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A Quantitative Estimation of Phytochemicals, Anti-Diabetic and Anti-Oxidant Activities of Crude Extracts of *Sphagneticola trilobata* (L.) and *Adathoda vasica* Linn.

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ABSTRACT

Due to therapeutic efficacy, *Sphagneticola trilobata* (roots), and *Adathoda vasica* (whole plant) were selected to explore anti-diabetic and anti-oxidant activities of their crude plant extracts. The plant samples were extracted with low to high polar solvents *viz.* n-hexane, ethyl acetate, methanol and water. Further crude extracts were screened for the presence of phytochemicals such as alkaloids, flavonoids, phenols etc. The quantitative estimation of phenols, flavonoids, alkaloids was performed by spectrophotometric approach. The evaluation of anti-oxidant activity of *Sphagneticola trilobata* was determined by DPPH method and ABTS method was applied to access the antioxidant activity of *Adathoda vasica.* The antidiabetic activity of crude extracts of *Sphagneticola trilobata* and *Adathoda vasica* was determined along with *Brassica oleraceae* by applying in *vitro* α-amylase inhibition method. The methanolic extract of *Sphagneticola trilobata* was significantly showed DPPH IC_{50} inhibition at 20 µg/mL, and *in vitro* α-amylase inhibition IC_{50} at 50 µg/mL. In *Adathoda vasica* methanol extract showed ABTS inhibition IC_{50} at 30 µg/mL, and *in vitro α-amylase* inhibition of IC_{50} at 25 µg/mL. It is concluded that methanolic extract of *Sphagneticola trilobata* can be potential source of anti-oxidant and methanolic extract of *Adathoda vasica* proven as good anti-diabetic agent.

1. Introduction

In the early days of human history so many medicinal plants have been identified and used to treat sick of the society. Locally many attempts were made to identify different parts of the plants for specific illness depending upon people's experience. Over generations human beings believe the healing power of plants and emphasize miraculous healing mechanisms of the plants [1]. The knowledge of medicinal plants was practiced among local communities and information passed to the people of all over the world. It has been widely known fact for centuries that derivatives of plant possess a wide range of biological activity [2]. The medicinal plants are referred to plants that are used for their therapeutic or medicinal values and contain a variety of chemical substances that act upon the human body. The use of the different parts of the plants are known to prevent, relieve and treat disease [3]. Medicinal herb is considered to be a factory of phytochemicals as it can produce multitude of Phytochemical like alkaloids, Flavonoids, phenolics, saponins etc.

Sphagneticola trilobata belongs to the family of *Asteraceae* and it is commonly known as the creeping- oxeye, Singapore daisy, trailing daisy, rabbits paw, widelia trilobata. It is widely cultivated as an ornamental ground cover. It is perennial herb up to 30 cm in height. It has rounded stems up to 40 cm long, rooting at nodes and with the flowering stems ascended. Leaves are fleshy, hairy, serrate, 4-9 cm long and 2-5 cm wide. The flowers are bright yellow ray florets of about 8-13 per head, rays are 16-15 mm long. It is grown best in sunny areas with well drained, moist soil at low elevation. It is noxious weed in agricultural land, along road sides, and urban waste places. This plant is anti-inflammatory, anti-microbial, analgesic, larvicidal, anti-diabetic, and anti-turmeric agent [4]. The stem, leaves, flowers, fruits, are used to treat reproductive problems, amenorrhea, dysmenorrhoea cold, fever, snake bites, insect bites etc., [5-7]. Roots showed in vitro antioxidant activity [8], the leaves and stem contains eudesmanolide, lactone, luteolin and kaurenoic acid [9,10], aerial part shows triterpenoids, diterpenoids, sterols, flavonoids, benzene derivatives [11].

Adathoda vasica Nees syn Adathoda zeylanica Medic, *Justicia adathoda* Linn, belongs to the family *Acanthaceae*. It is also called Vasa (Sanskrit), Malabarnut (English), and Addasaramu (Telugu). It is an ever green shrub found in many regions of India and throughout the world with 1-3 feet height with many long opposite branches, leaves are large and lance shaped, opposite and estipulate. The flowers are like spikes or panicles, small irregular zygomorphic, bisexual and hypogenous. The flowers are either white or purple in colour. Inflorescence in axillary, spicate cymes, densely flowered, peduncles short, foliaceous [12]. It is well known for its effectiveness in treating respiratory problems. The plant parts are extensively used for treating cold, cough, bronchitis, asthma as sedative and expectorant [13]. It is also used for multitude of disorders including leprosy, blood disorders, and heart troubles, loss of memory, leucodermas, jaundice, tumours, sore eye, and gonorrhea. Decoction of the leaves soothes the throat irritation and will help in loosen phlegm deposits in the air way. It is also been used to control both internal and external bleeding such as peptic ulcers, piles, bleeding gums. Due to its anti-implantation activity it should not be used while pregnant [14].

As per the knowledge of the author the selected plant samples were not explored for their anti-diabetic, anti-oxidant activities (in vivo) and also there was no report on the quantitative estimation of their phytochemicals. So, in the present investigation an attempt was made to estimate the phytochemicals, and evaluation of anti-diabetic and anti-oxidant activities of the crude extracts of *Sphagneticola trilobata* (L.) and *Adathoda vasica*.

2. Experimental Methods

2.1 Plant Samples

The studied plant samples *Sphagneticola trilobata* and *Adathoda vasica* are collected from Eluru of west Godavari District and Vijayawada of Krishna District of coastal Andhra Pradesh in India. The plant sample *Sphagneticola trilobata* and *Adathoda vasica* were identified and authenticated at the NISCAIR (CSIR), New Delhi. Authentic samples were deposited in the raw materials herbarium and museum Delhi (RHMD), reference numbers are NISCAIR/RHMD/consult/2016/2981-08-2 for *Sphagneticola trilobata,* NISCAIR/ RHMD/consult/2017/3044-71 for

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Adathoda vasica. The plant samples were cleaned and rinsed with water to remove any associated debris. The cleansed fresh materials were dried in shaded area and grounded to fine powder.

2.2 Plant Extractions

The dried sample powder of each plant were weighed and packed into a soxhlet apparatus and extracted with the solvents from low polar to high polar *viz.* n-hexane, ethyl acetate, methanol, and water. The extract was filtered through Whatman filter paper no-1 and then evaporated to dryness by using rotary evaporator. The final crude extracts were weighed and collected in an air tight container for further use. The weight of the crude extract obtained in each solvent is noted down in Table 1.

Table 1 Details of yields of crude extracts

S.No.	Compound	Weight in grams		% of extract value	
		Sphagneticola trilobata (roots)	*Adathoda vasica* (wholeplant)	*Sphagneticola trilobata* (roots)	*Adathoda vasica* (whole plant)
1	Sample	62.81	36.58	-	-
2	Hexane	0.39	1.39	0.62	3.80
3	Ethyl acetate	1.28	3.68	2.04	10.06
4	Ethanol	11.36	5.68	18.09	15.53
5	Water	8.42	5.25	13.41	14.35

2.3 Phytochemical Screening of the Plant Extractions

Phytochemical screening was conducted to the selected parts of the plants *Sphagneticola trilobata, Adathoda vasica* for presence of alkaloids, phenols, saponins, flavonoids, carbohydrates, glycosides, steroids, triterpenoids by using standard phytochemical procedures [15, 16].

2.3.1 Test for Steroids

Salkowski Test: Few drops of concentrated sulphuric acid are added to the plant extract, shaken and on standing; lower layer turns red in colour indicates steroids presence.

Liebermann Burchard's Test: To the extract, few drops of acetic anhydride is added and mixed well. 1 mL of concentrated sulphuric acid is added from the sides of test tube, a reddish brown ring is formed at the junction of two layers indicating the presence of steroids.

2.3.2 Tests for Triterpenoids

Salkowski Test: Few drops of concentrated sulphuric acid is added to the extract, shaken and on standing, lower part turns golden yellow colour indicating the presence of triterpenoids.

Lieberman Burchard's Test: To the extract, few drops of acetic anhydride is added and mixed well. 1 mL of concentrated sulphuric acid is added from the sides of test tube, a red ring indicates triterpenes.

2.3.3 Test for Saponins

Foam Test: Small amount of extract is shaken with little quantity of water, the foam produced persists for 10 minutes. It confirms the presence of saponins.

2.3.4 Test for Steroidal Saponin

The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for steroids.

Salkowski Test: Few drops of concentrated sulphuric acid are added to the plant extract, shaken and on standing; lower layer turns red in colour.

Liebermann Burchard's Test: To the extract, few drops of acetic anhydride is added and mixed well. 1 mL of concentrated sulphuric acid is added from the sides of test tube, a reddish brown ring is formed at the junction of two layers.

2.3.5 Tests for Triterpenoidal Saponin

The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for triterpenoids.

2.3.6 Tests for Alkaloids

Dragendroff's Test: The acid layer with few drops of Dragendroff's reagent (Potassium bismuth iodide) gives reddish brown precipitate.

Hager's Test: The acid layer when mixed with few drops of Hager's reagent (Saturated solution of picric acid) gives yellow coloured precipitate.

2.3.7 Test for Carbohydrates

Molischs's Test: The extract is treated with Molisch's reagent and conc. sulphuric acid along the sides of the test tube, a reddish violet ring shows the presence of carbohydrate.

Benedict's Test: The extract on heating with Benedict's reagent, brown precipitate indicates the presence of sugar.

2.3.8 Test for Flavonoids

Ferric Chloride Test: Alcoholic solution of extract reacts with freshly prepared ferric chloride solution and given blackish green colour.

2.3.9 Test for Glycosides:

Keller-Killiani Test: The extract and 0.4 mL glacial acetic acid are mixed with ferrous chloride and 0.5 mL of concentrated sulphuric acid. The acetic acid layer shows blue colour.

2.3.10 Test for Phenolic Compounds

Ferric Chloride Test: Treat the extract with ferric chloride solution then blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

Test for Chlorogenic Acid: Treat the test solution with aqueous ammonia and expose to air gradually, green colour is developed.

2.4 Quantitative Estimation of Phytochemicals

2.4.1 Total Phenolic Compounds

The total phenolics content in different solvent extracts was determined with the Folin-Ciocalteu's reagent (FCR). In the procedure, 1 mL of extract was mixed with 0.4 mL FCR (diluted 1:10 v/v). After 5 min 4 mL of sodium carbonate solution was added. The final volume of the tubes were made up to 10 mL with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenol content of the extract was expressed in terms of milligrams of catechol per gram of dry weight. Total phenolic compounds present in two crude extracts of *Sphagneticola trilobata (*roots*), Adathoda vasica(*whole *plant)* were measured from the calibration graph ($r^2 = 0.998$).

2.4.2 Total Flavonoid Content

Total flavonoid content was determined using aluminium chloride and quercetin as a standard. The plant extract (1 mL) was added to 3 mL distilled water followed by 5 % $NaNO_2$ (0.3 mL). After 5 min at 25 °C, $AlCl_3$ (0.3 mL, 10 %) was added. After further 5 min, the reaction mixture was treated with 2 mL of 1 M NaOH. Finally, the reaction mixture was diluted to 10 mL with water and the absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve ($r^2 = 0.999$) and the results were expressed as mg of quercetin equivalent per gram.

2.4.3 Total Alkaloid content

To 1 mL of extract 5 mL pH 4.7 phosphate Buffer was added and 5 mL BCG (bromo cresol green) solution mixture was shaken with 4 mL of chloroform. The extracts were collected in a 20 mL volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents. The total alkaloid content was calculated from a calibration curve ($r^2 = 0.998$) and the results were expressed as atropine equivalent per gram.

2.5 Study of Biological Activities

2.5.1 Measurement of Antioxidant Activity using DPPH Method

The antioxidant activity of the *Sphagneticola trilobata, Adathoda vasica* crude extracts was determined on the basis of their scavenging activity of the 1,1-dipheny 1,2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. 1 mL of each solution of different concentrations (1-500 µg/mL) of the extracts was added to 3 mL of 0.004 % ethanolic DPPH free radical solution. After 30 minutes the absorbance of the preparations were taken at 517 nm by a UV spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid concentrations (1-500 µg/mL) [17]. Then the % antioxidant activity was calculated by the following equation.

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$$\% \text{ Antioxidant Activity} = \frac{\text{Absorbance of blank-Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

2.5.2 Measurement of Antioxidant Activity using ABTS Method

This assay was based on the ability of different substances to scavenge *2,2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid* ($ABTS^{\cdot+}$) radical cation. The radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium per sulphate (1/1, v/v) and leaving the mixture for 4-16 hrs until the reaction was complete and the absorbance was stable. The $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.700 ± 0.05 at 734 nm for measurements. The photometric assay was conducted on 0.9 mL of $ABTS^{\cdot+}$ solution and 0.1 mL of tested samples and mixed for 45 sec; measurements were taken immediately at 734 nm after 15 min. The anti-oxidant activity of the tested samples was calculated by determining the decrease in absorbance at different concentrations by using the following equation: E= ((Ac-At)/ Ac) × 100; Where At and Ac are the respective absorbance of tested samples and $ABTS^{\cdot+}$, was expressed as µmol.

2.5.3 Measurement of Anti-Diabetic Activity

A mixture of 1 mL of alpha amylase 1 mL of plant extract in a test tube was incubated at 37 °C for 10 min. After pre-incubation, 1 mL of 1 % (v/v) starch solution was added to each tube and incubated at 37 °C for 15 min. The reaction was terminated with 2 mL DNSA reagent, placed in boiling water bath for 5 min, then cooled to room temperature, diluted, and the absorbance was measured at 546 nm [18]. The control reaction representing 100 % enzyme activity did not contain any plant extract. The % inhibition of alpha amylase by each plant extract can be calculated using the following formula.

$$\% \text{ Inhibition of } \alpha \text{ amylase} = \frac{\text{Enzyme activity of control} - \text{Enzyme activity of extract}}{\text{Enzyme activity of control}} \times 100$$

3. Results and Discussion

3.1 Phytochemical Screening

The Phytochemical screening of *the Sphagneticola trilobata*, *Adathoda vasica* crude extracts revealed the presence of phenolics, steroids, flavonoids and alkaloids, steroids, carbohydrates, saponins. The results are expressed as +ve for the presence and –ve for the absence of phytochemicals. The phytochemical screening results are shown in the Table 2.

In the Phytochemical screening of *Sphagneticola trilobata* methanolic extract shows positive for presence of phenols, flavonoids and aqueous extract shows positive for steroids, alkaloids, flavonoids, phenols, saponins, steroidal saponins, triterpenoids, triterpenoid saponins. In the phytochemical screening of crude extracts of *Adathoda vasica* methanol extracts shows positive results for biological compounds like steroids, carbohydrates, alkaloids, flavonoids, phenols and aqueous extract shows positive test for phenolics, flavonoids, alkaloids, steroids, steroidal saponins, triterpenoids, glycosides, and saponin.

Table 2 Phytochemical screening results of *Sphagneticola trilobata* and *Adathoda vasica*

S. No	Screening Tests	*Sphagneticola trilobata* (roots)			*Adathoda vasica* (whole plant)		
		Ethyl acetate Extract	Methanolic extract	Aqueous extract	Ethyl acetate Extract	Methanolic Extract	Aqueous Extract
1	Steroids						
	Salkowski	−v e	+ve	+ve	−v e	+ve	+ve
	Libermann	−v e	+ve	+ve	−v e	+ve	+ve
2	Triterpenoids						
	Salkowski	−v e	−v e	+ve	−v e	−v e	+ve
	Liberman tests	−v e	−v e	+ve	−v e	−v e	+ve
3	Saponins						
	Foam	−v e	−v e	+ve	−v e	−v e	+ve
4	Steroidal saponin						
	Salkowski	−v e	−v e	+ve	−v e	−v e	+ve
	Libermann	−v e	−v e	+ve	−v e	−v e	+ve
5	Triterpenoid saponin	−v e	−v e	+ve	−v e	−v e	+ve
6	Alkaloids						
	Dragondroffs	−v e	+ve	+ve	−v e	+ve	+ve
	Picric acid	−v e	+ve	+ve	−v e	+ve	+ve
7	Carbohydrates						
	Benedicts	−v e	+ve	+ve	−v e	+ve	−v e
	Molisch	−v e	+ve	+ve	−v e	+ve	−v e
8	Flavanoids						
	Ferric chloride (with alcoholic extract)	−v e	+ve	+ve	−v e	+ve	+ve
9	Glycosides	−v e	−v e	+ve	−v e	−v e	+ve
10	Phenols						
	$FeCl_3$	−v e	+ve	+ve	−v e	+ve	+ve
	Chlorogenic	−v e	+ve	+ve	−v e	+ve	+ve

3.2 Total Phenolic, Flavonoid, Alkaloid Contents

3.2.1 Quantitative Estimation of Phenolics

Phenolic compounds are of great importance as cellular support material because they form the integral part of cell wall structure and capture free radicals they contain hydroxyl groups these will donate hydrogen from their hydroxyl groups to free radicals and form stable phenoxy radicals and neutralize them thus preventing aging process. Phenolic compounds are more active due to lower bond dissociation energy of O-H. Higher phenolic content causes and higher cytotoxicity. Phenolics antioxidants mechanism includes termination of free radical reactions depending upon their activity with respect to ability to interfere with chain propagation reaction by rapid donation of hydrogen atom to lipid radical [19]. Bioactive polyphenols can protect the human body from the oxidative stress [20]. A higher phenolic compound shows anticancer activity by exhibit protective action against carcinogens as blocking agents.

The total phenolic contents in different solvent extracts of the *Sphagneticola trilobata, Adathoda vasica* was determined with the Folin-Ciocalteus reagent by using UV spectrophotometer and taking catechol as standard and the total phenolic content was expressed as mg of equivalent of catechol per gram. The total amount of phenolics present in different extracts is noted down in the Table 3. The methanolic extract of *Sphagneticola trilobata*, *Adathoda vasica* shows high quantity of phenolics of 68.79 mg/g, and 33.47 mg/g.

Table 3 Results of total amount of phenolics present in different extracts

S.No.	Compound	Absorbance λ_{max}		Amount (mg/g)	
		Sphagneticola trilobata (roots)	*Adathoda Vasica* (whole plant)	*Sphagneticola trilobata* (roots)	*Adathoda Vasica* (whole plant)
1	Hexane	-	-	-	-
2	Ethyl acetate	-	-	-	-
3	Methanol	0.582	0.328	68.79	33.47
4	Water	0.209	0.225	16.94	19.16

3.2.2 Quantitative Estimation of Flavonoids

Flavonoids are a group polyphenolic compounds contain a hydroxyl group in carbon position 3 and a carbonyl group in carbon position 4, poly hydroxylation of the A&B aromatic rings which influences the radical scavenging, inhibition of hydrolytic and oxidative enzymes [21]. The Flavonoids mode of action include quenching free radical, metal chelating, suppressing the enzymes associated with free radical scavenging, stimulation of internal antioxidant enzyme such as NADPH oxidise, inhibition of low density lipoproteins (LDL), inhibition of hydrolytic and oxidative enzymes (phospholipase A2, cyclooxygenase, lipoxygenase), and anti-inflammatory actions [22, 23]. They have ability to scavenge the reactive oxygen and chelate free radicals by donating hydrogen atom. Flavonoids also inhibit both cytosolic and membranal tyrosine kinase Integral membrane proteins, such as tyrosine 3-monooxygenase kinase Inhibition of these proteins results in inhibition of uncontrolled cell growth and proliferation can inhibit carcinogenesis. Flavonoids are also potent inhibitors of cell proliferation, angiogenesis, antithromboticity, infectious properties of the viruses [24]. Flavonoids are benzo-gamma pyrone derivatives of plant origin, which activate signalling metabolism and insulin sensitising in hepatic cells and adipose tissue leads to exert anti diabetic activity [25].

The total flavonoid content was expressed as mg quercetin equivalent to a gram. The total flavonoids present in the different extracts are depicted in Table 4.

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Table 4 Results of total content of flavonoids present in different extracts

S. No.	Compound	Absorbance λ_{max}		Amount found mg/g in extract	
		Sphagneticola trilobata (roots)	*Adathoda Vasica* (whole plant)	*Sphagneticola trilobata (roots)*	*Adathoda Vasica* (whole plant)
1	Hexane	-	-	-	-
2	Ethyl acetate	-	-	-	-
3	Methanol	0.958	0.589	81.59	47.10
4	Water	0.522	0.424	40.84	31.68

The methanolic extract of *Sphagneticola trilobata*, *Adathoda vasica* shows maximum quantity of flavonoids 81.59 mg/g, and 47.10 mg/g.

3.2.3 Quantitative Estimation of Alkaloids

Alkaloids are biologically active as stimulators, inhibitors, terminators of growth and controls endogenous security and regulation mechanism. Alkaloids plays important role in molecular interactions. Most alkaloids have a strong bitter taste and are very toxic for this reasons they are used by plant to protect themselves against herbivores, pests. Alkaloid represents a class of compounds which affects the central nervous system, reduces appetite, and also acts as antiparasitic, antimicrobial, antibacterial agents.

The total alkaloid content in different solvent extracts of the *Sphagneticola trilobata*, *Adathoda vasica*, was determined by using phosphate buffer and BCG (bromocresol green) and atropine as a standard. The total alkaloid content was expressed as mg atropine equivalent to a gram. The total amounts of alkaloids present in different extracts are noted down in Table 5.

Table 5 Results of total amount of alkaloids present in different extracts

S. No.	Compound	Absorbance λ_{max}		Amount found mg/g in extract	
		Sphagneticola trilobata (roots)	*Adathoda Vasica* (whole plant)	*Sphagneticola trilobata (roots)*	*Adathoda Vasica* (whole plant)
1	Hexane	-	-	-	-
2	Ethyl acetate	-	-	-	-
3	Methanol	-	0.391	-	42.22
4	Water	0.212	0.158	43.40	9.86

In the *sphagneticola trilobata* only aqueous extract shows the presence of alkaloids of amount 43.40 mg/g. The methanolic extract shows maximum quantity of alkaloids 42.22 mg/g.

3.3 Anti-Oxidant Activity

DPPH assay estimate the ability of sample to scavenge free radicals, which causes damage to natural macromolecules such as nucleic acid, polysaccharides, lipids. Natural antioxidants strengthen the endogenous antioxidant defenses against reactive oxygen species (ROS) and restore balance. Anioxidants delay autooxidation by inhibiting the formation of free radicals by scavenging species that initiates peroxidation, chelating metal to unable to generate reactive species, quenching and preventing the formation of peroxide, breaking autooxidation reaction, reducing localized o_2 concentration[26]. The presence of excess oxygen in the human body causes negative effects as it triggers radical chain reactions in the presence of reactive oxygen species. This causes aging and cell destruction. Antioxidants interrupt chain reactions to form radicals that can be removed from the human body and improving health. This protective effect is largely attributed to the presence of antioxidant phytochemical such as phenolics, flavonoids [27].

Table 6 DPPH activity of *Sphagneticola trilobata*

S. No	Concentration (µg/mL)	Ascorbic acid	*Sphagneticola Trilobata* (roots) *% of DPPH activity*		
			Ethyl acetate	Methanol	Water
1	5	12.94	-	1.07	-
2	10	22.35	-	8.45	0.96
3	15	37.11	-	22.46	7.27
4	20	56.26	2.57	56.68	18.93
5	25	75.94	4.92	76.36	43.85
6	30	77.32	8.23	77.75	52.30
7	50	89.84	19.57	79.14	65.45
8	100	91.98	41.07	88.02	72.40

The percentage of DPPH activity of *Sphagneticola trilobata* crude extracts with that of the standard ascorbic acid was shown in Table 6. The percentage of ABTS inhibition in the *Adathoda vasica* crude extracts with that of ascorbic acid shown in Table 7. In *Sphagneticola trilobata* methanol extract was found to possess an IC_{50} at 20 µg/mL in DPPH method. The standard Ascorbic acid shows DPPH IC_{50} at the 20 µg/mL (Fig. 1). In the *Adathoda vasica* the methanol extract was found to possess IC_{50} at the lower concentration of 30 µg/mL in ABTS method, while standard ascorbic acid shows ABTS IC_{50} at 20 µg/mL (Fig. 2).

Table 7 % ABTS inhibition in the *Adathoda vasica*

S. No.	Concentration µg/mL	Ascorbic acid	*Adathoda Vasica* (whole plant) *% of ABTS Activity*		
			Ethyl acetate	Methanol	Water
1	5	6.59	-	3.70	-
2	10	13.07	-	9.66	0.42
3	15	22.44	2.70	16.90	5.82
4	20	42.33	13.07	38.63	13.63
5	25	52.98	16.90	43.46	22.58
6	30	65.77	27.27	55.68	38.07
7	50	95.45	38.63	63.35	41.15
8	100	97.58	47.73	79.97	67.19

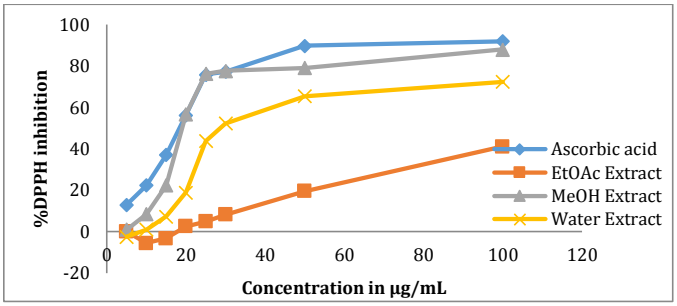

Fig. 1 % DPPH activity of *Sphagneticola trilobata*

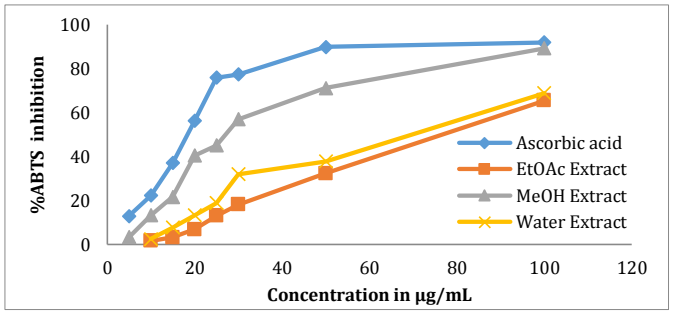

Fig. 2 % ABTS inhibition in the *Adathoda vasica*

3.4 Anti-Diabetic Activity

The anti-diabetic activity *Sphagneticola trilobata*, *Adathoda vasica, Brassica oleraceae* was determined with the application of *in vitro* α-amylase inhibition method using acarabose as a standard. Diabetes is considered a complex metabolic disorder resulting from insulin dysfunction. In diabetes the oxidative stress consists of the reduction in the antioxidant status and causes glycation of protein, inactivation of enzymes, alteration in structural function of collagen basement with age [28].

Table 8 α-amylase inhibition of *Sphagneticola trilobata, Adathoda vasica, Brassica oleraceae*

S. No	Conc. µg/mL	Standard drug Acarabose	*Sphagneticola Trilobata* (roots) % of inhibition			*Adathoda Vasica* (wholeplant) % of inhibition			*Brassica oleraceae* *% of inhibition*		
			EtOAc	MeOH	Water	EtOAc	MeOH	Water	EtOAc	MeOH	Water
1	5	14.41	-	14.08	-	-	11.17	-	-	6.59	4.35
2	10	26.93	0.45	20.56	2.90	-	21.67	3.57	3.46	10.50	7.70
3	15	39.55	7.82	26.03	9.38	1.45	29.38	9.27	7.70	18.99	11.17
4	20	53.63	14.75	33.63	18.66	9.27	41.23	18.88	14.07	29.83	16.98
5	25	70.39	20.11	42.90	26.14	15.30	54.97	30.61	20.55	38.21	20.55
6	50	78.88	25.25	48.27	35.08	25.25	63.91	41.34	30.16	53.85	38.88
7	100	83.80	42.79	59.44	52.40	35.08	77.54	59.55	43.79	74.41	64.35
8	200	90.05	52.29	88.38	63.57	44.02	88.49	70.61	63.91	78.43	76.98

Oxidative stress causes various tissue damages in the diabetic patients thus antioxidant can stimulates insulin secretion and glucose level will be decreases. The antioxidant protects the pancreatic beta cells against

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glucose toxicity too. The comparison of α-amylase inhibition of three plant crude extracts with the standard acarabose was presented in Table 8.

The antidiabetic activity *Sphagneticola trilobata*, *Adathoda vasica, Brassica oleraceae* was determined with the application of *in vitro* α-amylase inhibition method using acarabose as a standard. In the *Sphagneticola trilobata* methanolic extract shows α-amylase inhibition IC_{50} at 50 µg/mL (Fig. 3), methanolic extract of Adathoda vasica shows α-amylase inhibition at 25 µg/mL (Fig. 4), methanolic extract of *Brassica oleracea* used as a negative control showed α-amylase inhibition IC_{50} at 50 µg/mL (Fig. 5), while standard drug acarbose shows α-amylase inhibition IC_{50} at 20 µg/mL.

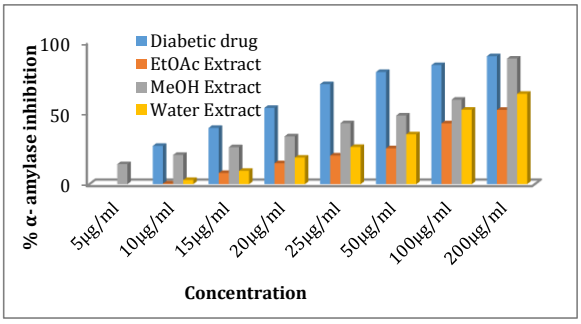

Fig. 3 α-amylase inhibition of *Sphagneticola trilobata*

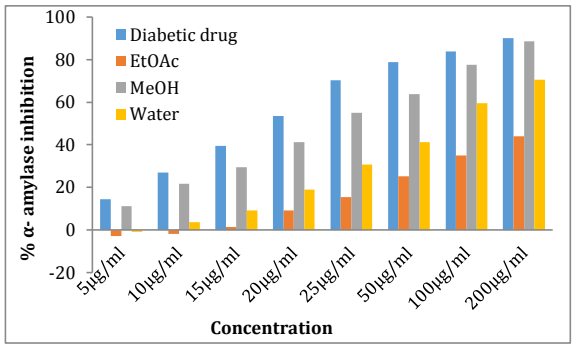

Fig. 4 α-amylase inhibition of *Adathoda vasica*

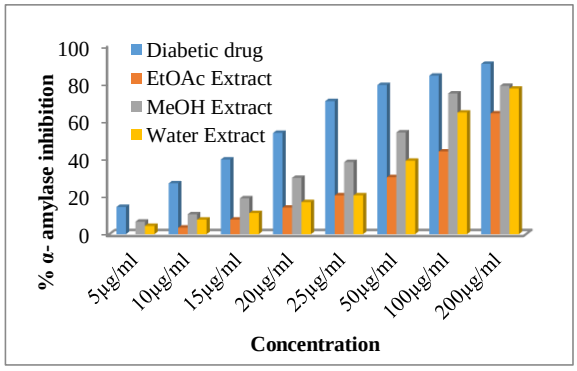

Fig. 5 α-amylase inhibition of *Brassica oleraceae*

4. Conclusion

This study resolutely proven the efficacy of methanolic extract of *Sphagneticola trilobata* had the greatest potential value as antioxidant when compared to *Adathoda vasica* antioxidant activity On the basis of response in terms of qualitative and quantitative estimation of phytochemicals followed by DPPH inhibition IC_{50} at 20 µg/mL. The methanolic extract of *Adathoda vasica* shows effective antidiabetic activity because it shows *α-amylase* inhibition IC_{50} at 25 µg/mL when compared to sphgneticola trilobata and *Brassica oleraceae.* The methanolic extract of Adathoda vasica can be better antidiabetic agent than methanolic extract of *Sphgneticola trilobata* and methanolic extract of *Brassica oleraceae* a negative control. Thus it could be concluded that *Sphagneticola trilobata (roots)* was possessed anti-oxidant potential and *Adathoda vasica (wholeplant)* proved as anti-diabetic agent.

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