International Journal of Phytomedicine 4 (2012) 543-551
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Comparative study of *in-vitro* antioxidant and antidiabetic activity of plant extracts of *Acacia arabica*, *Murraya koeingii, Catharanthus roseus* and *Rouwolfia serpentina*

Ram Aadil[1], Anand Barapatre[1], Namrata Rathore[1], Sandhya Pottam[1], Harit Jha*[1]

***Corresponding author:**

Harit Jha

[1]Department of Biotechnology
Guru Ghasidas Vishwavidyalaya
Koni, Bilaspur, 495009 (Chhattisgarh)
India
.

Abstract

Medicinal plants play very important role in preventing the progress of disease. In present studyfour different medicinal plants, *Acacia arabica, Murraya koeingii, Catharanthus roseus* and *Rouwolfia serpentina* commonly found in Chhattisgarh, (India), were screened for the potency of antioxidant and antidiabetic activity. Methanolic and aqueous extracts of *A. arabica* and *M. koeingii, C. roseus* and *R. serpentina* plant leaves were examined. Total phenolic content of extracts was analyzed by Folin-ciocalteu's method. Antioxidant activities of different extracts were evaluated by DPPH and H_2O_2 scavenging assay and putative Antidiabetic activity was determined by *in-vitro* glucose diffusion and alpha-amylase inhibition assay. The highest DPPH scavenging activity was found in methanolic extract of *C. roseus* while the highest hydrogen peroxide scavenging activity was found in aqueous extract of *M. koeingii*. In *in-vitro* antidiabetic test, highest -amylase inhibition was found in methanolic extract of *R. serpentina* and the highest diffusion rate of glucose was found in aqueous extract of *R. serpentina.* The plant extracts were further characterized by Liquid chromatography mass spectroscopy. The results suggest that these plants could serve as a source of natural antioxidants and antidiabetic agents with potential applications in pharmaceuticals industry.

Keywords: Antioxidant, Acacia arabica, Murraya koeingii, Catharanthus roseus, Rouwolfia serpentina,antidiabetic

Introduction

Herbs have been used in many domains including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics, smoking, and other industrial purposes. Since the prehistoric era, herbs have been the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century [1, 2]. Natural products represent a rich source of biologically active compounds and are an example of molecular diversity, with recognized potential in drug discovery and development [3].Medicinal plants contain large amounts of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases [4].

Plenty of herbal extracts have been reported for their antioxidant and antidiabetic activities for the treatment of diabetes [5]. The effect of these plants may delay the development of diabetic complications and correct the metabolic abnormalities. Many new bioactive drugs isolated from plants have been shown to have hypoglycemic effect [6].

Diabetes mellitus is one of the most common endocrine disorders, characterized by hyperglycemia with altered lipids, carbohydrates and protein metabolism [7]. Medicinal plants play an important role in the management of diabetes mellitus.Medicinal plants contain phenolic compounds that have been shown to exhibit bioactive properties, and in particular antioxidant effects. Herbal medicines have an ability to decrease the output of lipid peroxidation and scavenge free radicals and regulate carbohydrate metabolism [8].Herbal plants have significant anti-hyperglycemic activity and antioxidant property. The hyperglycemic activities were explained by the ability of water soluble plant compound to inhibit or delay the absorption of glucose in intestine to decrease glucose transport and metabolism in muscles or to stimulate insulin secretion Antioxidants present in plants and herbs prevent the development of the disease [9].

Identification and screening of pure, pharmaceutically active compounds from plants is a long and tedious process. Therefore, it is essential to have methods to purify and separation of compounds. Chemical characterization of plant extracts is allowed to identify the biologically active components. [10]. In order to

assess the free radical scavenging capacity of phyto-constituents, DPPH and hydrogen peroxide test are widely used. It is reproducible, reliable, easy and less time consuming methods. To assess the in vitro antidiabetic activity alpha amylase and glucose diffusion are commonly used methods.

Literature survey revealed that *M. koenigii* and *C. roseus* have antidiabetic activity [11].Alcoholic extract of flower and leaf of *Catharanthus roseus* has -amylase and -glucosidase inhibitory activity. The anti-oxidant property of the herb was also evaluated by its activity to inhibit lipid per oxidation. *C. roseus* extract of leaf and flower exhibit their anti-diabetic effect by inhibiting the enzymes which has a main role in carbohydrate metabolism like -amylase and -glucosidase [12]. The alkaloid present in *R. serpentina* play important role in treating hypertension.These alkaloids work by controlling nerve impulses along certain pathways that affect heart and blood vessels, lowering blood pressure [9].

Many natural products and herbal medicines have been recommended for the treatment of diabetes. Natural products, such as terpenoids, alkaloids, flavonoids, phenolics, and some others, have shown antidiabetic potential [13].Presence of higher levels of both flavonoid and phenolic contents in the extracts of *Withania somnifera* compared to *R. serpentina* show the higher free radical scavenging capacity [9]. *A. arabica* also has the antidiabetic property by initiating the release of insulin from pancreatic β cells in diabetic rats [14]. Kavishankar *et al.*, (2011) reviewed the diabetic properties of 136 medicinal plants including *A. arabica, C. roseus,* and *M. koeingii* [5].

The objective of present study is to extract active ingredients from A. arabica M. koeingii, C. roseus, and R. serpentina plant leaves and to check their antioxidant and antidiabetic activity by in-vitro methods.

Material and Methods

Collection and preparation of plant extract

Four plants *A. arabica* and *M. koeingii, C. roseus* and *R. serpentina* young healthy leaves were collected from the Guru Ghasidas Vishwavidyalya university campus, Bilaspur, Chhattisgarh India. Collected plant leaves were first washed several times with sterilized distilled water to remove the dust particles. The leaves were dried at room temperature and coarsely powder. 10g powder of all the plants sampleswasextracted using 100% methanol, 50% methanol and distilled water for 48 h in 200 ml at 120 rpm in rotary shaker at room temperature. The extraction was twice repeated and filtered through glass funnel and Whatman filter paper no. 1. Each filtrate was concentrated to dryness under reduced pressure using a rotary evaporator. Finally the dry extracts were lyophilized and stored for further analysis.

Phytochemical analysis

The extracts obtained were subjected to preliminary phytochemical screening for the presence alkaloids, flavonoids, tannins, saponins, proteins and sugar by the methods described by Harborne (1998) and Kokate (2001) [15,16].

Determination of total phenolic content

Total phenolic contents of plant extracts were determined by the Folin–Ciocalteu reagent method as described by Lister & Wilson (2001) [17]. A standard curve was first plotted using gallic acid as a standard. Different concentrations of gallic acid were prepared in 80% of methanol. 100 µl of sample was dissolved in 500 µl Folin–Ciocalteu reagent (1/10 dilution) and 1000 µl of distilled water was added. The solution were mixed and incubated at room temperature for 1 min. After that, 1500 µl of 20% sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken and then incubated for two hours in the dark at room temperature. The absorbance of all samples was measured at 760 nm using a Shimadzu 1800 UV–Vis spectrophotometer.

Antioxidant assay

DPPH radical scavenging activity

DPPH scavenging activity was measured using spectrophotometric method of Brand-Williams (1995), with slight modification [18].A solution of DPPH in methanol (6×10^{-5} M) was prepared freshly. 3 ml aliquot of this solution was mixed with 100 µl of the samples at different concentrations (50–250 µg/ml). The solutions in the test tubes were shaken and incubated in the dark light for 15 min at room temperature. The decrease in absorbance was measured at 517nm. Gallic acid was used as standard. The percentage inhibition of the radicals due to the antioxidantproperty of the isolated fractions was calculated using the formula,

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100,$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. Blank is the absorbance of the control reaction (containing allreagents except the test compound). The % scavenging activity of different concentration of isolated fraction was tabulated in Table.4

Hydrogen peroxide scavenging activity

The hydrogen scavenging activity was determined using the method given by Ruch *et al.*,(1989) with a slight modification [19].One millilitre of sample was mixed with 2.4 ml of 0.1 M phosphate buffer (pH 7.4), and then 0.6 ml of a 43 mM solution of $H_2O_2$was added. After40 min the absorbance was measured at 230 nm against a blank solution containing phosphate buffer without H_2O_2 for each sample. For each concentration, a separate blank sample was used for background subtraction. Gallic acid was used as standard. The percentage inhibition activity was calculated from:

$$\% \text{ inhibition activity} = [(A_0\ A_1)/A_0]\ 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance ofthe extract/standard.

Antidiabetic assay

Effect of plant extract on *in vitro* glucose diffusion

A simple model system was used to determine the effect of plant extracts on *in-vitro* glucose diffusion with slight modification. This method was described by Gallagher *et al*, (2003) [20]. *In-vitro* assay were carried out into dialysis tube (pore size is 2.4nm, Hi-Media). Reaction mixtures are consisting of 3 ml of plant extracts and 1 ml of 0.15 M NaCl containing 1.65 mM D-glucose. The dialysis tube was sealed at one end and placed in a centrifuge tube containing 35 ml 0.15 M NaCl. The tubes were placed on an orbital shaker water bath and incubated at 37 C for 3 h. Concentration of glucose within and outside the dialysis tubing was measured and tests without plant extracts were used as control. Glucose concentrations were determined by using DNS assay. Gallic acid was used as standard.
Inhibition percentage was calculated by following formula:

% inhibition = Control Glucose Concentration (Outside) – Test Glucose concentration (Outside)/ Glucose concentration Control 100

Alpha-amylase inhibition assay

The -amylase inhibition assay was performed by the method described by Hansawasdi *et al.*, (2000) [21]. 1ml starch solution (in1% phosphate buffer) was incubated with enzyme for 15 minutes at 37 C. In the test the 1 ml plant extract (50µg/ml) was added with enzyme solution. 2M NaOH solutions were used to stop the reaction. 1ml DNS solution was added and solution was kept in boiling water bath for 5 minutes. Tubes were cooled in running tap water volume was made up to 10 ml by adding distilled water and absorbance was taken at 560 nm using UV-VIS Spectrophotometer 1800 (Shimadzu). Gallic acid was used as standard and assay without plant extract used as control.
Inhibition percentage was calculated by following formula:

% inhibition = Absorbance $_{(control)}$-Absorbance $_{(test)}$/Absorbance $_{(control)}$ 100

Mass spectroscopy study

Four leaves extracts (AA3, MK3, CR1 and RS1) were used for Liquid Chromatography Mass Spectroscopy analysis. Powdered dried plant extracts were used for the detection of unknown compounds. Extracts were prepared by crushing 0.5 g of the powdered material in a mortar and pestle with 2 ml of HPLC grade methanol: water (1:1v/v). The extracts were then filtered through 2µM membrane filter and the filtrates were used for LC-MS analysis. Mass spectrums were recorded on Applied Bio system API 2000 LC-MS/MS. Each spectrum was recorded in range from 10 to 1800 m/z using positive polarity [2].

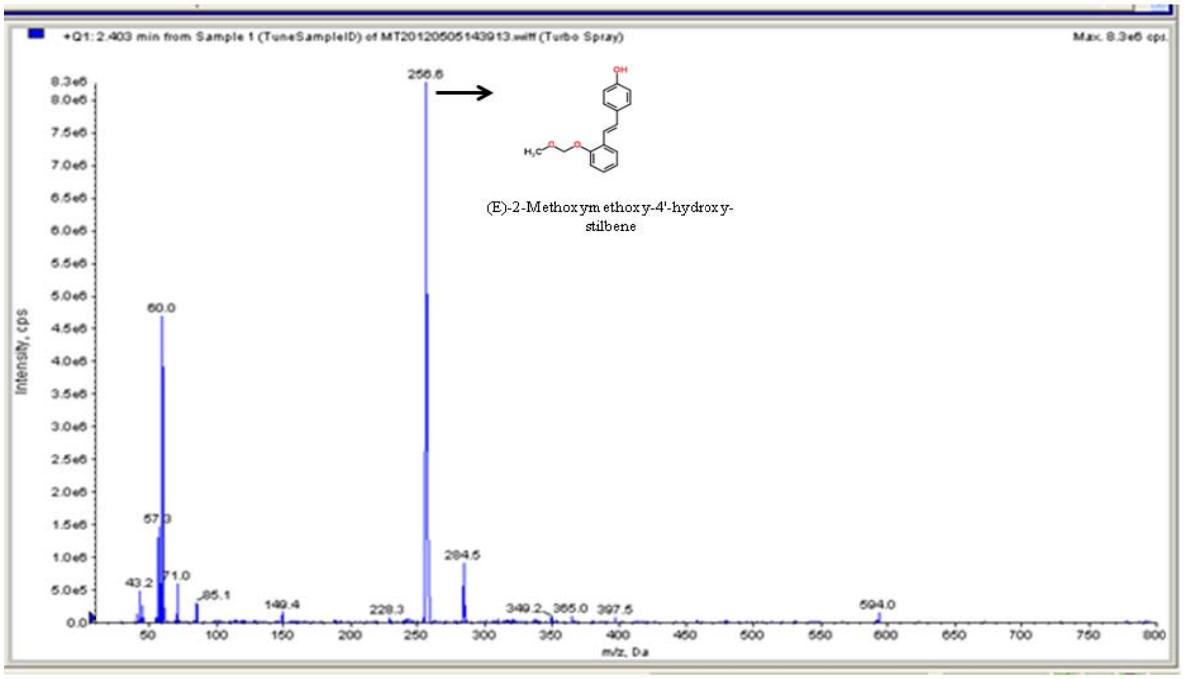


Figure 1. Mass spectrum of 100% methanol extracts of *C.roseus* (CR1)

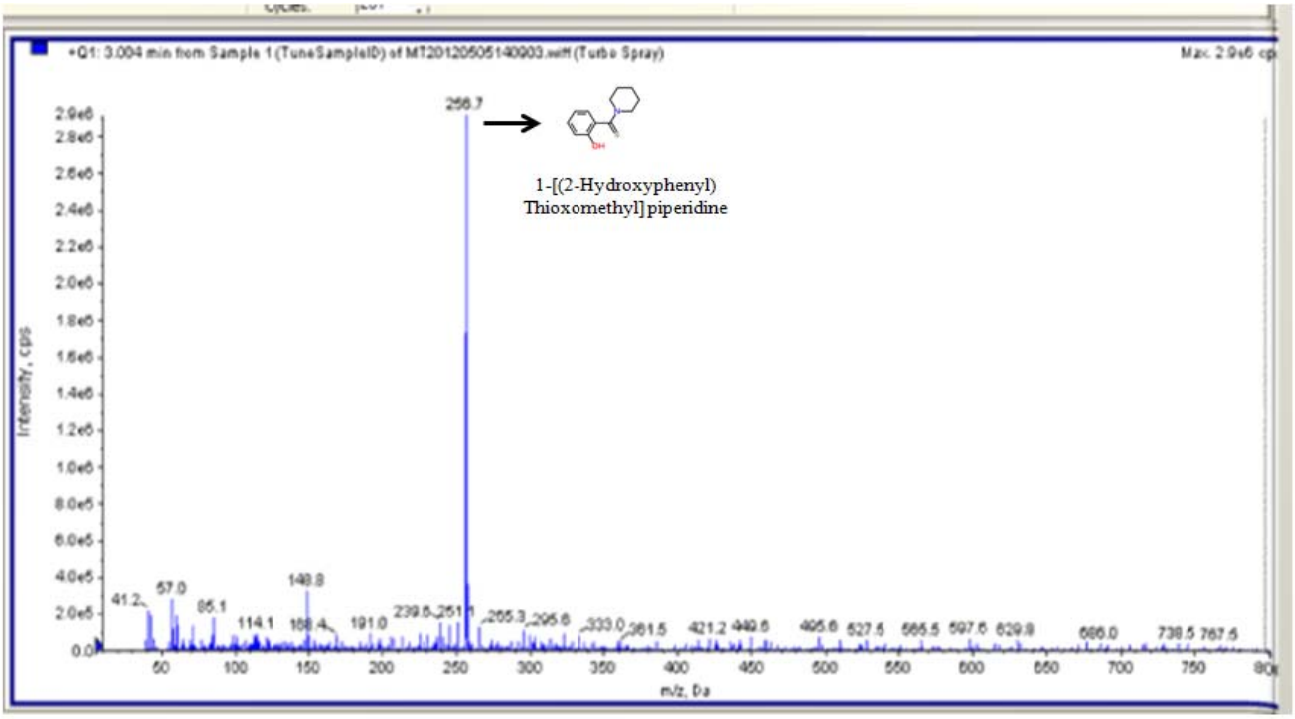


Figure 2. Mass spectrum of aqueous extracts of *M. koenigii* (MK3)

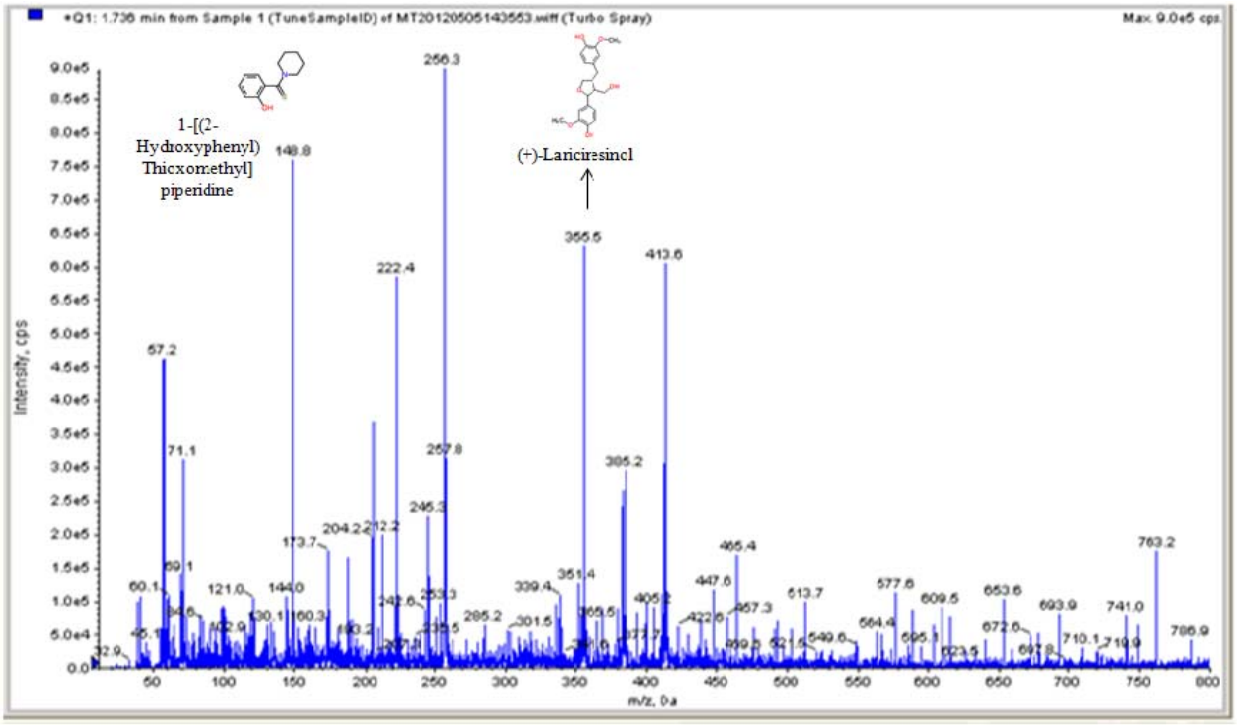


Figure 3. Mass spectrum of 100% methanol extracts of *Rouwolfia serpentina* (RS1)

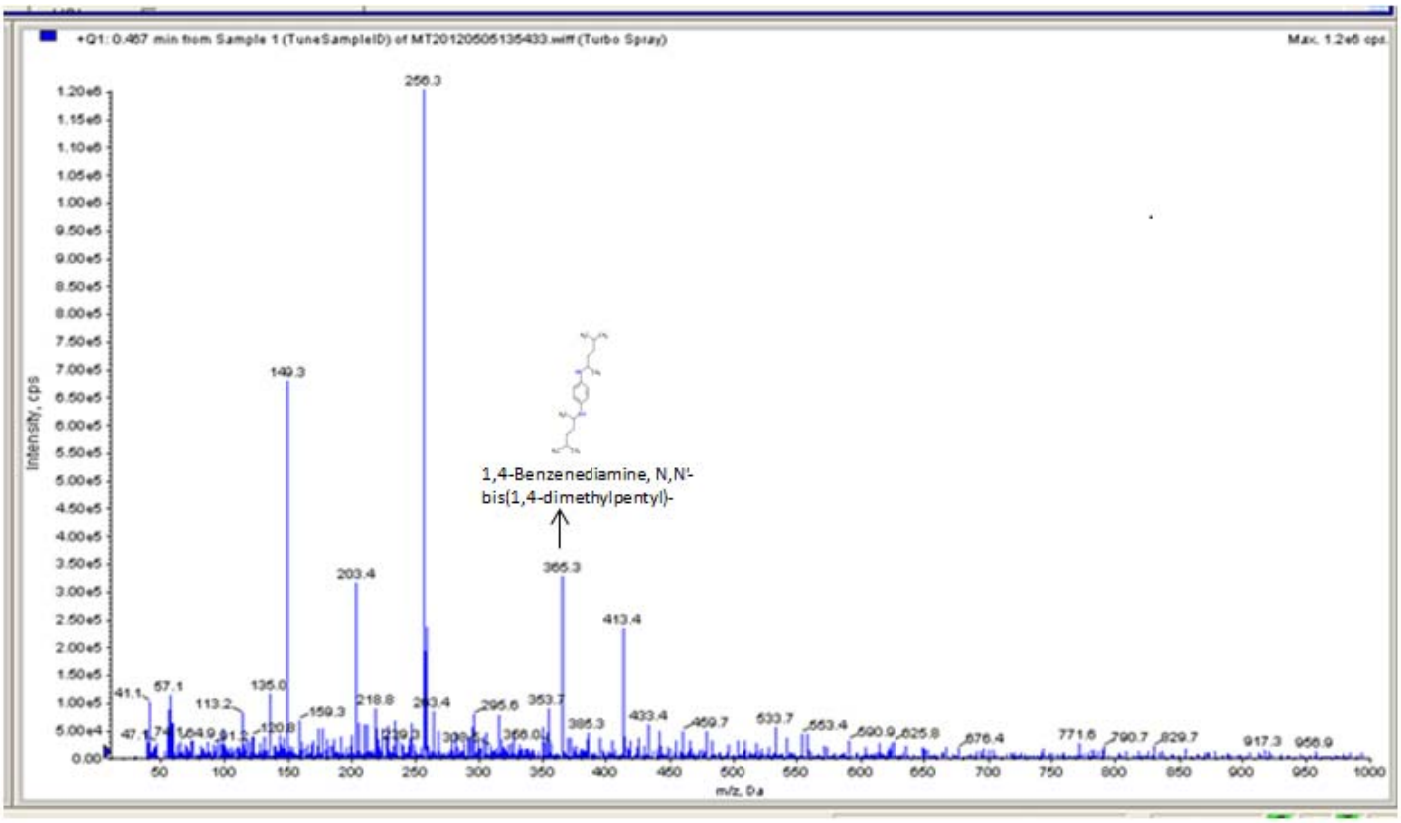


Figure 4. Mass spectrum of aqueous extracts of *Acacia arabica* (AA3)

Statistical analysis

Statistical analysis was performed using One-way analysis of variance (ANOVA) using Graph Pad PRISM software.Data were expressed mean± SD. All the experiments were performed in triplicate n=3.

Results and Discussion

Phytochemical analysis

As shown in table - 1 presence of alkaloid is shown by the all leaves extracts of the selected four plants. Saponin is present in the 50% methanol and aqueous extracts of *C.roseus*. All the extracts of *R. serpentina* showed the presence of saponin. Saponin is absent in the *extracts* of *A.arabia* and *M. koeingii.* Protein is present in all the extracts of *A.arabia and M. Koeingii. C.roseus* and *R. serpentina* did not show the presence of protein. Flavonoids and tannins are found in all the extracts of *A.arabia and M. koeingii, C.roseus* and *R. serpentina* extracts while presence of sugar is only shown by the extracts of *A.arabia and M. koeingii.* Alkaloids and flavonoids are reported to be the major component in the medicinal plants such as *Azadirachta-indica, Cajanus cajan*, *Aloe vera* [5].

Table 1: Preparation of Plants extracts

Name of samples	Solvent used	Abbreviations used for plant extracts	Weight used for extraction (g)	Weight of final extracts (g)
Acacia arabica	100% methanol	AA1	10	1.5
	50%methanol	AA2	10	1.1
	Distilled water	AA3	10	2.4
Murrayakoeingii	100% methanol	MK1	10	1.2
	50%methanol	MK2	10	0.9
	Distilled water	MK3	10	0.8
Catharanthusroseus	100% methanol	CR1	10	1.7
	50%methanol	CR2	10	1.9
	Distilled water	CR3	10	1.5
Rauwolfiaserpentina	100% methanol	RS1	10	1.2
	50%methanol	RS2	10	1.4
	Distilled water	RS3	10	0.6

Total phenolic content determination

As shown is table - 3 phenolic content was highest in aqueous extracts of *A. arabica*. 603.6 µg/ml followed by 50% methanol extracts of *A. arabica* i.e. 270 µg/ml while lowest phenolic contents

was found in 50% methanol extracts of *C.roseus* i.e. 50.6 µg/ml. In general, phenolic compounds are commonly found in plants and play several biological activities, including potential antioxidants and free radical scavenger [11, 22, 23].

Table 2: Phytochemical analysis

Samples	Alkaloid test	Saponin test	Protein test	Sugar test	Flavonoid test	Tannin test
AA1	+	-	+	+	+	+
AA2	+	-	+	+	+	+
AA3	+	-	+	+	+	+
MK1	+	-	+	+	+	+
MK2	+	-	+	+	+	+
MK3	+	-	+	+	+	+
CR1	+	-	-	-	+	+
CR2	+	+	-	-	+	+
CR3	+	+	-	-	+	+
RS1	+	+	-	-	+	+
RS2	+	+	-	-	+	+
RS3	+	+	-	-	+	+

Antioxidant assay

DPPH scavenging assay

Free radical scavenging activity of different extracts of all the four different plants was evaluated by two *in-vitro* methods: DPPH and H_2O_2assay. The antioxidant activity of the extracts mainly depends on the solvent used. Among the different extracts highest DPPH scavenging activity was observed in the 50%methanol extracts of *C.roseus,* (84.3%) while highest hydrogen peroxide scavenging capacity was observed in aqueous extracts of *M. koeingii* (92.4%). Phenolic compounds have been reported to be associated with antioxidant action in biological systems, mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [12].

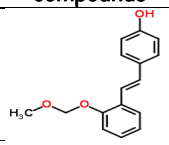
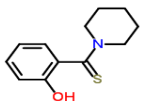
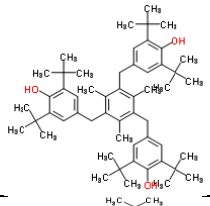
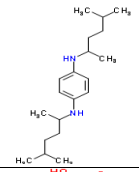
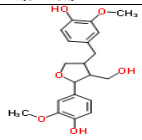
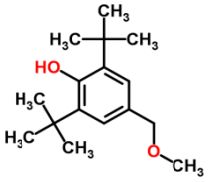
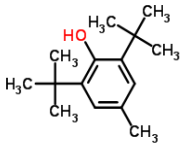
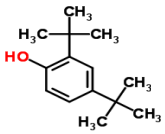
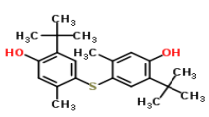
Hydrogen peroxide scavenging assay

The capacity to scavenge the hydroxyl radical of the all four plants extracts is shown in table.3, the aqueous extracts of *M .koeingii* (MK3) showed highest inhibition activity while 50% methanol extracts of *R.serpentina* showed the lowest hydroxyl radical scavenging activity. The cellular Hydrogen peroxide have the ability to cross the cell membrane and react with different metal ions to form hydroxyl radical, which damaged to cell. Therefore biologically asset for cells to control the amount of hydrogen peroxide [7]. The highest antioxidant activity were observed in 50% methanol extract of *C. roseus*, this activity is equivalent to activity of *C.roseus* shoot extract reported by Rasool *et al.*,(2011) [29]. Among the all plant extracts *C. roseus* and *M.koenigii* exhibited good scavenging activity. Bhutkar *et al* (2011) also reported the scavenging activity of both the plant leaves extract having good antioxidant activity [30]. The antioxidant activity of other two selected plant *A. arabica* and *R. serpentina* showed satisfactory free radical scavenging activity comparable to *C.roseus* and *M.koenigii*.

Table 3: Total phenol content and % inhibition of antioxidant and antidiabeticactivity of plant extracts

Plant extracts	Phenolic content (µg/mg)	DPPH Scavenging activity (%)	H_2O_2 Scavenging activity (%)	Glucose diffusion test (%)	-Amylase inhibition activity (%)
AA1	198.0	44.4	88.2	97.3	20
AA2	270.0	67.4	91.5	74.4	4.9
AA3	603.6	64.4	91.8	84.6	25.2
MK1	246.0	70.2	85.5	95.7	22.8
MK2	133.2	71.9	85.6	95.5	6.4
MK3	158.0	58.7	92.4	73.9	31.6
CR1	59.2	84.3	48.3	82.3	25.3
CR2	50.6	69.0	48.3	63.1	10.9
CR3	106.7	69.9	48.6	94.8	20.6
RS1	100.1	71.7	47.1	96.3	33.8
RS2	119.9	69.9	46.6	81.2	15.1
RS3	56.0	69.0	51.5	97.0	14.0

Table 4:Peaks assignment of compounds of LC-MS spectrum

Structure of compounds	Name of compound	Molecular formula	Library Peaks	Observed peaks m/z
	(E)-2-Methoxymethoxy-4'-hydroxy-stilbene	C16H16O3	256	255.6(CR1) 256.3 (RS1) 256.7 (MK3)
	1-[(2-Hydroxyphenyl)thioxomethyl]piperidine	C12H15NOS	221	222.4 (RS1) 221.3
	1,3,5-Trimethyl-2,4,6-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-benzene	C54H78O3	774	772
	1,4-Benzenediamine, N,N'-bis(1,4-dimethylpentyl)-	C20H36N2	304	301
	(+)-Lariciresinol	C20H24O6	360	361.5
	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(methoxymethyl)-	C16H26O2	250	250
	ButylatedHydroxytoluene	C15H24O	220	221.3
	Phenol, 2,4-bis(1,1-methylethyl)-	C14H22O	206	209.4
	Santonox	C22H30O2S	358	355.5

Antidiabetic assay

In- vitro glucose diffusion

Gallagher *et al.*(2003) reported that agrimony and avocado showed inhibitory effect on glucose diffusion whereas elder and nettle extracts did not significantly decrease glucose diffusion. They also reported that the plant extracts exhibited a concentration dependent inhibitory effect on glucose movement [20]. In the present work, four samples (CR2, MK3, RS1 and AA2) showed the significant, inhibitory effect on glucose diffusion outside the dialysis tube, while other extracts increase the movement of glucose across the dialysis tube. The highest inhibitory effect of 63% is shown by CR2 extract. Powdered seeds of *A.arabica* extract induces hypoglycemic effect by initiating release of insulin from pancreatic beta cells [10,26], Hypoglecemic and antioxidant activity of leaves extract of *C. roseus* and *M. koenigii* have been also reported [27]. Presence of different functional groups helps the compound to bind the glucose and other molecule which increase the retention time and control the diffusion of glucose molecules across the dialysis membrane [28].

Amylase inhibition assay

-amylase inhibition activity of 100%, 50% methanolic and aqueous extracts of all the four plants ranged between 4 and 33% with 100% methanolic extracts of *R.serpentina* exhibiting the highest inhibitory effect (Table.3). These activities are comparable to the range of values reported for certain medicinal plant such as *Phellodendron amurensen* and *Apocynum venetum* [11,24].

Mass spectroscopy analysis

Total nine compounds were identified by LC-MS analysis (Table.2) from 100% methenolic extract of *C.roseus*, aqueous extracts of *A.arabica* and *M.koeingii*, and 100 % methenolic *R. serpentina*. The mass spectrum of each compound was compared with that in NIST 11 library. The major compounds present in the extract were (E)-2-Methoxymethoxy-4'-hydroxy-stilbene, 1-[(2-Hydroxyphenyl)thioxomethyl]piperidine, 1,3,5-Trimethyl-2,4,6-tris(3,5-di-tert-butyl-4-hydroxybenzyl)-benzene,1,4-Benzenediamine,N,N'-bis(1,4-dimethylpentyl)-,(+)-Lariciresinol, 2,6-bis(1,1-dimethylethyl)-4-(methoxymethyl)-, Butylated Hydroxytoluene, Phenol, 2,4-bis(1,1-methylethyl)-, Santonox extract as identified by LC-MS were found in 100% methenolic extract of *C.roseus*, aqueous extracts of *A.arabica* and *M.koeingii*, and 100 % methenolic *R. serpentina* respectively. Some of the compounds were not identified and all of these were present in traces or less concentration. The extract may have some groups which may be involved in some other antioxidant and antidiabetic activities.

Conclusions

In the present work all the four plants exhibited potent free radical scavenging and antioxidant activity. Antidiabetic assay suggested that methanol extracts of *R. serpentina* could be good source of antidiabetic drugs. *A. arabica* and *R. serpentina* extract has high phenolic content, potent antioxidant activity and are able to regulate glucose migration and alpha amylase activity. Some of the molecules observed in LC-MS are-(+)-Lariciresinol, Butylated Hydroxytoluene, 2,4-bis(1,1-methylethyl)-, Santonox. These compounds may be the active component of the extracts and can be used as antidiabetic and antiageing lead molecules.

Authors' contributions

Keshaw Ram Aadil, has helped in writing the manuscript and performed LC-MS analysis.
Namrata Rathore and Sandhya Pottam have helped by collecting the plant samples and performed the phytochemical, antioxidant and antidiabetic analysis.
Anand Barapatre contributed in statistical analysis of data.
Harit Jha provided the concept for work, planned the experiments, analysed the results and prepared the manuscript.

Acknowledgements

The authors are thankful to the Head of the Department, Department of Biotechnology, Guru Ghasidas Vishwavidyalaya for providing facilities and infrastructure.

References

[1]. Chen JH and Ho CT. Antioxidant activities of caffeic acids and itsrelated hydrocinnamic acid compounds. Journal of Agricultural and Food Chemistry 1997, 45: 2374–2378.

[2]. Cuyckens F and Claeys. Mass spectrometry in the structural analysis of flavonoids. J. Mass Spectrom 2004, 39:1-15.

[3]. Khosh F and Khosh M. Natural approach to hypertension. Altern Med Rev 2001, 6(6):590-600.

[4]. Anderson KJ, Teuber SS, Gobeille A, Cremin P, Waterhouse AL, & Steinberg FM. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. Biochemical and molecular action of nutrients. Journal of Nutrition 2001, 131: 2837–2842.

[5]. Kavishankar GB, Lakshmidevi N, Murthy, M Prakash HS, Niranjana SR. Diabetes and medicinal plants-A review. Int J Pharm Biomed Sci 2011, 2(3): 65-80.

[6]. Modak M, Dixit P, Lodhe J, Ghaskadbi S, Devasgayam TPA. Indian Herbs and herbal drugs used for the Treatment of Diabetes. J Clin Biochem Nut 2007, 40(3):163-173.

[7]. Mishra KP, Ganju L, Sairam M, Banerjee PK, Sawhney RC, A review of high throughput technology for the screening of natural products. *Biomed Pharmacother* 2008, 62: 94–98.

[8]. Porter JR, Barrett TG. Monogenic syndromes of abnormal glucose homeostasis:clinical review and relevance to the understanding of the pathology of insulin resistance and b cell failure. J Med Genet 2005, 42:893–902.

[9]. Keshavkant S, Sukhdev T, Srinivasara o C, Naithani SC. Antioxidant activities, phenols and flavonoid contents of *Withania somnifera* and *Rauwolfia serpentine*, Indian Journal of Plant Physiology 2008,13(4):394-399.

[10]. Kumbhare MR, Guleha V2, Sivakumar. T2 Estimation of total phenolic content, cytotoxicity and in-vitro antioxidant activity of stem bark of Moringaoleifera. Asian Pacific Journal of Tropical Disease 2012, 144-150.

[11]. Lee Y, Kim H, Choi HS, Kang BH, Han YB, Kim SJ. "Effects of water extract of 1:1 mixture of Phellodendron cortex and Aralia cortex on polyol pathway and oxidative damage in lenses of diabetic rats. Phytother Res 1999,13(7): 555-560.

[12]. Saha MR, Hasan SMR, Akter R, Hossain MM, Alam MS, Alam MA, Mazumder MEH. In vitro free radical scavenging activity of methanol extracts of the leaves of *Mimusops elengi* Linn. Bangladesh J. Vet. Med. 2008, 6:197–202.

[13]. Imran M, Raja MM, Barith A, Asarudeen A. Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms Pleurotusflorida and Pleurotuseous. International Food Research Journal 2011, 18:579-582.

[14]. Sangala R, Kadati DR, Burra SD, Gopu J, Dubasi A. Evaliuation of Antidiabetic Activity of *Annona Squamosa* Linn seed in Alloxan-Induced Diabetic Rats. International Journal of Priclinical Researc 2011,2:100-106.

[15]. Harborne JB. Phytochemical methods to modern techniques of plant analysis. 3rd ed. New York, Chapman and Hall, 1998.

[16]. Kokate, CK. Pharmacohnosy. 16th Ed. Mumbai, India,Nirali Prakashan, 2001.

[17]. Lister E, Wilson P. Measurement of total phenolics and ABTS assay for antioxidant activity (personal communication), Crop Research Institute, Lincoln, New Zealand, 2001.

[18]. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity,LWT - Food Science and Technology 1995, 28(1): 25–30.

[19]. Ruch R, Cheng S, and Klauning J. Prevention of cytoxicity and inhibition of intercellular communication antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* 1989, 10:1003-1008.

[20]. Gallagher AM, Flatt PR, Duffy G, Abdel-Wahab YHA. The effects of traditional antidiabetic plants on *in vitro* glucose diffusion, Nutr Res 2003, 23:413–424.

[21]. Hansawasdi C, Kawabata J, Kasai T. - amylase inhibitors from Roselle (Hibiscus sabdariffa Linn.) tea. Biosci Biotechnol Biochem 2000, 64:1041-43.

[22]. Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK. Antidiabetic Agents from Medicinal Plants. Current medicinal chemistry 2006, 13:1203-1218.

[23]. Malathi V, Devi SS, Revathi K. Anti diabetic activity by the in vitro alpha amylase and alpha-glucosidase inhibitory activity of *catharanthus roseus.* The Bioscan, 2010, 5(4): 655-659.Yokozawa T, Nakagawa T. Inhibitory effects of Luobuma tea and its components against glucose-mediated protein damage. Food and Chemical Toxicology 2004, 42: 975-981.

[24]. McLauglin, JL, Chan g CJ, Smith Dl. Simple bench - top bioassays (brine shrimp and potato discs) for the discovery of plant anti - tumour compounds. In: Kinghorn AD, Balandrin MF (editors).Human medicinal agents from plants. Washington: American Chemical Society 1993, 112 -137.

[25]. Wadood A, Wadood N, Shah SA. "Effects of Acacia arabica and Carallumaedulis on blood glucose levels of normal and alloxan diabetic rabbits". J. Pak. Med. Assoc 1989, 39(8): 208-212.

[26]. Nammi S, Boini MK, Lodagala SD, Behara RB. The juice of fresh leaves of *Catharanthusroseus* Linn, reduces blood glucose in normal and alloxan diabetic rabbits. BMC Complement Altern Me 2003, 2: 3-4.

[27]. Khan BA, Abraham A, Leelamma S. Hypoglycemic action of *Murraya koenigii*(curry leaf) and *Brassica juncea* (mustard) mechanism of action. Ind J BiochemBiophys 1995, 32:106-108.

[28]. Rasool N, Rızwan K, Zubaır M, Naveed KR, Imranand Ahmed V. Antioxidant potential of different extracts and fractions of *Catharanthus roseus* shoots International Journal of Phytomedicine 2011,3:108-114.

[29]. Bhutkar MA, Bhise SB, Comparison of Antioxidant Activity of Some Antidiabetic Plants, International Journal of Research in Pharmaceutical and Biomedical Sciences 2011, 2 (3):982-987.