www.ayurpharm.com
ISSN: 2278-4772

Ayurpharm Int J Ayur Alli Sci., Vol. 3, No. 7 (2014) Pages 186 - 194

Research Article

STANDARDISATION OF A POLYHERBAL ANTI-DIABETIC AYURVEDIC MEDICINE DIAJITH

Rajalakshmy MR[1*], Asha K Maniyan[2], Sruthi CV[3], Sindhu A[4]

1. *Chemist, Research & Development Division, The Arya Vaidya Pharmacy (Coimbatore) Limited, Palakkad, Kerala, India.*
2. *Chemist, Research & Development Division, The Arya Vaidya Pharmacy (Coimbatore) Limited, Palakkad, Kerala, India.*
3. *Chemist, Research & Development Division, The Arya Vaidya Pharmacy (Coimbatore) Limited, Palakkad, Kerala, India.*
4. *General Manager, R&D and Technical, Research & Development Division, The Arya Vaidya Pharmacy (Coimbatore) Limited, Palakkad, Kerala, India.*

[Received: 15-06-2014; Revised: 02-08-2014; Accepted: 04-08-2014]

Abstract

Large number of herbal remedies is available in market for treating diabetes. Quality control and standardization of herbal medicines are two urgent needs of time. Diajith, a market available anti diabetic herbal drug, is taken for present study. This work aims to study quality aspects of Diajith and an attempt has been made to standardize the product. Physicochemical and phytochemical analysis was performed on three different batches of ingredient drugs and products using standard procedures. Quantitative determinations were done for total phenolic content, flavonoids and tannin content by UV-Visible spectrophotometric Method. Saponins were estimated by quantitative method. Thin layer chromatographic profiling was also performed on TLC Silica gel 60F254 aluminum plates for different extracts, Petroleum ether, ethyl acetate and methanol, of the product and also with standard Curcumin as marker compound**.** Results of physicochemical analysis conducted on three batches of raw herbs show slighter variations in all parameters. The physicochemical analysis and Quantitative estimations of active groups in three batches of Diajith yielded standardized values. The preliminary phytochemical analysis showed presence of similar active phytogroups in all three batches of Diajith thus by confirming its potency with about 38.90% phenols, 12.71% flavonoids, 4.03% tannins and 3.13% saponins. Thin layer chromatographic profiling of the Petroleum ether extracts, ethyl acetate extracts and methanol extracts were found similar in three batches of Diajith. The phytochemical finger print profile of the drug can serve as a guideline in ensuring the quality of the drug.

Keywords: Poly herbal; Anti-diabetic; Ayurvedic; Diajith; Standardization; Phytochemical analysis.

***Address for correspondence:**
Rajalakshmy MR,
Chemist, Research & Development Division, The Arya Vaidya Pharmacy (Coimbatore) Limited,
Kanjikode, Palakkad, Kerala, India – 678 621
E-mail: mrraji.suresh@gmail.com

Cite This Article

Rajalakshmy MR, Asha K Maniyan, Sruthi CV, Sindhu A. Standardisation of a polyherbal anti-diabetic Ayurvedic medicine Diajith. Ayurpharm Int J Ayur Alli Sci. 2014;3(7):186-194.

AYURPHARM IJAAS
www.ayurpharm.com
ISSN: 2278-4772
Ayurpharm Int J Ayur Alli Sci., Vol. 3, No. 7 (2014) Pages 186 - 194

INTRODUCTION

Diabetes mellitus is a carbohydrate metabolism disorder of endocrine system due to an absolute or relative deficiency of insulin secretion or both.[1] An Unhealthy life style has contributed to the increasing incidence of diabetes. The most prevalent form both in global and Indian scenario is the non insulin dependent diabetes mellitus (NIDDM type-2). Even though it is successfully managed by different systems of medicines herbal medicine has its acceptability due its effectiveness and reduced untoward effect. Though these herbal products have become increasingly popular, one of the impediments in the acceptance of these products is lack of standardisation. Quality assurance and standardisation of herbal medicines are the need of the hour.

Quality control and standardization of herbal medicine is a challenging task as herbal raw materials are prone to a lot of variations.[2][3][4] This variability of constituents in herbs usually occur due to environmental factors like, habitat climatic conditions, maturity of the officinal part, time of collection or harvesting and genetic variations. Standardisation of herbal medicine is the process of prescribing a set of standards or characteristics, constant parameters, qualitative, quantitative values that carry an assurance of quality, efficacy and reproducibility.[5]

Diajith an Ayurvedic medicine used for management of diabetes is taken for the present study. The ingredients of Diajith comprises of four potent Ayurvedic anti-diabetic drugs *Salacia reticulata, Tribulus terrestris, Curcuma longa and Emblica officinalis*.[6][7][8][9] Studies of the constituent ingredients confirm on their anti diabetic activity. Salacinol, a compound isolated from *Salacia reticulata* roots is reported to have anti diabetic activity.[10] Furastanol and spirostanol isolated from Tribulus terrestris are compounds having antidiabetic activity. Curlone and podocarpic acid are considered as the responsible compounds in *Curcuma longa*.[11] *Emblica officinalis* fruits are very popular anti diabetic drugs. The fruits in powder form or as water extractive and methanol extractive are reported to be very effective in the management of diabetes mellitus.[12][13] The present study aims at evaluating and standardizing the polyherbal anti diabetic formulation - Diajith, by qualitative, quantitative methods and thin layer chromatography profiling.

MATERIALS AND METHODS

Authenticated herbal samples of roots of *Salacia reticulata*, rhizomes of *Curcuma longa,* fruits of *Tribulus terrestris,* and *Emblica officinalis* were collected from the manufacturing unit. Chemicals used were of Analytical Reagent grade purchased from Nice and Merck Chemicals.

Physico chemical analysis of raw drugs

Physico chemical analysis was carried out on the raw drugs for parameters like Loss on drying, total ash, acid insoluble ash, water soluble extractive value, and alcohol soluble extractive value.[14]

Preparation of Diajith Choornam

All the four authenticated herbal ingredients were washed thoroughly with plenty of water and air dried at 60^0C. The dried samples were pulverized, sieved, and weighed. All the samples were then mixed thoroughly.[15] Three different batches of raw drugs and products were analysed for quality parameters.

Organoleptic analysis of Diajith

Organoleptic characters such as colour, smell, texture, and taste were observed.

AYURPHARM IJAAS
www.ayurpharm.com
ISSN: 2278-4772
Ayurpharm Int J Ayur Alli Sci., Vol. 3, No. 7 (2014) Pages 186 - 194

Physico chemical analysis of Choornam

Diajith was analysed for its physicochemical parameters such as Loss on drying, total ash, acid insoluble ash, water soluble extractive value, and alcohol soluble extractive value by standard methods.[16]

Qualitative Phytochemical analysis

Qualitative phytochemical tests for the identification of Carbohydrate, phenol, flavonoids, tannin, terpenoids, steroids, alkaloids, saponins, and Glycosides were carried out with alcohol extracts for three batches of Diajith by standard methods.[17][18] Phytochemical screening of the formulations is performed using the following reagents and chemicals: Fehlings solution A and B and Molisch's reagent for Carbohydrate, Phenols with 5% ferric chloride, flavonoids by lead acetate reagent, tannins with 10% alcoholic ferric chloride, steroids and terpenoids with concentrated sulphuric acid and chloroform, alkaloids with Dragendorff, Hager's, Wagners, and Mayer's reagent. Presence of saponins was checked by foam test and glycoside by Legal's test.

Quantification of chemical groups

The major bioactive groups of phytochemical, phenols, flavonoids, tannins and saponins were estimated by spectroscopy and other estimation methods as given below.

Determination of total phenolic content

The total phenolic content was determined using Folin-ciocalteu's reagent.[19][20] The methanol extract of Choornam was prepared. Appropriately diluted standard and samples were made up to 3.5ml with distilled water in a series of test tubes. These tubes were then treated with 0.5ml 2N Folin-ciocalteu's reagent and incubated for 3minutes at room temperature. The reaction was then neutralized by the addition of 1ml 20% sodium carbonate. The reaction mixture was then incubated at room temperature for 90minutes after which the absorbance was read at 760nm (Shimadzu UV Vis spectrophotometer, 1800) and the percentage phenolic content is calculated from the graph.

Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoids determination.[21] Methanol extract of the samples were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV/Visible spectrophotometer (Shimadzu UV Vis spectrophotometer, 1800) the calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 g in methanol.

Quantitative determination of tannin

The tannin content in Diajith was estimated by Folin-Denis Method.[20] Tannin like compounds reduces Phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution, the intensity of which is proportional to the amount of tannins present. The intensity is measured using UV-Vis spectrophotometer (Shimadzu UV Vis spectrophotometer, 1800) at700nm.The standard tannic acid is prepared by dissolving 100 mg of tannic acid in 100 ml of distilled water. To 100µl of appropriately diluted methanol extract, 0.5ml Folin-Dennis reagent 1ml sodium carbonate solution are added and diluted to 1ml.It was incubated for 30 minutes. The absorbance is read using Shimadzu UV-Vis spectrophotometer (1800). The percentage of tannin is calculated.

Quantitative Determination of Saponins

20g of the powder was extracted three times

AYURPHARM IJAAS
www.ayurpharm.com
ISSN: 2278-4772
Ayurpharm Int J Ayur Alli Sci., Vol. 3, No. 7 (2014) Pages 186 - 194

with distilled water and evaporated to get the residue. To 1g of the water extract taken in a round bottom flask. 10ml of 10% Hydrochloric acid in methanol was added. It was then refluxed on a water bath for three hours and then neutralized with sodium carbonate. To this was added 20ml distilled water by extraction with ethyl acetate. The ethyl acetate washings were combined and evaporated to dryness. The weight of saponins was noted and percentage was calculated

Thin layer chromatography profiling

Diajith samples were extracted successively using petroleum ether, ethyl acetate and methanol by maceration method. Each extracts were spotted in silica gel coated Aluminium backed readymade TLC Silica gel 60 F254 plates. Petroleum ether extracts of each batch were spotted and developed in TLC chamber. Solvent system used was Petroleum ether: Toluene (9:2). Ethyl acetate extracts were spotted and developed using Toluene: ethyl acetate: Formic acid (5:5:0.1) solvent system. Solvent system used for methanol extract was Ethyl acetate: Acetone: Methanol (4:3:1). Toluene: ethyl acetate: formic acid solvent system (5:4:1) was used for developing ethyl acetate extract of Diajith and marker compound Curcumin. All the plates were visualized using anisaldehyde sulphuric acid reagent.

RESULTS

The results of physicochemical analysis conducted on raw herbs are detailed in Table 1. There are slight variations among three samples in all parameters. Loss on drying gives a total measure of volatile matter and moisture content in the drug which shows less than 0.5% deviation from the mean value. Ash value is the measure of inorganic compounds in drugs. Acid insoluble ash is the inorganic matter such as silica, silicic acid etc. in the drugs. Both ash and acid insoluble ash content lies within ±0.5% deviation from mean value. The alcohol soluble extractive value and water soluble extractive value shows considerable variations compared to other parameters which show a variation of maximum ±0.94% from mean value.

Organoleptic characters are considered as important parameters as far as Ayurvedic and herbal products are considered. Diajith has characteristic colour and smell of the turmeric. The results are tabulated in Table 2.

Table 3 shows result of physicochemical analysis conducted on three batches of diajith. The ash value shows presence of good amount of inorganic matter in it. The acid insoluble matter is low. The active compounds in the medicine is quantified and represented as water soluble extractive value and alcohol soluble extractive value. Bulk density which depends on fineness and nature of the powder shows little variation. The pH of the three batches is in acidic range. Statistical analysis on the obtained results shows ±0.5 standard deviation from mean values.

Table 4 explains the results of preliminary phytochemical analysis carried out on three samples of Diajith. Except steroids all other analysed phytochemical groups shows their presence in the formulation and there is no variations among different batches. The active phytogroups such as total phenols, flavonoids, tannins and saponins were quantified and tabulated in Table 5.

The results reflect the presence of significant amount of active phytogroups which may be responsible for its activity. A batch to batch variation of ±0.15% was observed in estimations of total phenolic content, flavonoids, tannins and saponins. Figure 1 shows the TLC profiling of Petroleum ether (a), ethyl acetate (b) and methanol (c) extracts. Diajith was spotted with standard Curcumin and the finger printing profile was given as (d) in figure 1. Table 6 gives the Rf values obtained for each extracts.

AYURPHARM IJAAS
www.ayurpharm.com
ISSN: 2278-4772
Ayurpharm Int J Ayur Alli Sci., Vol. 3, No. 7 (2014) Pages 186 - 194

Table 1: Results of physicochemical analysis on raw drugs

Herbs	Sample	Loss on drying (%)	Ash (%)	Acid insoluble Ash (%)	Alcohol Soluble extractive value (%)	Water soluble extractive value (%)
Salacia reticulate	1	5.69	4.11	0.33	13.52	18.78
	2	5.79	4.23	0.29	12.98	18.56
	3	5.84	3.98	0.31	13.46	19.01
	Mean± SD	5.77±0.076	4.14±0.072	0.31±0.02	13.43±0.10	18.70±0.127
Tribulus terrestris	1	8.09	11.35	1.8	13.0	14.0
	2	9.12	10.22	1.64	11.96	12.35
	3	5.36	11.00	2.08	13.60	10.38
	Mean ±SD	7.91±0.33	10.85±0.47	1.90±0.15	13.18±0.35	12.91±0.94
Curcuma longa	1	5.16	3.50	1.04	10.72	10.96
	2	5.95	3.14	0.97	9.88	10.48
	3	6.48	4.10	1.18	10.29	11.52
	Mean ±SD	5.53±0.039	3.31±0.18	1.06±0.10	10.29±0.42	10.82±0.29
Emblica officinalis	1	10.64	2.99	0.28	23.0	48.86
	2	8.97	2.65	0.30	21.46	47.48
	3	9.51	3.02	0.32	22.68	45.76
	Mean ±SD	10.37±0..35	2.88±0.20	0.30±0..02	22.71±0.27	48.70±0.72

Table 2: Showing organoleptic characters of Diajith

Organoleptic characters	D1	D2	D3
Colour	Yellow	Yellow	Yellow
Smell	Characteristic smell of turmeric	Characteristic smell of turmeric	Characteristic smell of turmeric
Texture	Fibrous powder	Fibrous powder	Fibrous powder
Taste	Bitter & Astringent	Bitter & Astringent	Bitter & Astringent

Table 3: Results of Qualitative analysis on Diajith choornam

Sl. No.	Parameters	Results			
		D1	D2	D3	Mean±SD
1	Loss on drying (%)	4.72	3.76	4.67	4.38±0.54
2	Ash (%)	6.63	5.95	6.21	6.26±0.34
3	Acid insoluble ash	1.47	0.719	0.92	1.04±0.38
4	Alcohol soluble extractive (%)	12.2	10.03	14.29	12.17±0.91
5	Water soluble extractive (%)	19.28	20.65	21.52	20.48±0.82
6	Bulk density	0.56	0.55	0.55	0.55±0.005
7	pH	4.08	3.92	4.25	4.08±0.16

Table 4: Results of preliminary phytochemical analysis on three batches of Diajith

Sl. No.	Phytochemicals	DI	D2	D3
1	Carbohydrate	Present	Present	Present
2	Phenols	Present	Present	Present
3	Flavonoids	Present	Present	Present
4	Tannins	Present	Present	Present
5	Terpenoids	Present	Present	Present
6	Steroids	Absent	Absent	Absent
7	Alkaloids	Present	Present	Present
8	Saponins	Present	Present	Present
9	Glycosides	Present	Present	Present

AYURPHARM IJAAS
www.ayurpharm.com
ISSN: 2278-4772
Ayurpharm Int J Ayur Alli Sci., Vol. 3, No. 7 (2014) Pages 186 - 194

Table 5: Statistical analysis data for phytochemical estimations of three batches of Diajith

Sl. No.	Constituent	Sample		Population		t-value	p-value
		Mean	Std dev.	Mean	Std dev.		
D1		38.68	0.0082	38.90	0.09	29.86	0.00000
D2	Phenols	38.51	0.0079	38.90	0.09	180.19	0.00000
D3		39.5	0.0076	38.90	0.09	146.35	0.00000
D1		12.35	0.0070	12.71	0.01	106.30	0.00000
D2	Flavonoids	12.84	0.0060	12.71	0.01	26.14	0.00000
D3		12.95	0.0059	12.71	0.01	131.08	0.00000
D1		3.97	0.0068	4.03	0.06	11.19	0.00000
D2	Tannins	4.02	0.0058	4.03	0.06	19.17	0.00000
D3		4.1	0.0060	4.03	0.06	28.67	0.00000
D1		3.02	0.0038	3.13	0.12	87.00	0.00000
D2	Saponins	3.26	0.0040	3.13	0.12	45.93	0.00000
D3		3.12	0.0046	3.13	0.12	33.52	0.00000

t and p values shown above correspond to Sample pairs D1-D2, D2-D3, D3-D1.

Table 6: Showing Rf values of spots obtained for three extracts of Diajith

Sl No	Petroleum Ether extract	Ethyl acetate extract	Methanol extract
1	0.04	0.05	0.06
2	0.08	0.22	0.13
3	0.13	0.39	0.40
4	0.26	0.45	0.58
5	0.34	0.47	0.74
6	0.43	0.52	0.76
7	0.47	0.57	0.83
8	0.52	0.64	0.87
9	0.53	0.73	-
10	0.59	0.85	-
11	0.60	0.90	-
12	0.68	0.94	-
13	0.80	-	-
14	0.86	-	-

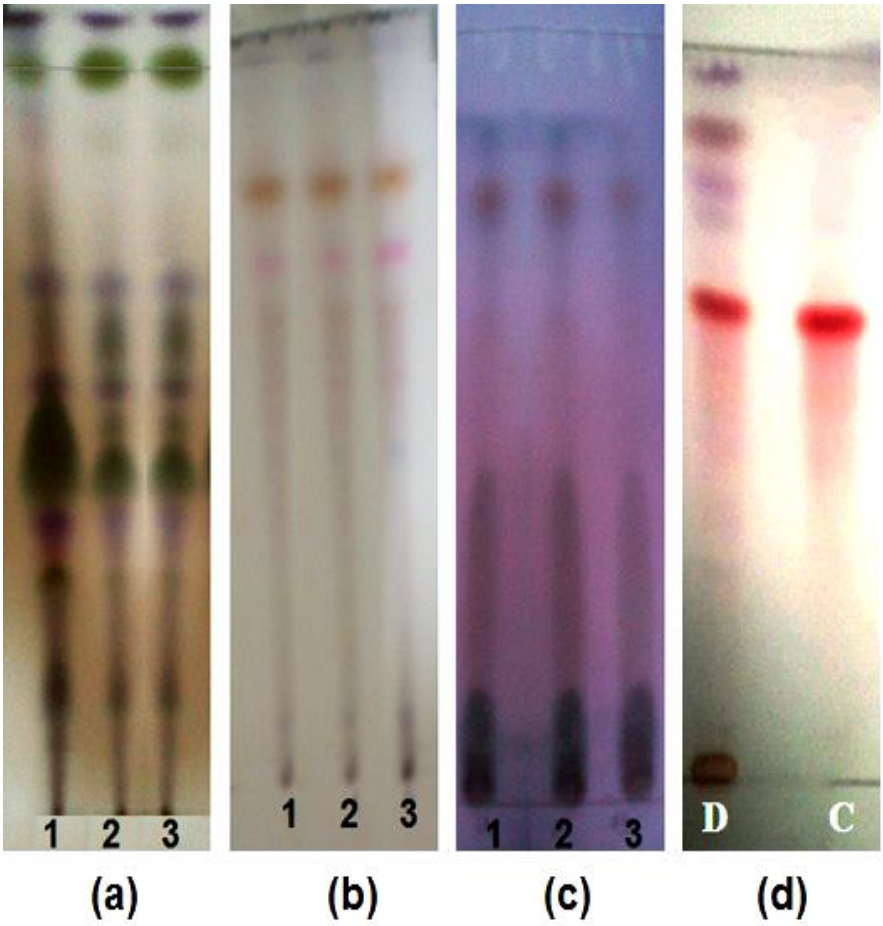
DISCUSSION

The present study reveals the qualitative aspects of the poly herbal formulation, Diajith and its ingredient herbs. The botanical authentication of the herbs for the three batches are done at the Pharmacognosy division of The Arya Vaidya Pharmacy (Coimbatore) Ltd. The authenticated samples of three batches of each herb were analysed and compared. Slight variations of less than ± 0.95% were observed among the three batches.

The variations in Loss on drying, ash value, acid insoluble ash value, water soluble and alcohol soluble extractive values may be attributed to natural variation. The three batches of each herb may be considered as standardized as three sets of parameters lie in a small range of variability.

AYURPHARM IJAAS
www.ayurpharm.com
ISSN: 2278-4772
Ayurpharm Int J Ayur Alli Sci., Vol. 3, No. 7 (2014) Pages 186 - 194

Figure 1: Showing TLC fingerprint



TLC fingerprint of extracts with (a) Petroleum ether, (b) Ethyl acetate, (c) Methanol, (d) Diajith with curcumin

Physicochemical parameters play important role in standardisation of herbal formulation. The results of which conducted on three batches of Diajith shows the loss on drying, ash and acid insoluble values in a comparable range with very small deviations from mean value. Low moisture content indicates higher stability of the formulation. Ash value indicates the amount of inorganic compounds in the medicine. Acid insoluble ash gives the amount of siliceous matter present in the sample. Water extractive values and alcohol extractive values of three samples are also comparable and these are important in the quality determination as these gives total of active constituents in the medicine. These two parameters show comparatively higher deviations. Bulk density depends on the fineness of particles and shows little variation. The lower density values indicate that the formulation is more bulky. pH value is observed to be in the acidic range indicating suitability for use.

Preliminary phytochemical profiling has special significance as it has direct bearing on the activity of the herbal drugs. The result of the preliminary phytochemical analysis shows the presence of a number of active components in the medicine. The therapeutic efficacy of the medicine may be attributed to the presence of these compounds. The three batches of formulation yielded presence of similar group of compounds in three batches.

AYURPHARM IJAAS
www.ayurpharm.com
ISSN: 2278-4772
Ayurpharm Int J Ayur Alli Sci., Vol. 3, No. 7 (2014) Pages 186 - 194

The quantitative estimations of some important and possible active components were carried out. The analytical quantitative tests yielded high percentage of phenolic content. This shows the potency of the formulation as this may impart high antioxidant and anti diabetic activity to the medicine. Flavonoids, a class of phenolic compounds were also present in the formulation in high amount. Tannins which are present abundantly in *Emblica officinalis* fruit also play its role in the efficacy of the medicine. Saponins which are considered as the active part in *Tribulus terrestris* are present in about 3 % in Diajith. Quantitative estimations yielded comparable values in three batches of samples indicating quality of raw drugs and method. Statistical analysis were carried out to find the t-values and p-values between different samples D1, D2, D3 for a 95% confidence level (ie significance for $p < 0.05$). The data is for 8 degrees of freedom.

Thin layer chromatographic finger printing was done as it serves as guideline to the phytochemical profile of the drug in ensuring the quality. It was carried on three extracts of three Diajith sample yielded exactly same fingerprinting pattern.The ethyl acetate extract of Diajith shows spot corresponding to Curcumin at Rf value 0.57.

CONCLUSION

The present study provides qualitative and quantitative aspects of an anti diabetic herbal drug Diajith. The analysis on three batches of the product yielded standardized values. The phyto chemical finger print profile of the drug can serve as a guideline in ensuring the quality of the drug.

REFERENCES

1. Alberti KG, Zimmet PZ. Definision, diagnosis and classification of diabetes mellitus and its complications part I: report of WHO consultation. Diabetic Medicine. 1998; 15: 539-553.
2. Kartik Chandra Patra, Surendra K Pareta, Ranjit K Harwansh, K Jayaram Kumar. Traditional approaches towards standardization of herbal medicine- A review. Journal of Pharmaceutical Science and Technology. 2010; 2: 372-379.
3. Songolin Li, Quanbin Han, Chunfeng Qiao, Jingzheng Song, Chuen Lung Cheng and Hongxi Xu. Chemical markers for the quality control of herbal medicine; An overview. Chinese Medicine Journal. 2008.3(7):1-16
4. Rathod S, Patel NM, Patel PM. A review on modification of analytical techniques in herbal research. International Journal of Research in Ayurveda and Pharmacy. 2011; 2: 1483-1485.
5. Kunle Oluyemisi F, Egharevha, Henry O, Abmadu PO. Standardisation of herbal medicine - A review. International J Biodiversity and conservation 2012; 4: 101-112.
6. Sivarajan VV, Indira B. Ayurvedic drugs and their plant sources. 1st ed. New Delhi: Oxford & IBH; 1994:169-171, 28.
7. Nadkarni KM. Indian Materia medica. 2nd ed. Bombay: Popular Prakashan; 1982: 414-415.
8. Akhtar M S, Ramzan A, Ali A, Ahamad M. Effect of amla fruit (*Emblica officinalis* Gaerth.) on blood glucose and lipid profile of normal subjects and type 2 diabetes patients. International Journal of food Science & Nutrition. 2011; 6: 609-616.
9. Ramamoorthy J, Vanathy, Meera R, Venketaraman SV, Devi P. Phytochemical investigation and anti-inflammatory activity of Salacia reticulate. J Chemical and Pharmaceutical Research 2010; 2: 618-625.
10. Mayasyuki Y, Toshio M, Hisashi M, Genzoh T, Osamu M. Absolute α-glucosidase inhibitor, salacinol with unique thiosugar sulfonium sulphate structure from the Ayurvedic traditional medicine salacia reticulate in Srilanka and India. Tetrahedron Letters1997; 38: 8367-8370
11. Junaid N, Priyanka P, Vikas G, Nalindupal K. Pharmacotherapeutics of Curcuma longa-A potent patent. Inter national Journal of Pharma professionals research 2010;1(1):24-33.
12. Sudha P, Remya R, Smita Z, Shobha B, Ameeta R. Evaluation of traditional Indian antidiabetic medicinal plants for human pancreatic amylase inhibitory effect invitro. Evidence- Based Complimentary and Alternative Medicine 2011; 2011:1-12.
13. Pallab Das Gupta and Amartya De, Diabetes mellitus and its Herbal treatment. International Journal of Research in Pharmaceuticals and Bio Medical Science 2012; 3: 706-721.

AYURPHARM IJAAS
www.ayurpharm.com
ISSN: 2278-4772
Ayurpharm Int J Ayur Alli Sci., Vol. 3, No. 7 (2014) Pages 186 - 194

14. Government of India. Ministry of Health and Family Welfare. The Ayurvedic Pharmacopoeia of India, part I, Vol.V, 1st ed. New Delhi: The controller of Publication; 2006. 213-214
15. Reddy RK, Kalpana A. Bhaishajya Kalpana Vijnanam. 3rd ed. Varanasi: Choukhamba Sanskrit Sanasthan; 2004. p.235-244.
16. Government of India. Ministry of Health and Family Welfare. The Ayurvedic Pharmacopoeia of India, part II, Vol.I, (Formulations). New Delhi: The controller of Publication; 2007. p.140-141.
17. Harborne JB. Phytochemical Methods: A guide to Modern techniques of plant analysis. 3rd ed, Newyork: Springer; 2008.p.41,60,92-129.
18. Rajalakshmy MR, Sindhu A. Preliminary phytochemical screening and antioxidant activity of an Ayurvedic formulation: Balarishtam. IJRAP 2011; 2:1645-1647.
19. Sadasivan S, Manickam A. Biochemical Methods. 3rd ed., New Delhi: New age international (p) Limited; 2008. p.203, 205.
20. Sruthi CV, Sindhu A. A comparison of the antioxidant property of five Ayurvedic formulations commonly used in the management of vata vyadhis. Journal of Ayurveda and Integrative Medicine 2012; 3: 29-32.
21. Chia chi chang, Ming hua yang, Hwei mei wen, Jiing chuan chern. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. J Food Drug Anal 2002; 10:178-82.

Source of Support: Nil Conflict of Interest: None Declared