

**Phytochemical Analysis And Antioxidant & Antidiabetic Activity Of  
Methanol Extracts In Diabetic Medicinal Plants Of *Andrographis  
Paniculata* And *Syzygium Cumini* (Leaves And Seed) Plant Materials.**

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**ABSTRACT**

**Objective:**

To investigate the Phytochemical and antioxidant & antidiabetic activity of different plant material extracts and to fortitude of their total phenolics and Reducing sugar level content.

**Methods:**

The extract was partitioned for possible antioxidant activities to check the free radical scavenging activity of DPPH, ABTS, FRAP. The Phytochemical analyses were checked & Antidiabetic activity ( $\alpha$ -Glucosidase and  $\beta$ -Galactosidase).

**Results:**

The different plant extract was prepared and checked the phytochemical activity specifically used for diabetic, as per phytochemical results mainly focused Alkaloids and Phenol, Acids are absence and Reducing sugar, Glycosides and protein. So, based on these results have confirmed 2 plant varieties of *Andrographis paniculata* and *Syzygium cumini*, *A. paniculate* & *S. cumini* were checked the antioxidant and diabetic activity. As per analysis report the total free radical Scavenging expression for *Andrographis paniculate* for DPPH =  $0.91 \pm 0.076$  and ABTS =  $0.76 \pm 0.083$  and FRAP = 0.07. & *Syzygium cumini* for DPPH = 0.043 and ABTS = 0.091 and FRAP = 0.08.

**Conclusion:**

The Natural and herbal plant's extract in combination can be performed the antioxidant and antidiabetic activity. Also, the extract was done phytochemical activity. Based on the results have to take up the future studies of HPLC, TLC, and product development.

**Keywords:**

Methanol Extract, Antidiabetic, Antioxidants, Plant materials.

**1. Introduction:**

The medicinal plant research has started in 1995 in these regions, India & the USA, also South Africa. This study displays which are herbal plant components have used for diabetic foot ulcers. Every year the diabetic patient's count has increased probably 12% to 15% in a year, and also is expected to hit 439 million adults by 2030. The Asian traditional medicinal plants have extended used as the main basis of the therapeutic drug's process, and the care on traditional medicine intensifying as their significant evidence that it can be a latent source for a drug to contest disease (10). The natural plant drug compounds are isolated from the medicinal plants and more efficient and might be a general consequence, have a traditional knowledge for the drug discovery (5). The Diabetic mellitus is a metabolic state and is caused abnormality level of carbohydrate, which is highly connected with low level of blood insulin (11), The low level of insulin has brought long-term damage to human organs, like the kidney, peripheral neuropathy and leg lower limbs, heart problems (15). In developed countries of Canada, USA has used medicinal plants for diabetic treatment. Many homegrown medicinal plants have used to be effectively managing the DM. The medicinal plants are easily available and very low side effects. The major source of drugs is currently derived directly from the traditional plant materials (2). This review study is reckoning some medicinal plant processing of antidiabetic activity. Thus, involved the particular plant part extraction. Generally, leaves are the very favorable storage spot for desired compounds and more than 55% of the plant's extracts for the treatment of diabetic.

**2.Plant Review:****2.1. Siritanangai leaf (*Andrographis paniculate*):**

The 3rd population trusts are highly regarded in the United States, India, Canada, and other nations, and Ayurvedic doctors of medicinal plants are thus utilised in primary healthcare hospital demands. Researchers employ and improve a variety of plant species based on advantageous chemical principles and a diversity of plant species. As a result, doing a

thorough literature search based on some plant species is necessary to keep up with the current level of knowledge. *Andrographis paniculata* (*A. paniculata*) was one of the first plant species employed in oriental and ayurvedic medicine. There are roughly 32 species in the genus *Andrographis*, which belongs to the Acanthaceae family. Only a few are well-known for their usage in traditional medicine to treat a variety of ailments. *A. paniculata* is the most important of these few. *A. paniculata*, often known as King of Bitters, is a branching annual plant that grows to a height of half to one metre. It's native to peninsular India and Sri Lanka, but it's also found in Southeast Asia, the United States, and Christmas Island. It is grown because of its well-known medicinal properties, and it thrives in a wide range of soil types. As a result, it is widely disseminated (7). In Asian countries, the plant has long been utilised in traditional medicine to treat a variety of infectious diseases and healing operations. In the plant world, this plant is also known as the King of Bitters. Researchers conducted a large-scale study using phytochemical analysis studies to determine what substances were present. It has been demonstrated that it retains a wide range of pharmacological properties (13) (6). The medicinal qualities, phytochemistry, and effects of its numerous extracts and components, such as anti-microbial, anti-oxidant & anti-infective, hepato-renal protective and liver enzymes, are the subject of this review.

## **2.2. Naval Plant seed (*Syzygium cumini*):**

*Syzygium cumini* belongs to the Myrtaceae family of myrtles, and it is endemic to tropical America and Australia. It is a genus plant that is found all over the world on a vast scale. Although the tree morphology of *S. cumini* is quite uneven. The genus has over a thousand restricted variety species, with a survey range extending from Africa and Madagascar to southern Asia. *S. cumini* is a medicinal herb that is widely utilised for a variety of treatments. Plants in this family are known to be high in unstable oils that have been used in medical applications (1), and *S. cumini* fruits and seeds have a long history of use as both nourishment and traditional medicines in various ethnobotanical practises throughout the tropical and subtropical medical world (16).

## **3. Methodology:**

### **3.1. The solvent extracts from plant parts of seed and leaves (*Syzygium cumini*) & (*Andrographis paniculate*):**

Each plant area's plant parts (leaves and seed) were manually separated and used by hand-picking method then dried in a hot air oven at 50 °C. This plant material was utilised in the

extractor of a Soxhlet apparatus after being dried into a good powder of the leaves and seed parts (20 g). Starting with Ethanol, Methanol, Aquas, Chloroform, and Acetone, the pure form of plant extract was carried out in a sequential solvent system with variable polarity solvents. In a 24-hour period, 250 ml of various solvents were used to extract the material. The extracts were lyophilized at 40 °C under reduced pressure in their respective solvent systems.

#### **4. Results & Discussion:**

##### **4.1. Phytochemical Analysis:**

The Plant extract have different type of compounds in those aerial parts of leaves and seeds and that are continuously used in the common principals. The various factors of geographical region and plant growth time & plant material drying time to processing method for the variety of chemical content. (8). Compared to the All extracts, specifically pointed the methanolic extract. The results were focused Alkaloids and Phenol, Acids are absence and the Reducing sugar, Glycosides and protein are presence also tannins and cardiac- glycosides are positive in *S.cumini* extract and in *A.paniculate* extract tannins and reducing sugar are present. Aquas and ethanol extract are the reducing sugar and protein Absent and glycosides present in siriyangai ethanol extract. In *S.cumini* Ethanol extract glycosides and protein are present and same in Aquas extract of *A.paniculate* extract shows Alkaloids and Acids are absent. Reducing sugar present and protein absent. In Naval Extract the Reducing sugar and protein also the glycosides are present. The chloroform and acetone extract shows absent in reducing sugar and protein. Based on the phytochemical analysis the ethanol and methanol extract were used for the further Antioxidant and Antidiabetic analysis.

##### **4.2. Antioxidant Activity:**

###### **4.2.1. DPPH Radical Scavenging assay**

The total free radical scavenging capacity of extracts from various plant samples was calculated using a slightly modified approach based on the stable DPPH radical, which has an

absorption maximum at 515 nm. 2.4 milligramme DPPH is dissolved in 100 mL methanol to make a radical solution. To 3.995 ml of methanolic DPPH, a test solution (5 l) was added. The mixture was vigorously shaken and stored at room temperature in the dark for 30 minutes. The reaction mixture's absorbance was measured spectrophotometrically at 515 nm. The absorbance of the DPPH radical in the absence of antioxidant, or blank, was also tested. All of the tests were carried out in duplicate. The following equation was used to calculate the ability to scavenge the DPPH radical.  $\text{DPPH Scavenged (\%)} = ((AB - AA)/AB) \times 100 \dots (1)$ , where, AB is absorbance of blank at  $t = 0$  min; AA is absorbance of the antioxidant at  $t = 30$  min. A calibration curve was plotted with % DPPH scavenged versus concentration of standard antioxidant (Trolox).

#### 4.2.2. ABTS radical scavenging assay

The ABTS radical cation decolorization assay was used to determine the free radical scavenging activity of plant materials. The reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) produced the  $\text{ABTS}^{\bullet+}$  cation radical, which was kept in the dark at room temperature for 12-16 hours before use. After diluting the  $\text{ABTS}^{\bullet+}$  solution with methanol, an absorbance of 0.700 at 734 nm was obtained. The absorbance was measured 30 minutes after the initial mixing of 5 l of plant extract with 3.995 ml of diluted  $\text{ABTS}^{\bullet+}$  solution. In each test, a suitable solvent blank was used. Every measurement was repeated at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula,  $\text{ABTS}^{\bullet+}$  scavenging effect (%) =  $((AB - AA)/AB) \times 100$  (2), where, AB is absorbance of ABTS radical + methanol; AA is absorbance of ABTS radical + sample extract/standard. Trolox was used as standard substance.

#### 4.2.3. Ferric reducing antioxidant power

The antioxidant capacity of medicinal herbs was determined spectrophotometrically using Benzie and Strain's method. The approach is based on the action of electron donating

antioxidants at low pH to reduce  $\text{Fe}^{3+}$  TPTZ complex (colourless complex) to  $\text{Fe}^{2+}$ -tripyridyltriazine (blue coloured complex). The change in absorbance at 593 nm is used to track this process. At  $37^\circ$ , the Ferric reducing antioxidant power (FRAP) reagent was made by combining 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl, and 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in a 10:1:1 proportion. Using a 1-5 ml variable micropipette (3.995 ml), freshly made working FRAP reagent was pipetted into 5 l of the correctly diluted plant sample and thoroughly mixed. When ferric tripyridyl triazine ( $\text{Fe}^{3+}$  TPTZ) complex was reduced to ferrous ( $\text{Fe}^{2+}$ ) form, a strong blue colour complex was generated, and the absorbance at 593 nm was measured against a reagent blank (3.995 ml FRAP reagent+5 l pure water) after 30 minutes at  $37^\circ$ . All of the tests were carried out in duplicate. The calibration curve was created by graphing the 593 nm absorbance vs different  $\text{FeSO}_4$  concentrations. The  $\text{FeSO}_4$  concentrations were then compared to the concentration of the typical antioxidant rolox. The FRAP values were calculated by comparing the change in absorbance in the test mixture with that obtained from increasing  $\text{Fe}^{3+}$  concentrations, and were expressed as mg of Trolox equivalent per gm of material.

### 4.3. ANTIDIABETIC ACTIVITY

#### 4.3.1. $\alpha$ -glucosidase inhibition assay.

The effect of aqueous and organic extracts of *Andrographis paniculata* and *Syzygiumcumini* aerial parts on the activity of  $\alpha$ -glucosidase enzyme in vitro was determined using Lordan's technique. A volume of 150 l of the tested extract was combined with 100 l of 0.1M phosphate buffer (pH = 6.7) containing a solution of  $\alpha$ -glucosidase (0.1U/mL) and preincubated at  $37^\circ\text{C}$  for 10 minutes. The reaction mixtures were then incubated for 30 minutes at  $37^\circ\text{C}$  with 200 l of p-nitrophenyl—D-glucopyranoside (p-NPG) (1Mm) as substrate. The reaction was stopped with 1 mL of 0.1M  $\text{Na}_2\text{CO}_3$ , and the absorbance was measured at 405 nm with a spectrophotometer. The inhibiting impact of  $\alpha$ -glucosidase was

tested using acarbose as a positive control. The inhibition of  $\alpha$ -glucosidase is determined using the formula below, which is reported as a percentage of inhibition:

$$\% \text{ of inhibition} = \frac{[(\text{DO Control} - \text{DO Control blank}) - (\text{DO Sample} - \text{DO Sample blank})] * 100}{(\text{DO Control} - \text{DO Control blank})}$$

**Control:** Consists of 150  $\mu\text{L}$  of phosphate buffer plus 100  $\mu\text{L}$  of  $\alpha$ -glucosidase enzyme solution

**Control blank:** Consists of 250  $\mu\text{L}$  of phosphate buffer

**Sample:** 150  $\mu\text{L}$  of the extracts of different concentrations plus 100  $\mu\text{L}$  of the  $\alpha$ -glucosidase enzyme solution

**Sample blank:** 150  $\mu\text{L}$  of the extracts of different concentrations plus 100  $\mu\text{L}$  of phosphate buffer.

The results are expressed as  $\text{IC}_{50}$  (concentration necessary to inhibit 50% of the enzymatic activity).

#### 4.3.2. $\beta$ -galactosidase inhibition assay.

A method previously described was used to determine the inhibitory activity of  $\beta$ -galactosidase. Specifically, 150  $\mu\text{L}$  of extract or acarbose at various concentrations were incubated for 10 minutes at  $37^{\circ}\text{C}$  with 100  $\mu\text{L}$  of  $\beta$ -galactosidase enzyme solution (1U/mL) in 0.1 mM phosphate buffer (pH 7.6). The mixture was then incubated for 30 minutes at  $37^{\circ}\text{C}$  with 200  $\mu\text{L}$  of the substrate o-nitrophenyl—D-galactopyranoside (oNPG) (1mM). By adding 1 mL of  $\text{Na}_2\text{CO}_3$ , the reaction was brought to a halt (0.1M). In parallel, a negative control with no extract was carried out. As a reference standard, different amounts of quercetin were utilised. At 410 nm, the absorbance is measured. The outcome is given as a percentage of inhibition, which was computed using the procedure below:

$$\% \text{ of inhibition} = \frac{[(\text{DO Control} - \text{DO Control blank}) - (\text{DO Sample} - \text{DO Sample blank})] * 100}{(\text{DO Control} - \text{DO Control blank})}$$

**Control:** Consists of 150  $\mu\text{L}$  of phosphate buffer plus 100  $\mu\text{L}$  of  $\beta$ -galactosidase enzyme solution

**Control blank:** Consists of 250  $\mu\text{L}$  of phosphate buffer

**Sample:** 150  $\mu\text{L}$  of the extracts of different concentrations plus 100  $\mu\text{L}$  of the  $\beta$ -galactosidase enzyme solution

**Sample blank:** 150  $\mu\text{L}$  of the extracts of different concentrations plus 100  $\mu\text{L}$  of phosphate buffer.

The result was also expressed as  $\text{IC}_{50}$  (concentration necessary to inhibit 50% of the enzymatic activity)

## RESULTS AND DISCUSSION

Medicinal plants (Botanical names)	TEAC (mg/gdw) <sup>a</sup>			(Total phenolic) <sup>b</sup>	(Total flavonoid) <sup>c</sup>
	DPPH	ABTS	FRAP		
<i>Andrographis paniculate</i>	0.91 $\pm$ 0.076	0.76 $\pm$ 0.083	0.68 $\pm$ 0.07	0.59 $\pm$ 0.03	0.94 $\pm$ 0.06
<i>Syzygium cumini</i>	0.48 $\pm$ 0.043	0.85 $\pm$ 0.091	0.59 $\pm$ 0.08	0.55 $\pm$ 0.07	1.34 $\pm$ 0.08

Table 1: to determine the DPPH, ABTS & FRAP and Total Phenol control and total

Flavonoid control

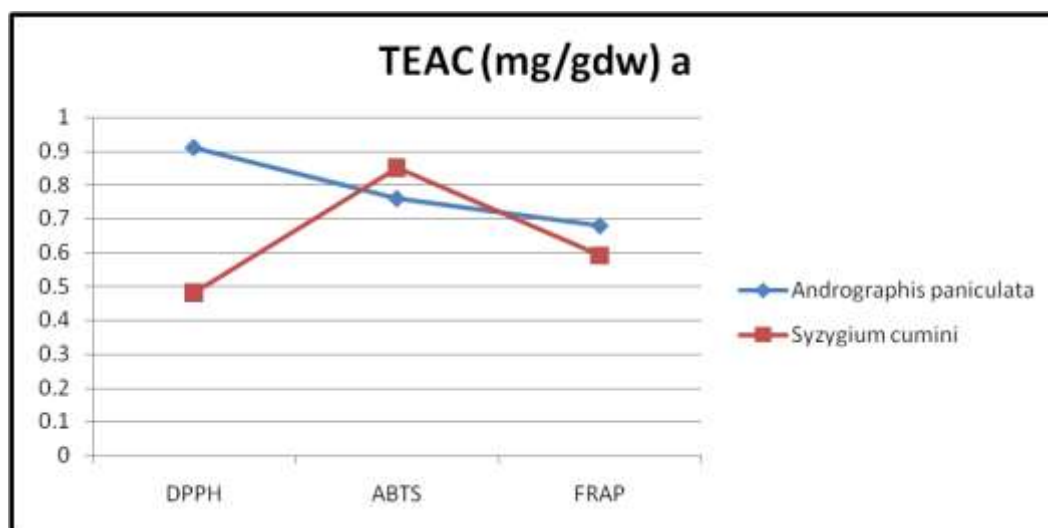
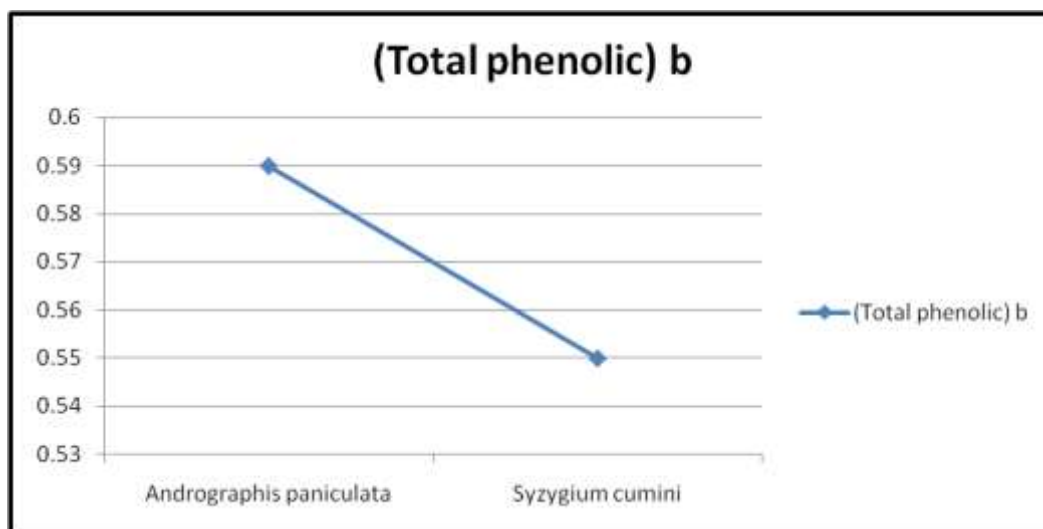
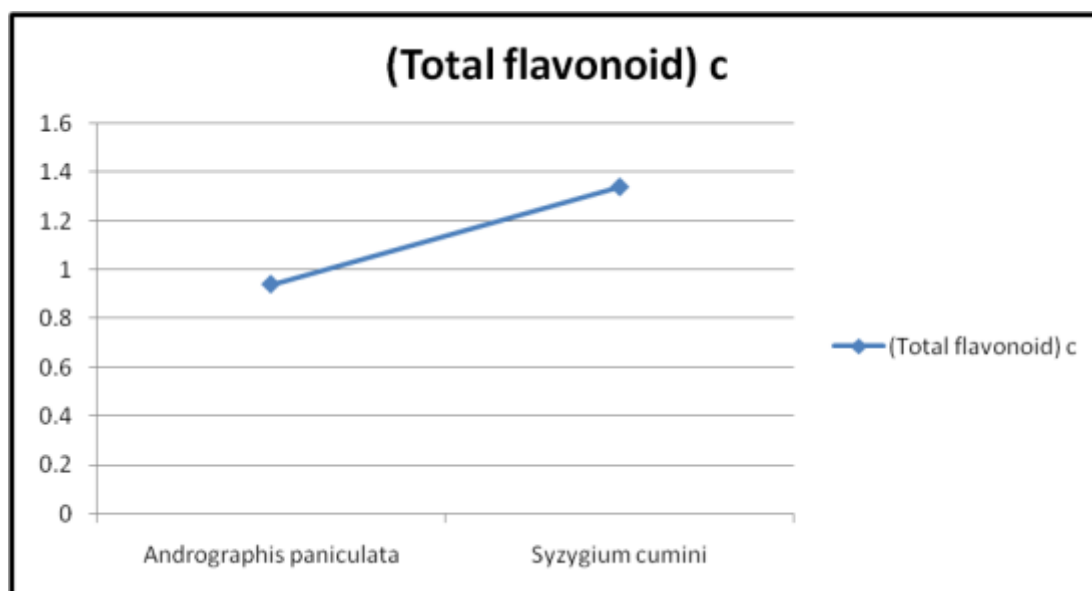




Table 2: TEAC of ration for *A.paniculate* and *S.cumini*Table 3: The total phenolic control for *A.paniculate* and *S.cumini*Table 3: The total flavonoid control for *A.paniculate* and *S.cumini*.

Extracts	Medicinal plants (Botanical names)	$\alpha$ -glucosidase (IC <sub>50</sub> in $\mu\text{g/ml}$ )	$\beta$ -galactosidase (IC <sub>50</sub> in $\mu\text{g/ml}$ )
Methanol extract	<i>Andrographis paniculate</i>	145.75 $\pm$ 1.54	498.76 $\pm$ 81.95
	<i>Syzygium cumini</i>	143.86 $\pm$ 1.65	691.54 $\pm$ 178.54
Quercetin	<i>Andrographis paniculate</i>	214.75 $\pm$ 1.87	356.76 $\pm$ 123.76
	<i>Syzygium cumini</i>	243.81 $\pm$ 1.17	754.54 $\pm$ 197.65

Acrabose	<i>Andrographis paniculate</i>	321.35±2.16	556.46±163.45
	<i>Syzygium cumini</i>	256.71±1.89	775.47±297.65

Table 4: The antidiabetic checked in both  $\alpha$ -glucosidase (IC<sub>50</sub> in  $\mu\text{g/ml}$ ) and  $\beta$  galactosidase (IC<sub>50</sub> in  $\mu\text{g/ml}$ ).

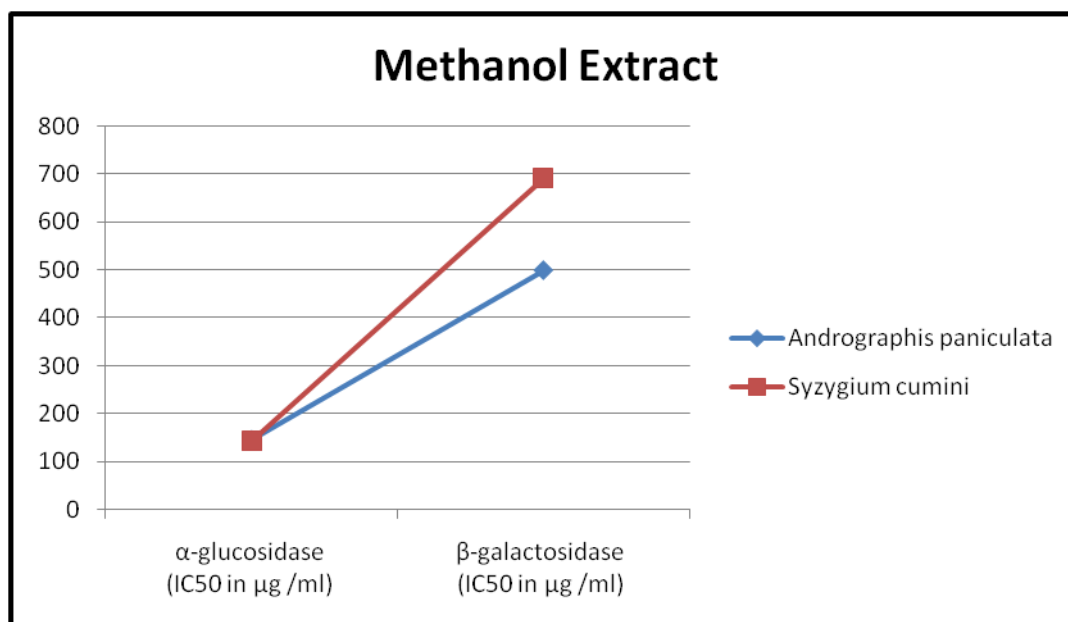


Table 5: for both methanol extract fusion of  $\alpha$ -glucosidase and  $\beta$  galactosidase per  $\mu\text{g/ml}$  the *S. cumini* & *A. paniculate*

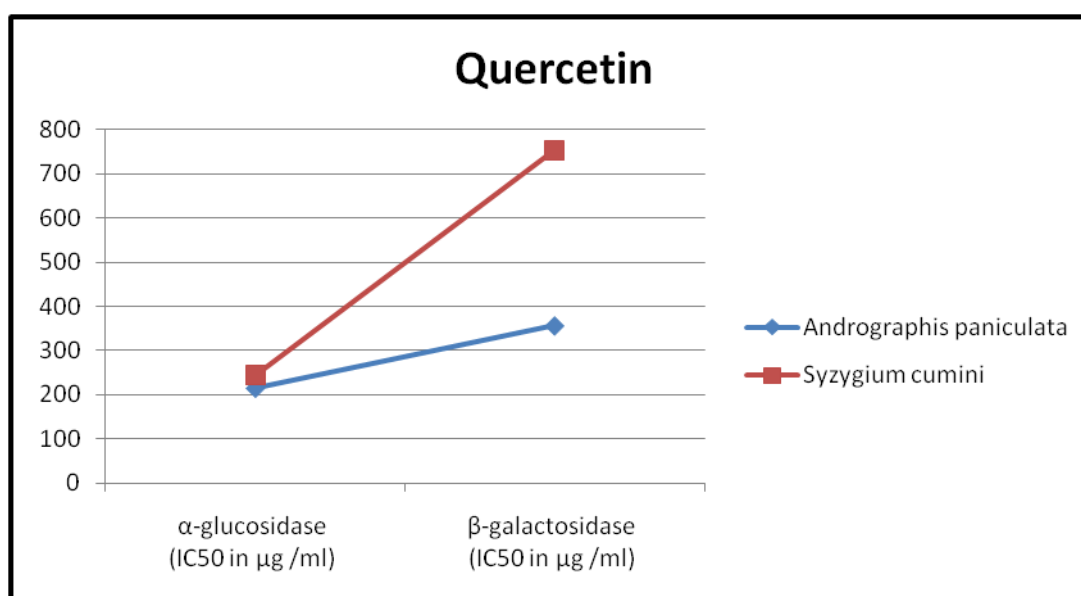


Table 6: Shows the activity of Quercetin the red and blue colour indicates the *S. cumini* and *A. paniculate*

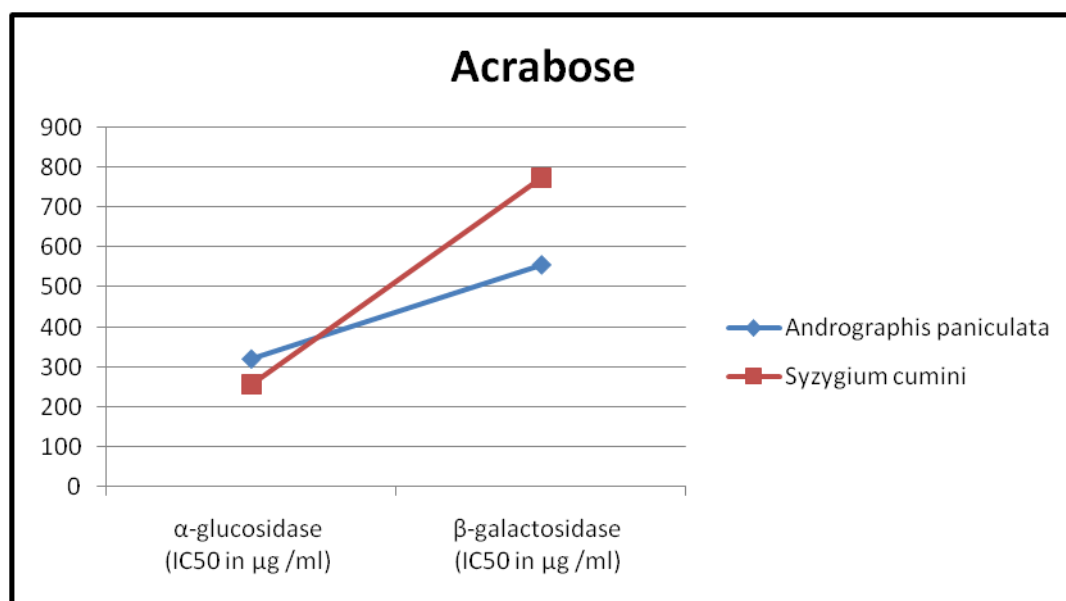


Table 7: Shows the activity of Acrabose for both plant of *S. cumini* and *A. paniculata*

## 5. Discussion & Conclusion:

*Andrographis paniculata* and *Syzygium cumini* is normally used to treat the wound healing & to treat many diseases especially diabetic and related problems. Medicinal plants are had huge level compounds which are deliberate the plant characteristics. Now a days, the medical society and siddha research studies related with diabetic were used the leaves and seeds and other plant portions. Likewise, based on these facts, the role of *A.paniculata* and *S.cumini* have used in various ayurvedic and homeopathy treatments. and also, this plant has recommended that the further research development studies & had better be done on these plants for the drug discovery.

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## References:

1. Acylated flavonol glycosides from *Eugenia jambolana* leaves. Mahmoud II, Marzouk MS, Moharram FA, El-Gindi MR, Hassan AM *Phytochemistry*. 2001 Dec; 58(8):1239-44.

2. Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber CC, Flores-Saenz JL. Study of the anti-hyperglycemic effect of plants used as antidiabetics. *J Ethnopharmacol* 1998; 61: 101-110.
3. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as measurement of “antioxidant power” The FRAP assay. *Anal Biochem* 1996;239:70-6.
4. Bouabid, K.; Lamchouri, F.; Toufik, H.; Sayah, K.; Cherrah, Y.; Faouzi, M.E.A. Phytochemical screening and in vitro evaluation of alpha amylase, alpha glucosidase and beta galactosidase inhibition by aqueous and organic *Atractylisgummifera*L. extracts. *Plant Sci. Today* 2018, 5, 103-112, <http://dx.doi.org/10.14719/pst.2018.5.3.393>.
5. K. Chandrakant, G. Arun, K. Satyajyoti, and K. Shefali, “Drug discovery from plant sources: an integrated approach,” *AYU (An International Quarterly Journal of Research in Ayurveda)*, vol. 33, no. 1, pp. 10–19, 2012.
6. Khare CP. *Andrographis paniculata*. In: Khare CP, editor. *Indian medicinal plants, an Illustrated Dictionary*. New Delhi, India: Springer; 2007. p. 2
7. Latto SK, Khan S, Dhar AK, Chaudhry DK, Gupta KK, Sharma PR. Genetics and mechanism of induced male sterility in *Andrographis paniculata* (Burm.f.) Nees and its significance. *Curr Sci*. 2006;91:515–519.
8. Li WK, Fitzloff JF. HPLC-PDA determination of bioactive diterpenoids from plant materials and commercial products of *Andrographis paniculata*. *J Liq Chromatogr Relat Technol*. 2004;27:2407–2420.
9. Lordan, S.; Smyth, T.J.; Soler-Vila, A.; Stanton, C.; Ross, R.P. The  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory effects of Irish seaweed extracts. *Food Chemistry* 2003, 141, 2170–2176, <http://dx.doi.org/10.1016/j.foodchem.2013.04.123>.
10. M. Mathew and S. Subramanian, “In vitro screening for anti-cholinesterase and antioxidant activity of methanolic extracts of ayurvedic medicinal plants used for cognitive disorders,” *PLoS One*, vol. 9, no. 1, Article ID e86804, 2014.
11. Maiti R, Jana D, Das UK, Ghosh D. Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin induced diabetic rats. *J Ethnopharmacol* 2004; 92: 85-91.
12. Maruhn, D. Rapid colorimetric assay of beta-galactosidase and N-acetyl-beta-glucosaminidase in human urine. *Clin Chim Acta* 1976, 73, 453-61, [http://dx.doi.org/10.1016/0009-8981\(76\)90147-9](http://dx.doi.org/10.1016/0009-8981(76)90147-9).

13. Mishra SK, Sangwan NS, Sangwan RS. *Andrographis paniculata* (Kalmegh): a review. *Pharmacognosy Rev.* 2007;1:283–298.
14. Ordon-Ez AA, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of *Sechiumedule* (Jacq.) Swart extracts. *Food Chem* 2006;97:452-8.
15. Pari L, Saravanan R, "Antidiabetic effect of diasulin, an herbal drug, on blood glucose, plasma insulin and hepatic enzymes of glucose metabolism in hyperglycaemic rats". *Diabetes Obes. Metab.*, 6, (2004), 286-292.
16. Reynertson KA, Basile MJ, Kennelly EJ. Antioxidant potential of seven myrtaceous fruits. *Ethnobot Res Appl.* 2005; 3:25–35.
17. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods Enzymol* 1999;299:152-78.