.12 |
| ***MEAS+ MESC (1:1)*** | 25µg/ml | 0.49± 0.20 |
| | 50µg/ml | 0.41± 0.10 |
| | 75µg/ml | 0.28± 0.10 |
| | 100 µg/ml | 0.15± 0.12 |
| **SG** | 25µg/ml | 0.15± 0.11 |
| | 50µg/ml | 0.04± 0.12 |
| | 75µg/ml | 0.03± 0.11 |
| | 100µg/ml | 0.02± 0.12 |

Table 6: The percentage inhibition of MEAS, MESC, MEAS+MESC (1:1) and SG at 25, 50, 75 and 100 µg/ml

| Test sample | Concentration | Avg. (n=3)± std. dev. |
|---|---|---|
| **Enzyme + substrate** | --- | 0 |
| ***MEAS*** | 25µg/ml | 0.88 |
| | 50µg/ml | 12.54 |
| | 75µg/ml | 38.32 |
| | 100µg/ml | 65.24 |
| ***MESC*** | 25µg/ml | 0.69 |
| | 50µg/ml | 7.56 |
| | 75µg/ml | 25.21 |
| | 100µg/ml | 49.8 |
| ***MEAS+ MESC (1:1)*** | 25µg/ml | 0.92 |
| | 50µg/ml | 16.42 |
| | 75µg/ml | 43.26 |
| | 100µg/ml | 68.24 |
| **SG** | 25µg/ml | 92 |
| | 50µg/ml | 93 |
| | 75µg/ml | 95 |
| | 100 µg/ml | 98 |

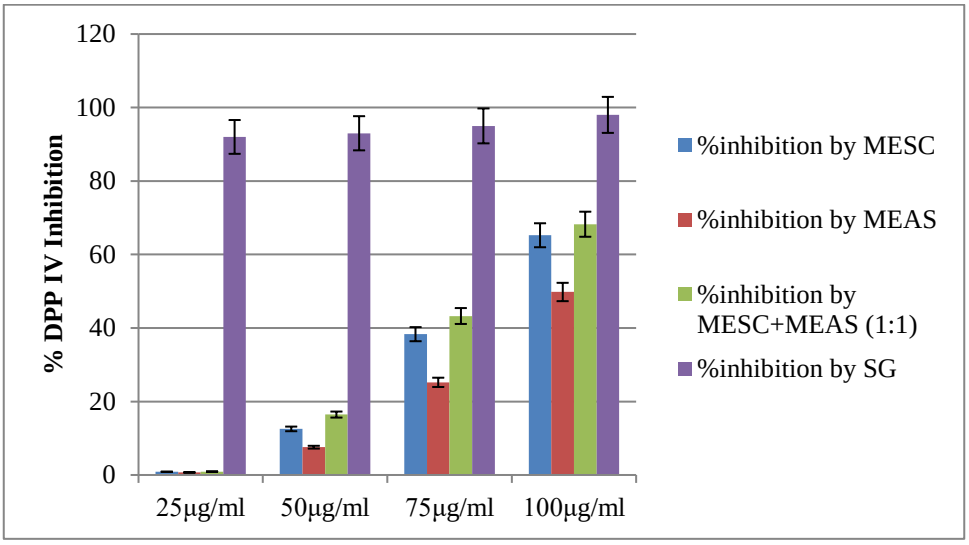



Figure 8: The percentage inhibition of MEAS, MESC, MEAS+MESC (1:1) and sitagliptin at 25, 50, 75 and 100 µg/ml.

Percentage inhibition of DPP-4 enzyme by MEAS, MESC, MEAS+MESC (1:1), and sitagliptin, was determined and the results are shown in Table 5, 6 and Figure 8.

## 4. Conclusion

Synergistic effect of the methanolic extract of *Swertia chirata* (MESC) and *Allium sativum* (MEAS) alone and in combination (1:1) was screened for the antioxidant and antidiabetic potential and found out that the combination (1:1) of methanolic extract of *Swertia chirata* (MESC) and *Allium sativum* (MEAS) has shown synergistic activity in comparison to the alone might be due to the combination of multiple phytochemicals with together. These results suggest that polyherbal formulation for the treatment of diabetes mellitus (DM) can be developed for the combination of these plants in future after *in-vivo* activities.

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