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SCIENTIFIC VALIDATION OF ANTIDIABETIC PLANTS OF SIKKIM HIMALAYA

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ABSTRACT

There are enormous medicinal plants in Sikkim Himalaya. Folk healers of Sikkim are using many medicinal plants to treat diabetic patients. Most of the plants are not scientifically validated. Aim of the present work was to give scientific validation of those antidiabetic plants. 52 medicinal plants were brought from the folk healers of Sikkim who claimed that they collect the plants from different parts of Sikkim Himalaya. Antidiabetic activity of the plant leaves were determined by *in vitro* assays of alpha amylase and alpha glucosidase by standard methodologies. Results showed that 44 medicinal plants out of 52 can inhibit alpha amylase and alpha glucosidase activities. Animal experiments followed by clinical trial are now needed to confirm the antidiabetic activity of the plants. Anti diabetic compounds may also be isolated from these plant leaves.

KEYWORDS: Antidiabetic plants, Sikkim Himalaya, folk healers of Sikkim, alpha amylase, alpha glucosidase.

1. INTRODUCTION

Diabetes mellitus, a chronic metabolic non-communicable disease, is increasing rapidly. In 1980 there were approximately 108 million diabetic patients in the world but in 2011 the number has been increased to 366 million. It is predicted that by 2030 the figure would have risen to 552 million. Presently highest population of diabetics are found in China, India, USA, Brazil, Mexico and Indonesia and half of diabetics of the world are from China, India and USA. In India diabetes is increasing so rapidly that the number of adults with diabetes is expected to reach 87 million by the year 2030. This high incidence of diabetes mellitus took several lives till today. Only in 2011 diabetes mellitus was the cause of death of about 3 million people worldwide.[1]

Sikkim is the smallest mountain state of India with a geographical area of only 7,096 sq.km. Sikkim is also one of the least densely populated Indian states, with only 86 persons per square kilometre. According to 2011 Census, Sikkim had only 6.11 lakh population which subsequently increased to 6.32 lakh in the year 2013. Such a small state has high incidence of diabetes. As per survey report of National Program for prevention and control of cancer, diabetes, cardiovascular diseases and stroke (NPCDCS), Sikkim has the highest number (13.67 per cent) of diabetics followed by Karnataka (9.36 per cent), Punjab (9.36 per cent), Gujarat (9.10 per cent) and Andhra Pradesh (7.42 per cent). Majority of diabetics of Sikkim are suffering from type 2 diabetes.[2] Many of them take medicinal plants from the folk healers to keep diabetes under control. Medicinal plants of Sikkim origin, not scientifically validated, but being used in type -2 diabetes mellitus are also listed in the literature.[3]

Type – 2 diabetes mellitus is characterized by postprandial hyperglycemia. One of the therapeutic approaches, therefore, is to reduce postprandial hyperglycemia.[4] This can be done by inhibiting carbohydrate splitting enzymes. Such enzymes are alpha amylase and alpha glucosidase which hydrolyze complex carbohydrates of food to free sugars. Inhibition of these enzymes could reduce hydrolysis of complex carbohydrate thereby postprandial hyperglycemia in diabetic patients may remain under control.[5] Medicinal plants are therefore investigated to check their antidiabetic activity through the inhibitions of alpha amylase and alpha glucosidase and many plants were found having the activity.[6]

In the present work leaves of 52 plants of Sikkim Himalaya were investigated for their possible alpha amylase and alpha glucosidase inhibitory activities.

2. METHODOLOGY

2.1 Collection of plant materials

52 plant leaves were collected from the folk healers of different districts of Sikkim. These leaves are being used by them in the treatment of diabetic patients. Plant leaves were authenticated by the taxonomist of the department of Botany of the University of North Bengal, Siliguri. Voucher specimens were kept in the department Biochemistry, North Bengal Medical College Sushrutanagar, Siliguri, Dist. Darjeeling, West Bengal, India for

future references. Scientific names of the collected plant leaves, their common names, families, part used and their identification numbers are listed in Tables 1 and 2.

Table – 1: Showing scientific and local names, families, part used and identification numbers of the collected plants from the folk healers of Sikkim.

Scientific name of the plants / Local name	Family	Part used	Identification No.
Abroma augustaa / Ulatkamal	Sterculiaceae	Leaves	NBMC/Bio/2011/022
Abutilum indicum / Ghantiphool	Malvaceae	Leaves	NBMC/Bio/2011/023
Acorus calamus / Bhojo	Araceae	Leaves	NBMC/Bio/2011/024
Aegle marmelous / Bael	Rutaceae	Leaves	NBMC/Bio/2011/025
Allium sativum/ Lasun	Liliaceae	Leaves	NBMC/Bio/2011/026
Aloe barbadensisc / Ghew kumara	Liliaceae	Leaves	NBMC/Bio/2011/027
Asparagus racemosusa /Kurilo	Liliaceae	Leaves	NBMC/Bio/2011/028
Actinodaphne hookeri / Runchepat	Lauraceae	Leaves	NBMC/Bio/2011/029
Bacopa monnieri / Brahmi	Scorphulariaceae	Leaves	NBMC/Bio/2011/030
Boenninghausenia al-biflorad/ Chirbirpatay	Rutaceae	Leaves	NBMC/Bio/2011/031
Berberis aristata / Sano chutro	Berberidaceae	Leaves	NBMC/Bio/2011/032
Cannabis sativa / Ganjah	Urticaceae	Leaves	NBMC/Bio/2011/033
Campylandra aurantiacad / Nakima	Liliaceae	Leaves	NBMC/Bio/2011/034
Costus speciosus / Betlaure	Zingiberaceae	Leaves	NBMC/Bio/2011/035
Catharanthus roseuse / Sada bahar	Apocynaceae	Leaves	NBMC/Bio/2011/036
Calamus rotanga / Bet	Arecaceae	Leaves	NBMC/Bio/2011/037
Cinnamomum tamalad / Sinkauli	Lauraceae	Leaves	NBMC/Bio/2011/038
Cissampelos pareiraa / Batulpatay	Lymaceae	Leaves	NBMC/Bio/2011/039
Cinnamomum tamala / Sinkauli	Lauraceae	Leaves	NBMC/Bio/2011/040
Cissampelos pareiraa / Batulpatay	Menispermaceae,	Leaves	NBMC/Bio/2011/041
Daucas carota / Gajor	Apiaceae	Leaves	NBMC/Bio/2011/042
Dalbergia sissoo / Sisau	Liguminosae	Leaves	NBMC/Bio/2011/043
Dalbergia latifolia / Sati saal	Liguminosae	Leaves	NBMC/Bio/2011/044
Eichhorria crassipes / Indra Kamal	Pontederiaceae	Leaves	NBMC/Bio/2011/045

Table – 2: Showing scientific and local names, families, part used and identification numbers of the collected plants from the folk healers of Sikkim.

Scientific name of the plants / Local name	Family	Part used	Identification No.
Ficus racemosa / Dumri	Moraceae	Leaves	NBMC/Bio/2011/046
Gynocardia odorataa / Gantay	Flacourtiaceae	Leaves	NBMC/Bio/2011/047
Girardiana heterophyllab / Bhangre sisnu	Urtica-ceae	Leaves	NBMC/Bio/2011/048
Hedera helix / Dudela	Araliaceae	Leaves	NBMC/Bio/2011/049
Ipomoea batatas / Sakarkand	Convolvulaceae	Leaves	NBMC/Bio/2011/050
Jatropha urens / Hatticane	Euphorbiaceae	Leaves	NBMC/Bio/2011/051
Kaempferia rotunda / Bhuichampa	Zingiberaceae	Leaves	NBMC/Bio/2011/052
Kydia calycina / Kubinde	Malvaceae	Leaves	NBMC/Bio/2011/053
Litsea cubebag / Siltimmur	Lauraceae	Leaves	NBMC/Bio/2011/054
Morus alba / Mul-berry	Moraceae	Leaves	NBMC/Bio/2011/055
Momordica chrantiaa / Karela	Cucurbitaceae	Leaves	NBMC/Bio/2011/056
Musa sapientum / Bankera	Scitaminaceae	Leaves	NBMC/Bio/2011/057
Nardostachys jatamansia / Jatamansi	Valerianaceae	Leaves	NBMC/Bio/2011/058
Ocimum sanctum / Tulsi	Labiateae	Leaves	NBMC/Bio/2011/059
Oroxylum indicum / Totola	Bignoniaceae	Leaves	NBMC/Bio/2011/060
Panax pseudoginseng / Panch patay	Araliaceae	Leaves	NBMC/Bio/2011/061
Picrorhiza kurrooa / Kutki	Scrophulariaceae	Leaves	NBMC/Bio/2011/062
Paederia foetida / Birilahara	Rubiaceae	Leaves	NBMC/Bio/2011/063
Potentilla fulgens / Banmula	Rosaceae	Leaves	NBMC/Bio/2011/064
Quercus lanatad / Banj	Fagaceae	Leaves	NBMC/Bio/2011/065
Syzygium cuminiie / Kyamuna	Myrtaceae	Leaves	NBMC/Bio/2011/066
Saraca asoca / Asok	Caesalpiniaceae	Leaves	NBMC/Bio/2011/067
Swertia pedicellata / Chireto	Gentianaceae	Leaves	NBMC/Bio/2011/068
*Stephania glabra*b / Tamarkay	Gentianaceae	Leaves	NBMC/Bio/2011/069
Swertia chirayita / Chireto	Menispermaceae	Leaves	NBMC/Bio/2011/070
Trigonella foenum-graecum/ Methi	Fabaceae	Leaves	NBMC/Bio/2011/071
Urtica dioica / Sisnu	Urticaceae	Leaves	NBMC/Bio/2011/072
Zingiber officinale / Adua	Zingiberaceae	Leaves	NBMC/Bio/2011/073

2.2 Test drug

Leaves of the plants were washed thoroughly under tap followed by distilled water. Leaves were then shed dried and powered. The powder, used as test drug, was stored desiccated at 4 ^{0}C until further use.

2.3 Alpha amylase inhibition assay

Alpha amylase inhibition assay of the test drug was carried out by the method described by Deguchi *et al.*[7] with slight modifications. 400 µl of 0.1 M sodium phosphate buffer (pH 7.0), 500 µl of 1% starch solution, 100 µg/ml of the test drug dissolved in DMSO and 50 µl of pancreatic α-amylase (Sigma, St. Louis, USA) solution (2 U/ml) were mixed and incubated at 37 °C for 10 min. 3 ml of 3,5-dinitrosalicylic acid (DNS) color reagent was then added. The mixture was kept in a boiling water bath for 5 min and then diluted with 20 ml of distilled water. The absorbance was recorded at 540 nm. Control sample was prepared accordingly without test drug and acted as a negative control. Acarbose was used as positive control. Inhibition capacity of test drug and acarbose were calculated as following:

Inhibition Percentage (%) = 1 − DO sample/DO control × 100.

All tests were done for five sample replications.

2.4 Alpha glucosidase inhibition assay

The α-Glucosidase inhibitory activity of the test drug was determined according to the method of Dong *et al*.[8] with slight modifications. Mixture of 100 µg/ml of the test drug in DMSO, 2.5 mM p-nitrophenyl-α-glucopyranoside (pNPG), and 0.3 U/ml of α-glucosidase in phosphate buffer, pH 6.9 was prepared. DMSO, enzyme and substrate are present in the control tube. Acarbose replaced the test drug in positive control tube. The inhibition capacity of test drug and acarbose were calculated by the following formula.

Inhibition Percentage (%) = 1 − DO sample /DO control × 100.

All tests were carried out for five sample replications.

2.5 Statistical calculation

This was done by SPSS 20. The statistical significance of enzyme inhibitions between test drugs and acarbose, the known inhibitor, was evaluated with Duncan's multiple range test (DMRT). 5% was considered to be statistically significant.[9]

3. RESULTS

Results are given in Tables 3 and 4. Acarbose, known inhibitor of alpha amylase and alpha glucosidase, in the concentration of 100 µg/ml showed 61.5% and 63.7% inhibitions in alpha amylase and alpha glucosidase activities respectively in *in vitro* experiment.

Leaves of *Abroma augusta, Aegle marmelous, Allium sativum, Aloe barbadensisc, Cannabis sativa, Campylandra aurantiacad, Costus speciosus, Girardiana heterophyllab, Nardostachys jatamansia* and *Paederia foetida*a in the concentration of 100 µg/ml showed inhibition of alpha amylase and alpha glucosidase activities in the range of 52.4 – 58.4%.

41.2-49.9% inhibitions of alpha amylase and alpha glucosidase activities were noted by the leaves of *Abutilum indicum, Acorus calamus, Bacopa monnieri, Calamus rotanga, Cinnamomum tamala, Cissampelos pareira*a, *Gynocardia odorata, Ipomoea batata, Momordica chrantia, Musa sapientum,* Ocimum sanctum, *Picrorhiza kurrooa, Quercus lanata, Urtica dioica* and *Zingiber officinale* in concentration of 100 µg/ml.

Actinodaphne hookeri, Berberis aristata, Ficus racemose, Panax pseudoginsengd and *Potentilla fulgens* leaves in the dose 100 µg/ml showed alpha amylase and alpha glucosidase inhibitory activities in the range of 32.7 – 39.2%.

22.7 – 28.5% inhibitions of alpha amylase and alpha glucosidase activities were noted by the leaves (concentration, 100 µg/ml) of *Asparagus racemosusa, Boenninghausenia al-biflorad, Catharanthus roseuse, Cinnamomum tamala, Morus alba, Oroxylum indicum, Syzygium cuminii*e and *Trigonella foenum-graecum*. *Cissampelos pareiraa* and *Litsea cubebag* leaves in 100 µg/ml concentration showed inhibitions of alpha amylase and alpha glucosidase activities in the ranges of 17.8 – 19.8%.

Leaves of *Daucas carota, Dalbergia sissoo, Dalbergia latifolia, Eichhorria crassipes, Hedera helix, Jatropha urens, Kaempferia rotunda and Kydia calycina,* however, did not show alpha amylase and alpha glucosidase inhibitory activities. All the above studies were done under *in vitro* condition.

Table – 3: Showing alpha amylase and alpha glycosidase inhibition activities of the plants under study.

Scientific name of the plants / Local name	Family	Alpha amylase inhibition (%)	Alpha glucosidase inhibition (%)
Abroma augusta / Ulatkamal	Sterculiaceae	55.5±1.2	52.9±1.0
Abutilum indicum / Ghantiphool	Malvaceae	46.2±1.4	47.8±1.1
Acorus calamus / Bhojo	Araceae	43.6±1.7	42.9±1.6
Aegle marmelous / Bael	Rutaceae	52.4±2.1	53.4±2.0
Allium sativum/ Lasun	Liliaceae	57.8±2.5	58.4±2.3
Aloe barbadensisc / Ghew kumara	Liliaceae	56.3±2.0	55.7±2.6
Asparagus racemosusa /Kurilo	Liliaceae	22.7±0.9	23.2±0.5
Actinodaphne hookeri / Runchepat	Lauraceae	37.1±0.8	39.2±0.6
Bacopa monnieri / Brahmi	Scorphulariaceae	48.5±2.0	49.9±2.1
Boenninghausenia al-biflorad/ Chirbirpatay	Rutaceae	28.5±2.3	26.8±2.6
Berberis aristata / Sano chutro	Berberidaceae	33.2±2.5	34.6±2.9
Cannabis sativa / Ganjah	Urticaceae	56.2±2.4	54.2±2.3
Campylandra aurantiacad / Nakima	Liliaceae	50.4±1.2	52.4±1.0
Costus speciosus / Betlaure	Zingiberaceae	57.2±1.1	55.2±1.8
Catharanthus roseuse / Sada bahar	Apocynaceae	22.7±1.3	23.4±1.6
Calamus rotanga / Bet	Arecaceae	41.2±1.4	43.2±1.5
Cinnamomum tamala / Sinkauli	Lauraceae	47.8±1.2	46.3±1.7
Cissampelos pareiraa / Batulpatay	Lymaceae	19.8±0.7	18.5±0.5
Cinnamomum tamala / Sinkauli	Lauraceae	26.6±0.3	25.4±0.6
*Cissampelos pareira*a / Batulpatay	Menispermaceae,	44.5±0.4	45.3±0.8
Daucas carota / Gajor	Apiaceae	0	0
Dalbergia sissoo / Sisau	Liguminosae	0	0
Dalbergia latifolia / Sati saal	Liguminosae	0	0
Eichhorria crassipes / Indra Kamal	Pontederiaceae	0	0
	Acarbose ………	**61.5**±1.2	**63.7**±1.4

Results are mean values ±SE

Table – 4: Showing alpha amylase and alpha glycosidase inhibition activities of the plants under study.

Scientific name of the plants / Local name	Family	Alpha amylase inhibition (%)	Alpha glucosidase inhibition (%)
Ficus racemosa / Dumri	Moraceae	33.3±1.7	35.7±0.4
Gynocardia odorata / Gantay	Flacourtiaceae	43.1±0.9	44.5±1.2
Girardiana heterophyllab / Bhangre sisnu	Urtica-ceae	57.2±1.3	55.9±1.5
Hedera helix / Dudela	Araliaceae	0	0
Ipomoea batata / Sakarkand	Convolvulaceae	43.7±0.5	44.5±0.6
Jatropha urens / Hatticane	Euphorbiaceae	0	0
Kaempferia rotunda / Bhuichampa	Zingiberaceae	0	0
Kydia calycina / Kubinde	Malvaceae	0	0
Litsea cubebag / Siltimmur	Lauraceae	17.8±1.1	19.1±1.6
Morus alba / Mul-berry	Moraceae	25.5±1.6	26.8±1.0
Momordica chrantia / Karela	Cucurbitaceae	45.5±1.3	43.3±1.4
Musa sapientum / Bankera	Scitaminaceae	49.2±1.9	48.4±1.3
Nardostachys jatamansia / Jatamansi	Valerianaceae	54.1±1.7	52.3±1.5
Ocimum sanctum / Tulsi	Labiateae	46.5±1.9	47.7±0.9
Oroxylum indicum / Totola	Bignoniaceae	27.7±1.3	26.5±1.5
Panax pseudoginsengd / Panch patay	Araliaceae	33.9±0.7	32.7±0.8
Picrorhiza kurrooa / Kutki	Scrophulariaceae	42.4±0.9	43.7±0.7
Paederia foetida / Birilahara	Rubiaceae	53.1±1.3	55.2±1.5
Potentilla fulgens / Banmula	Rosaceae	38.1±1.6	37.6±1.7
Quercus lanata / Banj	Fagaceae	47.8±1.5	45.9±1.4
Syzygium cuminiie / Kyamuna	Myrtaceae	27.5±1.3	26.4±1.1
Saraca asoca / Asok	Caesalpiniaceae	42.4±1.6	44.2±1.7
Swertia pedicellata / Chireto	Gentianaceae	50.7±1.5	52.4±1.8
*Stephania glabra*b / Tamarkay	Gentianaceae	44.7±1.2	42.9±1.5
Swertia chirayita / Chireto	Menispermaceae	56.1±1.8	53.4±1.7
Trigonella foenum-graecum/ Methi	Fabaceae	24.6±1.0	26.1±1.1
Urtica dioica / Sisnu	Urticaceae	45.5±1.3	47.1±1.5
Zingiber officinale / Adua	Zingiberaceae	42.8±1.7	46.9±1.8

Results are mean values ±SE

4. DISCUSSION

Alpha amylase activity of medicinal plant is known in literature. Nandhakumar *et al*. studied *in vitro* assay of alpha amylase inhibitory activity of Indian medicinal herb and observed high alpha amylase inhibitory activity in *Acalypha indica*.[10] Nickavar and Yousefian noted alpha amylase inhibitory activity of few Iranian plants.[11] Ali *et al.* demonstrated high alpha amylase inhibitory activity of some Malaysian plants used to treat diabetes.[12] Kobayashi and his coworkers noted alpha amylase inhibitory activity of some Mongolian plants.[13] Funke and Melzig observed alpha amylase inhibitory activity in many Brazelian medicinal plants.[14] Even it is reported in the literature that the terrestrial and marine natural products have alpha amylase inhibitory activity.[15] In the present study we confirmed alpha amylase inhibitory activity of 44 medicinal plants which are being used by the folk healers of Sikkim in the treatment of diabetic patients. The activities are statistically significant and comparable to that of the alpha amylase inhibitory activity of acarbose, a known inhibitor of alpha amylase.

Medicinal plants are also known for alpha glucosidase inhibitory activity. Alpha glucosidase inhibitory activity of selected Philippine plants was recorded by Lawag *et al.*[16] Plants used in traditional Chinese medicine for treatment of diabetes mellitus also showed alpha glucosidase inhibitory activity.[17] Ye *et al.* observed alpha-glucosidase inhibition from a Chinese medical herb (Ramulusmori) in normal and diabetic rats and mice.[18] Wongsa *et al.* noted potential inhibition against alpha glucosidase of culinary herbs of Thailand.[19] Screening of alpha glucosidase inhibitory activity of some Indonesian medicinal plants used in treatment of diabetes mellitus was undertaken by Mun'im and coworkers who observed that the plants had high alpha glucosidase inhibitory activity.[20] In the present study we screened 52 medicinal plants which are being used in the treatment of diabetic patients by the folk healers of Sikkim. 44 medicinal plants showed strong alpha glucosidase inhibitory activity. Values were statistically significant and comparable to that of acarbose.

Alpha amylase and alpha glucosidase inhibitory activities of acarbose and 52 medicinal plants under study are presented in figure – 1 & Figure – 2 respectively. Alpha amylase inhibitory activity of acarbose (A) was 61.5%. 12 plants of 'B' group, 17 plants of 'C' group, 5 plants of 'D' group, 8 plants of 'E' group and 2 plants of 'F' group had mean value of alpha amylase inhibitory activity 54.1%, 45.3%, 35.6%, 25.2% and 18.8% respectively. 8 plants of 'E' group did not exert alpha amylase inhibitory activity. Alpha glucosidase inhibitory activity of acarbose (A) was 63.7%. 12 plants of 'B' group, 17 plants of 'C' group, 5 plants of 'D' group, 8 plants of 'E' group and 2 plants of 'F' group had mean value of alpha glucosidase inhibitory activity 55.3%, 46.4%, 36.4%, 25.0% and 18.8% respectively. 8 plants of 'E' group, however, did not show alpha glucosidase inhibitory activity.

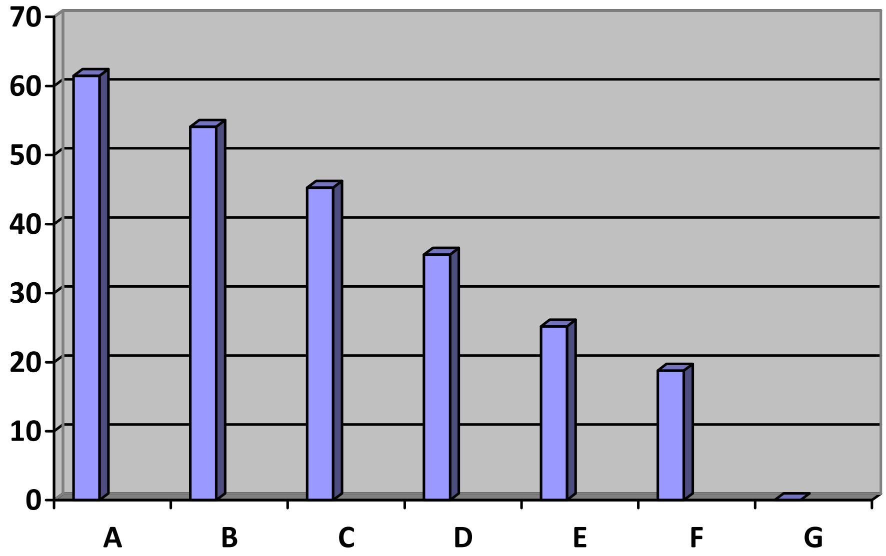

Figure – 1: Showing alpha amylase inhibitiory activity (% inhibition) of acarbose and 52 medicinal plants under study.

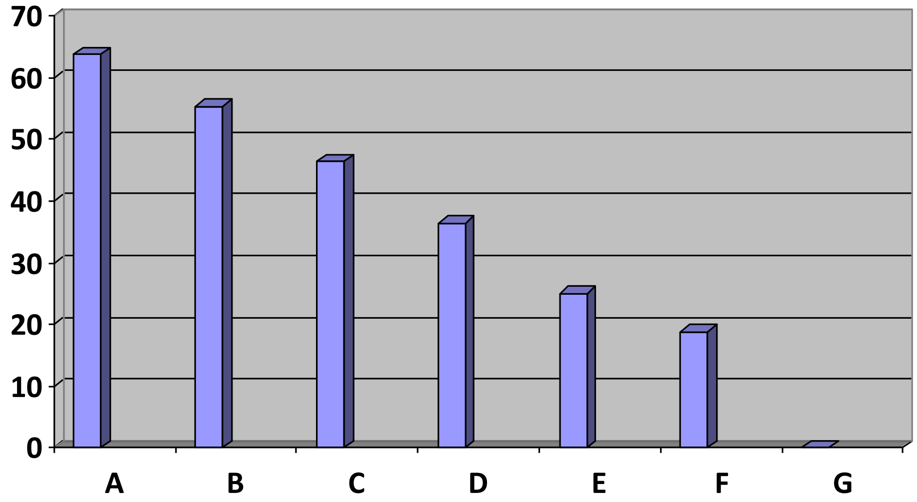

Figure – 2: Showing alpha glucosidase inhibitiory activity (% inhibition) of acarbose and 52 medicinal plants under study.

A: Acarbose B: 12 plants C: 17 plants D: 5 plants E: 8 plants F: 2 plants G: 8 plants

The present work is the scientific validation of 44 medicinal plants being used in the treatment of diabetes by the folk healers of Sikkim. Due to having alpha amylase and alpha glucosidase inhibitory activities the plant leaves possess anti diabetic property. This anti diabetic property of the plant leaves now need experimentations on animal diabetes followed by clinical trial. Presently work is going on in this direction in our laboratory.

5. CONCLUSION

The present work is the scientific validation of 44 medicinal plants of Sikkim origin as antidiabetic plants. The work, therefore, opens new avenues to the future researchers to work on these medicinal plants in experimental diabetes as well as in clinical cases. Further, work may be done on isolation of antidiabetic compound(s) from these plant leaves.

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