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Antioxidant and Enzyme Inhibitory Activities of Ethanol Extracts of Selected Medicinal Plants from South Punjab, Pakistan

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ABSTRACT

Natural products are promising source of drugs in pharmaceutical industry, used for the treatment of diseases and serves as a lead molecule for the synthesis of potent drugs. Therefore, current research work was planned to investigate *in-vitro* antioxidant, antidiabetic and hypolipidemic potential. For this purpose, different parts of *Albizia lebbeck* L., *Mangifera indica* L., *Vitis vinifera* L., *Syzigium jabolanum* L., *Abelmoschus esculentus* L., *Calotropis procera* L., *Grewia asiatica* L., *Morus alba* L., and *Leucaena leucophala* L. were collected from South Punjab. After collection, these parts were dried and extraction was performed by maceration. Total phenolics and total flavonoids contents were measured. Antioxidant potential, in-vitro α-glucosidase, α- amylase, lipase, lipoxygenase and urease inhibition assay was performed. Result showed that *Calotropis procera* (leaves) had maximum phenolic content of 66 mg/g equivalent of gallic acid and had maximum flavonoid content of 76mg/g equivalent of quercetin. *Ficus carica* (leaves) showed maximum antioxidant potential of 86%. *Vitis vinifera* (leaves) showed inhibitory concentration of 50 for lipoxygenase assay. *Abelmoschus esculentus* (aerial) showed maximum percentage inhibition of 87% for α-glucosidase inhibition assay. *Vitis vinifera* (leaves) showed maximum inhibition of 79% for α- amylase inhibition assay. *Calotropis procera* (leaves) had maximum percentage inhibition of 83% for lipase assay. *Abelmoschus esculentus* (aerial part) showed inhibitory concentration of 37 for urease assay. It is concluded from the aforementioned results that *Calotropis procera* leaves showed strong DPPH inhibition, lipase inhibition and urease inhibition potential. *Vitis vinifera* leaves had significant lipoxygenase inhibition, lipid modulating and antidiabetic potential whereas *Calotropis procera* leaves possessed promising lipid-modulating properties. Therefore, these plants may be helpful in the development of novel therapeutic approaches for dyslipidemia and related metabolic disorders.

Keywords: α-Amylase, Lipase, Phenolic content, Urease

INTRODUCTION

Oxidative stress led to inflammation which causes cellular damage and release pro-inflammatory agent such as bradykinin, cytokinin and prostaglandin that leads to various vascular diseases such as diabetes, obesity and cardiovascular problems. This oxidative stress occurred due to environmental factors, metabolic process and lifestyle changings [1]. Oxidative stress contributes to insulin resistance, pancreatic β-cell dysfunction and increased the chances of diabetes, causes lipid peroxidation which leads to hyperlipidemia [2]. Antioxidants play a vital role in combating diseases by neutralizing the free radicals and reducing oxidative stress, therefore, shows protection against chronic diseases such as cancer, inflammation, cardiovascular disease and neurodegenerative disorder [3]. Natural products like green tea, black tea, walnuts, almonds, cinnamon, ginger, fenugreek, neem, chia seed and turmeric are rich source of polyphenol, flavonoids and phytochemicals which possesses antioxidant potential. These compounds are used to neutralize the free radicals, modulate systemic inflammation and also reduced the sensitivity of insulin [4]. Phenolic compounds possess antioxidant, anti-inflammatory potential, therefore, used for the treatment of various disease such as cancer, cardiovascular disease, neurodegenerative disease and infectious diseases [5]. Lipoxygenase involved in production of leukotriene by oxidation of polyunsaturated fatty acid. These leukotrienes are signaling molecule that promote the inflammation. Lipoxygenase inhibitors block the production of leukotrienes therefore used for the treatment of various disease such as inflammation, cancer and cardiovascular disease [6]. α-glucosidase inhibitor prohibited the conversion of carbohydrates into glucose by competing the enzyme in the intestinal mucosa [7]. α-amylase inhibitor inhibits the conversion of starch and carbohydrates to glucose in gastrointestinal tract. Inhibitions of α-amylase and α-glucosidase may decrease the absorption of glucose, and reduce the postprandial high blood glucose level [8]. Lipase enzyme involved in the breakdown of fats into glycerol and fatty acid. When excessive fatty acid stored in the adipose tissue, it leads to weight gain and ultimately to the obesity. Inhibition of lipase enzyme reduced the absorption of fat in adipose tissue and reduces the chances of obesity [9]. Urease causes the hydrolysis of urea into carbon dioxide and ammonia. Excessive urease causes gastric ulcer, stomach cancer, kidney stones, urinary tract infection and ammonia toxicity. Inhibition of urease enzyme prevent ammonia toxicity and used for the treatment of gastric cancer and urinary tract infection [10].

Albizia lebbeck L. commonly known as sharin belonging to family Fabaceae, contains fatty acids, phenolic compounds, flavonoids, alkaloids and saponin. It is used for the treatment of boils, eye infection, cough, flu, lung disorder and abdominal tumor [11]. *Mangifera indica* L. commonly known as mango, belongs to family Anacardiaceae, contains carbohydrates, proteins, fats, lipids, steroids, flavonoids, terpenoids, tannin and organic acids. It is used for the treatment of diarrhea, bleeding hemorrhoids, scabies, diphtheria and rheumatoid

arthritis [12]. *Vitis vinifera* L. commonly known as grapes, belongs to family Vitaceae, contains vitamins, terpenoids, flavonoids, enzymes, lipids and carbohydrates. It is used to treat the discomfort of legs due to problem in blood circulation in the veins and relieve the itching and burning associated with hemorrhoids [13]. *Syzigium jabolanum* L. commonly known as jamun, belonging to family Myrtaceae, contains alkaloids, glycosides, tannins, flavonoids, anthocyanin and phenolic compounds. It is used for chronic diarrhea and have cardioprotective, antioxidant and antihyperglycemic properties[14]. *Abelmoschus esculentus* L. commonly known as lady finger, bhindi, belongs to family Malvaceae, containing carbohydrates, vitamins, oxalic acid and phenolic compounds. It is used for the treatment of inflammation, wound healing and liver detoxification. It possess antioxidant, antibacterial, antidiabetic and anti-obesity properties [15]. *Calotropis procera* L. commonly known as oak, belongs to family Apocynaceae, contains tannin, fatty acids, flavonoids, phenolic compounds, terpenoids and glycosides. It possesses anti-inflammatory, antiseptic, wound healing, antidiarrheal and astringent potential [16]. *Grewia asiatica* L. commonly known as falsa, belonging to family Malvaceae, contains flavonoids, proteins, tannin, vitamin C and anthocyanins. It possess antioxidant, anticancer, antimicrobial, analgesic and radioprotectant activities [17]. *Morus alba* L. commonly known as shatoot belongs to family Moraceae, contains vitamin, quercetin, volatile oil, antioxidant. It possesses antidiabetic, anticancer, antioxidant, and anti-dopaminergic effect [18]. South Punjab region of Pakistan has diverse ecosystem that favors the growth of various plant species. These plants possess economic potential, biodiversity, stress adaptability, and medicinal potential [19]. In current research work different plants from south Punjab were selected, suggesting that their bio-waste parts for antioxidant, antidiabetic and hypolipidemic potential.

MATERIALS AND METHODS

Collection and Drying of Crude Drugs

Albizia lebbeck L. (seed), *Mangifera indica* L. (leaves), *Vitis vinifera* L. (leaves), *Syzygium jabolanum* L. (leaves and seed), *Abelmoschus esculentus* L. (aerial part), *Leucaena leucocephala* L. (aerial part), *Calotropis procera* L. (leaves), *Grewia asiatica* L. (leaves) and *Morus alba* L. (leaves) were collected in march 2025 from the vicinity of District Muzaffar-Garh, part of south Punjab, Pakistan. The plants, *Albizia lebbeck* L. having voucher number R.R. Stewart F. W. Pak 261(2), *Mangifera indica* L. having voucher number R.R. Stewart F. W. Pak 65(6), *Vitis vinifera* L. having voucher number R.R. Stewart F. W. Pak 46(5), *Syzygium jabolanum* L. having voucher number R.R. Stewart F. W. Pak 223(5), *Abelmoschus esculentus* L. having voucher number R.R. Stewart F. W. Pak 501(3), *Leucaena leucocephala* L. having voucher number R.R. Stewart F. W. Pak 41(2), *Calotropis procera* L. having voucher number R.R. Stewart F. W. Pak 646(7), *Grewia asiatica* L. having voucher number R.R. Stewart F. W. Pak 546(6) and *Morus alba* L. having voucher number R.R. Stewart F. W. Pak 72(3). These collected parts were washed with tap water, cut into small pieces and were dried under-shade for ten days. After drying, dried samples were grinded in pestle and mortar to a course powder, weighed and packed in tightly closed polystyrene bags for further analysis [20].

Extraction of Crude Drugs

Powdered drug (250. g) of each plant material mentioned in table 1 were soaked separately in 1:3 ratio of ethanol in a tightly closed glass container. These bottles were placed at room temperature for 3-7 days with occasional shaking. After that this mixture was filtered by using Wattman filter No.1 separately and dried them in rotary vacuum evaporator (Rotavapor R-200, Buchi) at temperature of 45 °C, rotation speed of 4 rpm and pressure of 0.07MPA or 20 in Hg. The marc was re-macerate in ethanol and procedure was repeated thrice for each extract. Then the final semisolid extract of each plant material were weighed, labelled and stored in bio-medical freezer (Sanyo biomedical freezer, MDF-U333, Japan) at temperature of -20 °C for further analysis [21]. Results are mention in table 1.

Estimation of Total Phenolic Content

Total phenolic content was estimated by Folin-ciocalteu assay method. In this method 0.5ml of plant extract (1.0 $mgmL^{-1}$, 0.5 $mgmL^{-1}$, 0.25 $mgmL^{-1}$) was added in 2.5ml of Folin-ciocalteu reagent (10%), and then mixed this mixture for 30 seconds. Then allowed the mixture to stand for about 10 minutes at temperature of 25 °C. After that 2.0ml anhydrous sodium carbonate (7.5%) was mixed with the mixture and again vortexed for 30 seconds these test tubes were incubated for 30 minutes in water bath at 40°C so that reaction started and color developed. After this absorbance was taken at 765nm by using spectrophotometer. Gallic acid (Sigma Aldrich, USA) was used as a positive control at different concentrations (1.0 $mgmL^{-1}$, $mgmL^{-1}$, 0.5 $mgmL^{-1}$, 0.25 $mgmL^{-1}$) [22]. TPC was expressed in mg/g of gallic acid (GAE/g) equivalent by using standard equation based on calibration curve

$Y=0.0052x$; R^2 0.9846 Equation No. 1 ⟶

Estimation of Total Flavonoid Content

Aluminium chloride colorimetric method was used for the determination of total flavonoid content by using spectrophotometer. The mixture was prepared by adding 0.5ml of plant extract (1.0 $mgmL^{-1}$, 0.5 $mgmL^{-1}$,0.25 $mgmL^{-1}$), distilled water of 2.0ml in test tube then add 0.15 ml of 5.0 % of sodium nitrite. Then allowed this mixture to stand for five minutes at room temp, and 0.15ml of $AlCl_3$ was added and incubated it again at room temperatures for 5 minutes. 1 ml of NaOH (4%) was mixed with solution and finally made the solution to 5ml by adding distilled water, then this mixture was vortexed, incubated for 15 minutes at room temperature and observe a change in color. Quercetin (Quercetin Q4951, Sigma Aldrich, USA) was used as a positive control at different concentrations (1.0 $mgmL^{-1}$, $mgmL^{-1}$, 0.5 $mgmL^{-1}$, 0.25 $mgmL^{-1}$) [23]. The absorbance was finally measured at 420 nm. The flavonoid content was expressed as mg/g quercetin equivalent by using the calibration given below

$Y=0.0029x$; $R^2=0.997$ Equation No 2 ⟶

DPPH Radical Scavenging Assay

The antioxidant assay was performed by DPPH method, this method was described by Olajuyigbe and Afolayan (2011), with a slight modification, in this method, methanolic DPPH solution (0.135mM) was prepared. Different concentrations of plant extract and ascorbic

acid (Sigma Aldrich, USA), used as a standard drug (2-10 $mgmL^{-1}$) were prepared. Then 1.0ml of each concentration of plant extract and ascorbic acid were taken separately in tubes and added 1.0ml of methanolic DPPH solution in each tube. The reaction mixture was mixed properly and then incubated the reaction mixture in dark for 30 minutes at room temperature. Then measured the absorbance of the reaction mixture at 517 nm by using spectrophotometer. Methanolic DPPH was used as a blank [24]. The assay was performed in triplicates. RSA of plant extracts and standard were calculated by using following formula

$$\%\text{Inhibition} = \frac{\text{Abs (control) - Abs (test)} \times 100}{\text{Abs (control)}} \quad \text{Equation No. 3}$$

While, Absorbance of control represents the absorbance of radical and DPPH Absorbance of sample show the absorbance of plant extract and DPPH radical.

Anti-inflammatory Assay

The reaction mixture (5 mL) of 0.2mL of egg albumin (source fresh hen's egg), 2.8mL of phosphate buffered saline (pH 6.4) and add 2ml of extract solution (100 μgmL^{-1}). Similar volume of double distilled water served as control. The mixtures were incubated at (37°C±2) for 15min and then heated at 70°C for 5min. Then cool it and measured the absorbance at 660nm. Quercetin (Sigma Aldrich, USA) (100 μgmL^{-1}) was used as standard [25]. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\%\ \text{Inhibition} = 100 - (\text{OD test well} / \text{OD control}) * 100 \quad \text{Equation No. 4}$$

α-Glucosidase Assay

α-Glucosidase inhibitory activity of plant extract was estimated by standard method of Naki et al (2005) with minor amendment. In this activity a 96-well plate microplate reader was used and reaction mixture was prepared by adding 50 ul of phosphate buffer, having strength 100Mm, Ph 6.8, 10 ul of α-Glucosidase, consisting of 1U/ml, and 20 µL plant extract of varying concentration ranging from 0.1- 0.5 $mgmL^{-1}$ was added. It was incubated for 15 minutes at 37 ℃ After this P-NPG (5mM) of 20 ul was mixed as a substrate and then further incubated for 20 minutes at 37 ℃. This reaction was stopped when 50 ul of sodium carbonate anhydrous (0.1M) was added. Acarbose (Glucobay 50 by Bayer Pakistan) was used as a standard (0.1-0.5 $mgmL^{-1}$) [23, 24]. Blank control was prepared without test sample or standard. The absorbance was measured at 405nm by using microplate reader [26]. The percentage inhibition was calculated by using following formula

$$\%\text{Inhibition} = \frac{\text{Absorbance (control) - Absorbance (test)} \times 100}{\text{Absorbance (control)}} \quad \text{Equation No. 5}$$

While, Absorbance of control represents the absorbance of blank without sample.
Absorbance of sample show the absorbance of plant extract and standard.

α-Amylase Assay

Phosphate buffer of 40.0 mM (pH 6.9) was prepared by mixing 45 mL of sodium monobasic solution (6.24 g of $NaH_2PO_4.2H2O$ in 1L distilled water) and 55 mL of sodium dibasic solution (7.12 g of $Na_2HPO_4.2H_2O$ in 1L distilled water) and make the final volume of 200 mL with distilled water. α amylase was prepared by adding 3.246 mg in 100 mL of phosphate buffer pH 6.9. Acarbose (Standard drug) solution and plant extract of different concentrations (1.0-10.0 $mgmL^{-1}$) was prepared. In this procedure, 60 ul of each phosphate buffer (negative control / blank), test sample and positive control acarbose (Glucobay 50 by Bayer Pakistan) with concentration of 1.0-10.0 $mgmL^{-1}$ was mixed with 30 µl of enzyme (35 U/mg). Mixed and incubated at 37°C for 10 minutes. After incubation added 125 µl of starch as a substrate and further incubated at 37°C for 8 minutes. Then measure the absorbance of each sample at 405 nm spectrophotometrically [26]. The control reaction was performed without test sample

Percentage inhibition was calculated as

$$\%\text{Inhibition} = \frac{\text{Absorbance (control) - Absorbance (test)} \times 100}{\text{Absorbance (control)}} \quad \text{Equation No. 6}$$

While, Absorbance of control represents the absorbance of blank without sample
Absorbance of sample show the absorbance of plant extract and standard.

Lipase Assay

The different concentration (0.2$mgmL^{-1}$, 0.4$mgmL^{-1}$ and 0.8 $mgmL^{-1}$) of each plant extract was prepared in ethanol and enzyme (procaine lipase enzyme) was prepared by dissolving 25 mg of enzyme in 25 ml of 10% DMSO. Similarly, substrate (p- nitrophenyl butyrate) was prepared by dissolving 20.9 mg in 2.0ml of acetonitrile. After that 0.2ml of extract and 0.1ml of enzyme were mixed and final volume were made 1.0ml with tris HCl. Then incubate at 37°C for 15 min in water bath. After incubation, 100 µl of substrate was added and incubated at 37°C for 15 minutes. The different concentration (0.2$mgmL^{-1}$, 0.4$mgmL^{-1}$ and 0.8$mgmL^{-1}$) of Orlistat (Orlis 120 mg capsule by Ferozsons, Pakistan) (positive control) was prepared similarly to plant extract. Then absorbance was measured at the wavelength 405nm [27].

Percentage inhibition was calculated by using following formula

$$\%\text{Inhibition} = \frac{\text{Absorbance (control) - Absorbance (test)} \times 100}{\text{Absorbance (control)}} \quad \text{Equation No. 7}$$

Urease Assay

Indophenol method was used for quantification of urease and ammonia. 40 µl buffer of pH 8.2 (EDTA 1mM, urea 100 mM, $K_2HPO_4$0.01 M, 0.01 M $LiCl_2$), 10 µl of enzyme (5U/ml) and 10µl of extract were incubated in a 96 well plate at 37ºC for 10 min. Furthermore, solutions of 40µl of alkali reagent (NaOH 0.5% w/v, NaOCl 0.1%) and 40µl of phenol reagent having sodium nitroprusside 0.005% w/v and phenol 1% w/v was added to each well. Thiourea (Eisen Golden Laboratories, USA) was used as standard inhibitors of urease. Experiments were performed in triplicate. Absorbance was measured at 625nm. Bio-TekELx 800 microplate reader is used to perform this analysis [28]. Percent inhibition was calculated by using following equation,

% inhibition = 100 - (OD test well/ OD control) * 100. Equation No. 8 ⟶

RESULTS

Table 1 Percentage yield of Extraction by Ethanol

Sr.No	Plant	Part	Weight after drying (kg)	Extract (gm)	Percentage (%)
1.	*Syzigium jabolanum* L.	Leaves	3	51	1.7
		Seed	2	48	2.5
2.	*Vitis vinifera* L.	Leaves	3	62	2
3.	*Abelmoschus esculentus* L.	Aerial part	3	30	1
4.	*Leucaena leucophala* L.	Aerial part	2	25	1.2
5.	*Albizia lebbeck* L.	Seed	1	20	2
6.	*Calotropis procera* L.	Leaves	2	55	2.7
7.	*Morus alba* L.	Leaves	3	60	2
8.	*Mangifera indica* L.	Leaves	2	45	2.2
9.	*Grewia asiatica* L.	Leaves	2	52	2.6

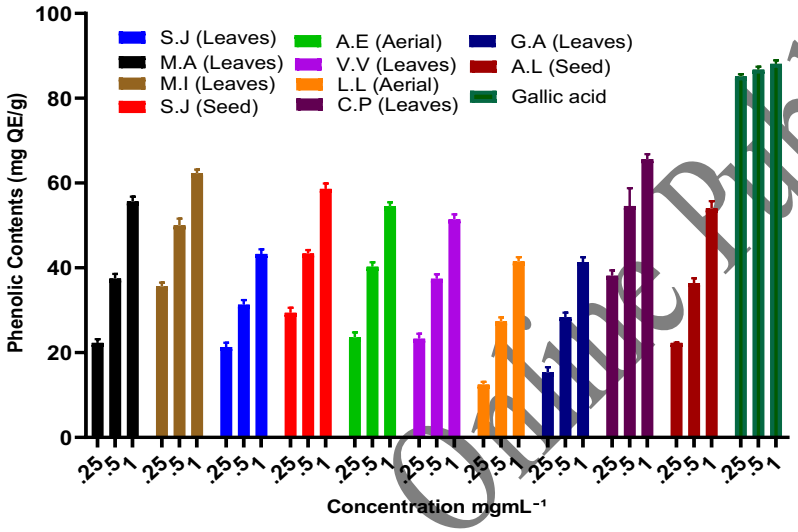

Fig. 1 Total phenolic contents of ethanolic extract of selected medicinal plants
*Whereas V.V (*Vitis vinifera*), S.J (*Syzigium jabolanum*), C.P (*Calotropis procera*), M.A (*Morus alba*), M.I (*Mangifera indica*), G.A (*Grewia asiatica*), L.L (*Leucaena leucophala*), A.E (*Abelmoschus esculentus*), A.L(*Albizia lebbeck*).

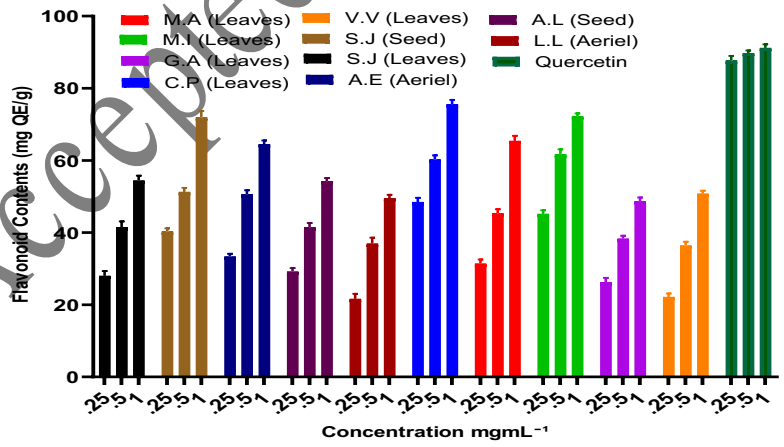

Fig. 2 Total flavonoid contents of ethanolic extract of selected medicinal plants
*Whereas V.V (*Vitis vinifera*), S.J (*Syzigium jabolanum*), C.P (*Calotropis procera*), M.A (*Morus alba*), M.I (*Mangifera indica*), G.A (*Grewia asiatica*), L.L (*Leucaena leucophala*), A.E (*Abelmoschus esculentus*), A.L(*Albizia lebbeck*).

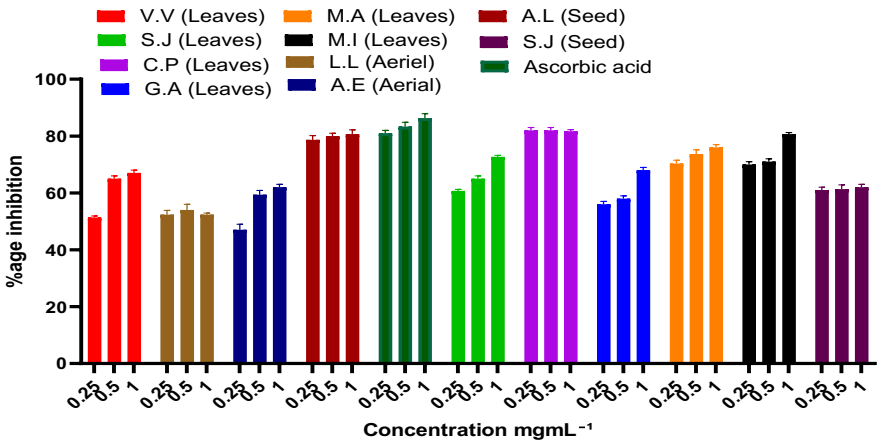

Fig. 3 DPPH assay of ethanolic extract of selected medicinal plants
*Whereas V.V (*Vitis vinifera*), S.J (*Syzigium jabolanum*), C.P (*Calotropis procera*), M.A (*Morus alba*), M.I (*Mangifera indica*), G.A (*Grewia asiatica*), L.L (*Leucaena leucophala*), A.E (*Abelmoschus esculentus*), A.L(*Albizia lebbeck*).

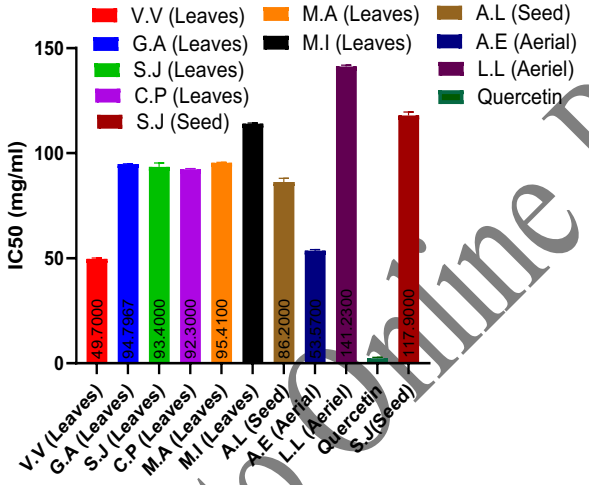

Fig. 4 Lipoxygenase inhibition assay of ethanolic extract of selected medicinal plants
*Whereas V.V (*Vitis vinifera*), S.J (*Syzigium jabolanum*), C.P (*Calotropis procera*), M.A (*Morus alba*), M.I (*Mangifera indica*), G.A (*Grewia asiatica*), L.L (*Leucaena leucophala*), A.E (*Abelmoschus esculentus*), A.L(*Albizia lebbeck*).

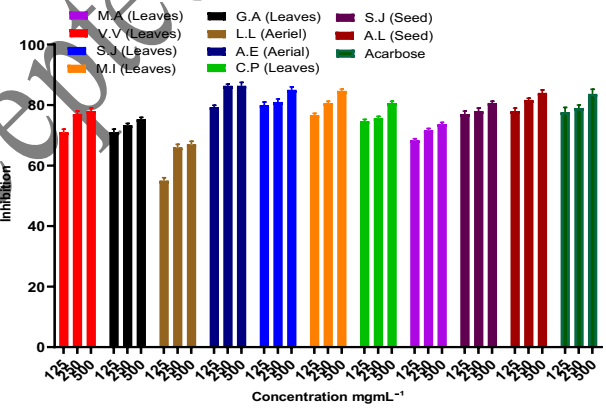

Fig. 5 Alpha glucosidase inhibition assay of ethanolic extract of selected medicinal plants
*Whereas V.V (*Vitis vinifera*), S.J (*Syzigium jabolanum*), C.P (*Calotropis procera*), M.A (*Morus alba*), M.I (*Mangifera indica*), G.A (*Grewia asiatica*), L.L (*Leucaena leucophala*), A.E (*Abelmoschus esculentus*), A.L(*Albizia lebbeck*).

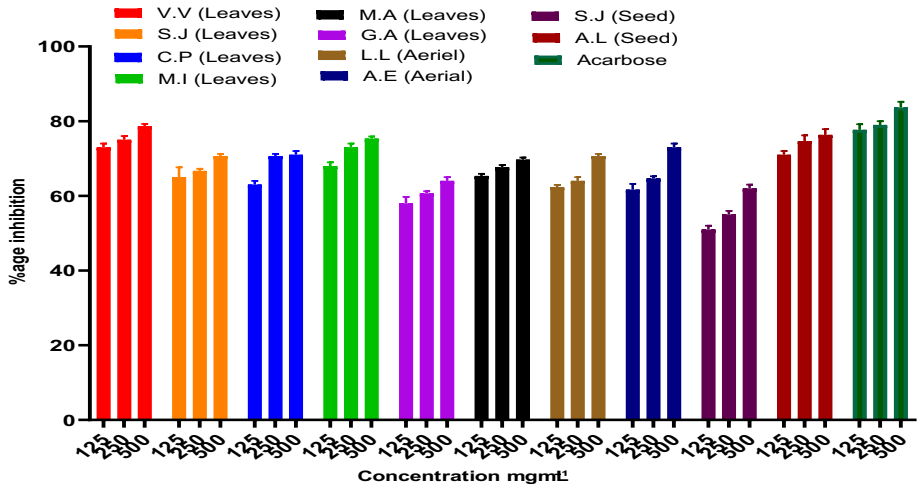

Fig. 6 Alpha amylase inhibition assay of ethanolic extract of selected medicinal plants
*Whereas V.V (*Vitis vinifera*), S.J (*Syzigium jabolanum*), C.P (*Calotropis procera*), M.A (*Morus alba*), M.I (*Mangifera indica*), G.A (*Grewia asiatica*), L.L (*Leucaena leucophala*), A.E (*Abelmoschus esculentus*), A.L(*Albizia lebbeck*).

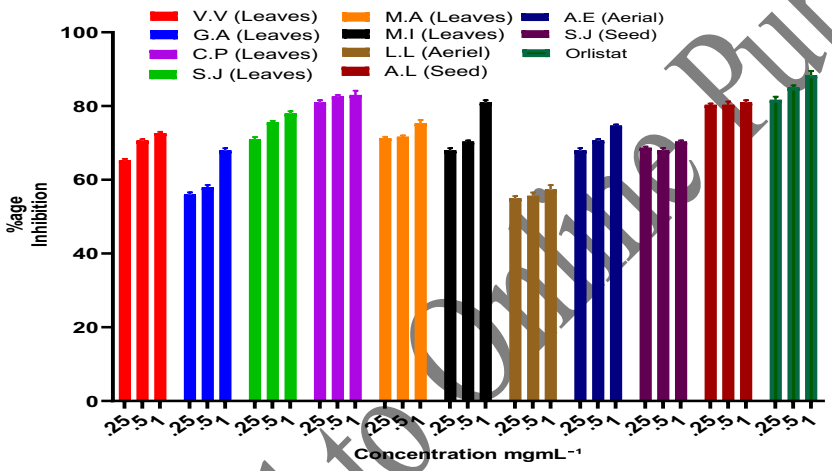

Fig. 7 Lipase inhibition assay of ethanolic extract of selected medicinal plants
*Whereas V.V (*Vitis vinifera*), S.J (*Syzigium jabolanum*), C.P (*Calotropis procera*), M.A (*Morus alba*), M.I (*Mangifera indica*), G.A (*Grewia asiatica*), L.L (*Leucaena leucophala*), A.E (*Abelmoschus esculentus*), A.L(*Albizia lebbeck*).

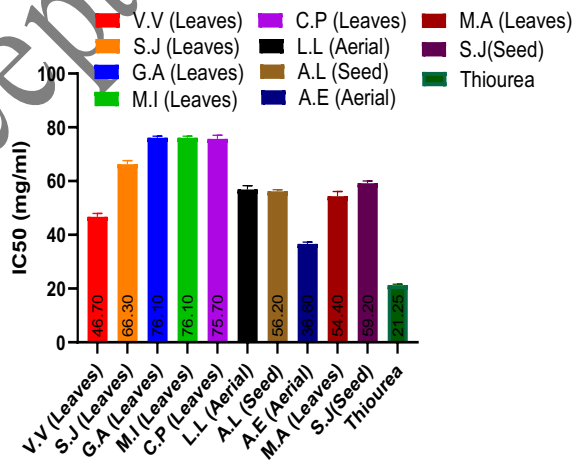

Fig. 8 Urease inhibition assay of ethanolic extract of selected medicinal plants
*Whereas V.V (*Vitis vinifera*), S.J (*Syzigium jabolanum*), C.P (*Calotropis procera*), M.A (*Morus alba*), M.I (*Mangifera indica*), G.A (*Grewia asiatica*), L.L (*Leucaena leucophala*), A.E (*Abelmoschus esculentus*), A.L(*Albizia lebbeck*).

DISCUSSION

Separation of secondary metabolites based on solubility depends on extraction principal and nature of the crude drug. In current research work extraction was carried out by maceration method using ethanol due to its polar nature [29]. *Calotropis procera* (leaves) has 2.7% yield which is highest percentage among the other drugs.

Phenolic compounds possess antioxidant and anti-inflammatory potential, therefore, used for the treatment of various disease such as cancer, cardiovascular disease, neurodegenerative disease and infectious diseases [5]. In current research work, total phenolic contents of selected plants are in order of *Calotropis procera* (leaves) > *Mangifera indica* (leaves) > Syzigium jabolanum (seed) >*Morus alba* (leaves) >*Abelmoschus esculentus* (aeriel) >*Albizia lebbeck* (seed) >*Vitis vinifera* (leaves) >*Syzygium jabolanum* (leaves) >*Grewia asiatica* (leaves) > *Leucaena leucocephala* (aerial part) with Gallic acid as standard, having phenolic content of 89mg/g. Similarly, the sequence of flavonoid contents are in the following order, *Calotropis procera* (leaves) > *Mangifera indica* (leaves) > *Syzigium jabolanum* (seed) > *Morus alba* (leaves) > *Abelmoschus esculentus* (aeriel) > *Albizia lebbeck* (seed) > *Syzygium jabolanum* (leaves) > *Vitis vinifera* (leaves) >*Grewia asiatica* (leaves) >*Leucaena leucocephala* (aerial part) with Quercetin as standard drug having flavonoids content of 92 mg/g. The results are shown in figures 1 and 2.

DPPH radical scavenging assay is easy and well-known method. When DPPH react with antioxidant, antioxidant donate electron to DPPH forming a stable molecule and neutralize its free radical nature [30]. Ascorbic acid has ability to neutralize the free radicals and also regulate the level of reactive oxygen species as they formed therefore used as standard. There is a positive relation between total phenolic content and DPPH assay because phenolic compounds such as flavonoids contain hydroxyl group which donate electron or hydrogen atom and neutralize the free radicals. Therefore, more phenolic content will lead to more DPPH inhibition and more antioxidant potential will be observed [31]. The extracts of selected medicinal plants have antioxidant potential in the following order, *Calotropis procera* (leaves) > *Albizia lebbeck* (seed) >*Mangnifera indica* (leaves) > *Morus alba* (leaves) > *Syzygium jabolanum* (leaves) > *Grewia asiatica* (leaves) > *Vitis vinifera* (leaves) >*Abelmoschus esculentus* (aeriel part) >Syzigium jabolanum (seed) >*Leucaena leucocephala* (aerial part). Ascorbic acid shows 88% percentage inhibition. The results are shown in graph 3.

Lipoxygenase enzyme is involved in the oxidation of polyunsaturated fatty acid and leads to the production of leukotriene. These are basically the signaling molecule that promote the inflammation. Lipoxygenase inhibitor block the enzyme's activity and block the production of leukotriene which are used for the treatment of various disease such as inflammation, cancer and cardiovascular disease [6]. There is a positive dose dependent relationship between total phenolic content and lipoxygenase assay because phenolic compound such as phenolic acid or flavonoids have tendency to bind with active site of LOX leading to enzyme inhibition [32]. In the current research work, the plants show IC_{50} values for lipoxygenase inhibition in the following order, *Vitis vinifera* (leaves) > *Abelmoschus esculentus* (aeriel) > *Albizia lebbeck* (seed) > *Syzygium jabolanum* (leaves)> *Calotropis procera* (leaves) >*Grewia asiatica* (leaves) > *Morus alba* (leaves) >Syzigium jabolanum (seed) >*Mangnifera indica* (leaves) > *Leucaena leucocephala* (aerial part). Plants show low IC_{50} that suggest its potent lipoxygenase inhibition. These results are given in graph 4.

α-glucosidase inhibitor competes with enzyme in the intestinal mucosa, inhibits the activity of glucosidase enzyme and prohibited the conversion of carbohydrates into glucose [7]. α- amylase inhibitor inhibits the conversion of starch and carbohydrates to glucose in gastrointestinal tract. Inhibitions of these enzyme may reduce the chances of degradation of carbohydrate which may decrease the absorption of glucose, and reduce the postprandial high blood glucose level [33]. In current study, the plants for α-glucosidase inhibition are in the following order, *Abelmoschus esculentus* (aerial part) >*Syzygium jabolanum* (leaves)>*Albizia lebbeck* (seed) > *Mangifera indica* (leaves) > *Calotropis procera* (leaves) > *Syzygium jabolanum* (seed) >*Grewia asiatica* (leaves) >*Morus alba* (leaves) > *Leucaena leucocephala* (aerial part) >*Vitis vinifera* (leaves). The results are shown in graph 5. Similarly, the potential of plant extract for α-amylase inhibition is in the following order, *Vitis vinifera* (leaves) >*Albizia lebbeck* (seed) >*Mangifera indica* (leaves) >*Abelmoschus esculentus* (aerial part) >*Calotropis procera* (leaves) >*Syzygium jabolanum* (leaves) >*Leucaena leucocephala* (aerial part) >*Morus alba* (leaves) > *Grewia asiatica* (leaves) >*Syzygium jabolanum* (seed). Acarbose used as a standard drug which show percentage inhibition 85%. The results are shown in graph 6.

Lipase enzyme involved in the breakdown of fats into glycerol and fatty acid which cause the absorption of fatty acid in the body. When excess fatty acid stored in the adipose tissue leads to weight gain and ultimately to obesity. Inhibition of lipase enzyme reduced the absorption of fat in adipose tissue and ultimately reduced the chances to have obesity [9]. There is a positive relationship between total phenolic content and lipase assay because some phenolic compounds such as quercetin, catechin are potent lipase inhibitor by binding the active site of lipase enzyme and increased percentage inhibition [34]. Plants show lipase inhibition in the following order, *Calotropis procera* (leaves) >*Albizia lebbeck* (seed) >*Mangifera indica* (leaves) >*Syzygium jabolanum* (leaves) > *Morus alba* (leaves) > *Abelmoschus esculentus* (aerial part) >*Vitis vinifera* (leaves) >*Syzygium jabolanum* (seed) > *Grewia asiatica* (leaves) > *Leucaena leucocephala* (aerial part). Orlistat, standard lipase inhibitor shows highest percentage inhibition that is 90%. These results suggest that certain plants may have potential therapeutic application for the treatment of obesity. The results are given in graph 6.

Urease enzyme is involved in hydrolysis of urea into carbon dioxide and ammonia. Excessive urease activity leads to significant consequences and health issues. Urease produce Helicobacter pylori bacteria which cause gastric ulcer, stomach cancer, kidney stones, urinary tract infection and ammonia toxicity. Inhibition of urease enzyme reduced the level of ammonia and prevent ammonia toxicity. Furthermore, urease inhibitor affects the survival of *H.pylori* bacteria in the stomach and used for the treatment of gastric cancer and urinary tract infection [10]. There is dose dependent relationship between total phenolic content and urease assay. Many phenolic compounds such as phenolic acid, flavonoids bind to the active site of urease enzyme or reduce its potential to convert urea into ammonia by interacting with its metal cofactor leading to higher inhibition [35]. Plant shows urease inhibition in the following order, *Abelmoschus esculentus* (aerial part) >*Vitis vinifera* (leaves) >*Morus alba* (leaves) >*Albizia lebbeck* (seed) > *Leucaena leucocephala* (aerial part) > *Syzygium jabolanum* (leaves) > *Syzygium jabolanum* (seed) > *Grewia asiatica* (leaves) *Mangifera indica* (leaves) > *Calotropis procera* (leaves). Thiourea is used as standard, that is potent inhibitor of urease. The results are given in graph 8.

CONCLUSION

It is concluded from the above mention results that *Calotropis procera* (leaves) show good antioxidant, lipid-modulating and urease inhibition potential. *Vitis vinifera* (leaves) show significant lipoxygenase, α-amylase, glucosidase inhibition and lipid-modulating properties.

These results suggest that further studies are needed to explore the toxicity and utilization of these biowastes for the drug development.

Conflict of Interest

The authors declare that they have no conflict of interest.

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