Formulation and Evaluation Study of Polyherbal Formulations towards Antidiabetic, Antilipidemic and Water Intake

Dr. Sunil J. Aher [1]*, Poonam Patil [2], Ashish Vinod Dashpute [3], Dr. Kiran C. Mahajan [4], Dr. Vijaykumar S. Wakale [5], Dr. Supriya C. Joshi [6]

[1] Department of Pharmaceutics, SRES's Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, India. suniljaher@gmail.com

[2, 3] Department of Pharmaceutical Quality Assurance, SRES's Sanjivani College of PharmaceuticalEducation and Research, Kopargaon, Maharashtra, India

[4] Department of Pharmaceutics, SGMSPM's Sharadchandra Pawar College of Pharmacy, Dumbarwadi (Otur), Tal- Junnar, Dist.- Pune, Maharashtra, India

[5] Department of Pharmaceutical chemistry, Samarth Institute of Pharmacy, Belhe, Tal- Junnar, Dist.- Pune, Maharashtra, India

[6] Department of Pharmaceutics, Indira College of Pharmacy, Vishnupuri, Nanded, Maharashtra, India

Abstract: Present investigation has to undertake for estimation of Antidiabetic, antilipidemic and water intake study in Polyherbal formulation. Total flavonoid content was64.01 mg/ml which is catechin equivalent, while Total phenolic content was 142.20 mg/ml, it shown strong anti-oxidant properties are indicated by the relatively high gallic acid content. The polyherbal juice's DPPHactivity was found to be 68.45% and for polyherbal formulations A and B to be 55.04% and 58.85%. The RC50 value for the DPPH scavenging assay was reported to be 6.0 g/ml. Quantitative Analysis of Phytoconstituents in Formulation was carried out using preliminary phytochemical assays and HPTLC.

Keywords: Polyherbal juice, standardisation, DPPH, antioxidant, and anti-diabetic.

1. INTRODUCTION

Ayurveda is among the most traditional supplemental medical systems in existence.It demonstrates a close resemblance to the WHO's current concept of health and takes the most systematic approach for diseases [1][2]. The polyherbal composition consists of a mixture of herbs and plant material. It has been determined how effective antioxidants are in several conventional treatments. Free radicals have contributed to disorders like hyperlipidaemia, atherosclerosis, diabetes mellitus, arthritis, ischemia and the ensuing damage to numerous tissues, gastritis, and cancer. Radiation, toxins, poisons, fried foods, physical stress, and free radicals are immune system-depleting factors. Alterations in gene expression that result in abnormal proteins are another. Antioxidants are key elements with the ability to shield organisms from harm brought on by oxidative stress produced by free radicals. The redox characteristics of phenolics,add significantly to their antioxidant capabilities. [3]

2. MATERIALS AND METHODS

Collection, Authentication and Identification:

Methi (Fenugreek,Trigonellafoenum-graecum), Gurmar (Gymnemasylvestre), Alovera (Aloe barbadensis miller),Amla (Indian gooseberry), Tulsi (Holy Basil, Ocimumtenuiflorum), Cranberry (Vaccinium subg. Oxycoccus), TeaPlant

(Camelliasinensis), Ashwagandha(Withaniasomnifera), KiwiFruit (Actinidia deliciosa),Triphala was purchased from local market.

Chemicals:

Alloxan, Vildagliptin and Metformin (Micro Labs Ltd., Bangalore), as well as DDPH and ascorbic acid had all been purchased from Merck Limited in Mumbai.

3. ANALYSIS OF PLANT MATERIALS PURSUANT TO WHO GUIDELINES:

Preliminary phytochemical screening:

To identify different components, polyherbal juice underwent a preliminary phytochemical screening. [4][5]

Physical-chemical standards in accordance with WHO recommendations

Ashes values, extractive values, moisture content, and drying losses can all be used to detect adulteration and help set standards for herbal products. [6] [7]

Formulation of Polyherbal Juice:

Table 1: Polyherbal Juice Formulation

SR. No.	Ingredients	Quantity (gm)
1	Methi (which raises healthy HDL cholesterol levels while decreasing harmful LDL and triglyceride levels in the blood)	68.66gm
2	Kiwi Fruit (Maintain A Healthy Blood Pressure)	68.66 gm
3	Cranberry (Enriched with Vitamin C)	68.66 gm
4	Aloe Vera (lowers blood sugar levels)	68.66 gm
5	Gurmar (Regulates the Sugar Level in The Body)	68.66 gm
6	Ashwagandha (Lowers Blood Sugar and Fat)	68.66 gm
7	Emblica(Immunity-Boosting properties)	68.66 gm
8	Tulsi (Supports respiratory system)	68.66 gm
9	Triphala (Manage Blood Glucose Levels, Intestinal Infections)	68.66 gm
10	Green tea (effects on type 2 diabetes, liver disease, and weight loss)	170 gm
11	Vehicle	Q.S.1000ml

Each plant was ground into a powder and then put through a 72-hour cold maceration process with distilled water as the solvent. Filtering and adding sodium benzoate as a preservative were the next steps. The combination was then placed into a container for storage after that.[8]

Marketed Anti-diabetic Formulation A andB contain the following ingredients:

Marketed Formulation A: Jamun, Bel Patra, Amla, Methi, Karela, Kutki, Giloy, Vijaysar, Tulsi, Gudmaar, and Neem

Marketed Formulation B: Jamun, Bel Patra, Amla, Methi, Karela, Giloy, and Vijaysar

METHODS: [10-22]

0.1 gm of gallic acid was dissolved in 100 ml of ethanol to produce a gallic acid stock solution (1000 µg/ml). From this stock solution, various standard gallic acid dilutions were created. Gallic acid solutions of 20, 40, 60, 80, 100, 120, and 140 g/ml, 5.0 ml of tenfold diluted Folin-Ciocalteu reagent and 4.0 ml of sodium carbonate solution (75 g/l) were utilized to create a calibration curve. The absorbance was determined at 760 nm after 20°and 40 minutes. The absorbance was measured to determine the total phenolic compound using the formula [9,10]

$C1 \times \frac{V}{m} \frac{V}{m}$

The total amount of flavonoids:

The total amount of flavonoid has been determined using a colorimetric approach. After adding 1.5 ml of distilled water to dilute the 0.5 ml aliquot of methanolic extract with 1 ml of a 5% $NaNO_2$ solution and 1 ml of a 10% aluminium chloride solution were added. After the mixture had stood for 10 minutes, 1 ml of 1 M sodium hydroxide was added, bringing the total to 5 ml of distilled water. In contrast to the control, the solution was thoroughly mixed, and the absorbance at 510 nm was calculated right away.[10][11]

Antioxidant activity:

1 ml of juice in methanol was combined with the specified amount of DPPH solution. The reaction mixture was kept in the dark and at room temperature for 30 minutes. At 517 nm, the absorbance of the mixture was determined spectrophotometrically. The standard utilized was ascorbic acid. The potential ability of a chemical to scavenge DPPH radicals was calculated [12][13]

HPTLC

Preparation of Standard Gallic acid solution:

A proper measurement of 10 mg of gallic acid into a volumetric flask is followed by the addition of 100 ml of methanol, which raises the solution's final concentration to 100 µg/ml, to create the standard gallic acid solution. [14][15] Polyherbal juice and standard gallic acid were spotted on pre-coated silica gel 60 F plates. Ethyl acetate: toluene formic acid (20:45:20:05) was utilized as the mobile phase. The plates had been placed in the twin chamber for saturation for 15 minutes. After process the plates were air dried and scanned at 280 nm for gallic acid.[16]

Infrared Spectroscopy:

To disperse the liquid between the polished sodium chloride plates and clamp them together, the formulation was placed in between the plates. Peaks in the spectrum were detected [15][17]

Particle size analysis:

To measure the particle size of the formulation by acetone as the solvent. The method described above produces the particle size distribution's range and the mean particle diameter.[16]

Analysis of heavy metals

Heavy metals were checked by using inductively Coupled Plasma Atomic Emission Spectroscopy.[18]

Hyperglycaemia study on Experimental Animals:

Wistar rats of either sex, weighing 180–200 g, were purchase. They were kept in cages that measured 40 cm by 26 cm by 18 cm and could only hold three animals for a week. Each animal's tail was marked with a permanent marker, and labels with relevant information were adhered to the cages to distinguish each cage. Animals were housed in cages that were 30–70% relative humidified and maintained at 24 + 2°C. It was 12:12 during the light and dark cycle. All animals had unrestricted access to water and a standard pelleted laboratory animal diet [19]. Rats were given a single dosage of Alloxan monohydrate, dissolved (120 mg/kg/i.p.) in sterile saline, to cause experimental diabetes. To confirm the beginning of diabetes, blood samples were obtained 48 hours later, and glucose levels were assessed. Only animals with hyperglycaemiawere used in the experiment. [20-22]

Dosing for formulation:

For administration, suspension-based formulations were used. Animals were denied nourishment for two hours before the injection. Herbal juices were administered orally by gavage in a single dosage with a syringe attached to a cannula of the proper size. The formulation volume was 0.2 ml for a 150 g rat. The precise amount body weight measured after administering the medication. so far, the animals were fasted for an hour.

Groups of animals:

Group determination of experimental animals as follows:

Table 1: Groups of Animal for study

Group Name	Type of Animals
A	Normal
B	Control treated with vehicle (distilled water)
C	Standard treated with Vildagliptin & Metformin (10 mg/kg)
D	Sample A treated with polyherbal formulation (1.5 ml/kg)
E	Sample B treated with market formulation A (1.5 ml/kg)
F	Sample C treated with market formulation B (1.5 ml/kg)

Determination of blood Sugar:

One hour after the final dose was administered, blood samples were taken via the retro-orbital plexus. Experimental animals were made sleepy using chloroform to make drawing blood easier. Using common diagnostic kits, blood sugar was measured by an Autoanalyzer.

Statistical Findings:

The results were then tested using Dennett's t-test with P 0.05 was used asfor statistical significance. [21,22]

4. RESULTS AND DISCUSSION

Phytochemical Analysis:

It was determined whether polyherbal juice included a variety of phytoconstituents. A phytochemical examination reveals the existence of therapeutically effective phytoconstituents as well as flavonoids, phenols, alkaloids, carbohydrates, and saponins.

Physicochemical Standardization:

The following physiochemical parameters meet standards:

a. Loss on drying: 8.0%

b. Total Ash: 7.8%

c. Water Soluble Ash: 30.10%;

d. Alcohol Soluble Extractive: 35.05%.

Determination of Total phenolic content (TPC):

Total phenolic content was 142.20 mg/ml, it shown strong anti-oxidant properties are indicated by the relatively high TPC.

Determination of Total Flavonoid Content (TFC):

The total flavonoid amount has been determined to be found to be 64.01 mg/ml which is the amount catechin equivalent.

DPPH Activity:

The polyherbal juice's DPPHactivity was found to be 68.45% and for polyherbal formulations A and B to be 55.04% and 58.85%. The RC50 value for the DPPH scavenging assay was reported to be 6.0 g/ml.

HPTLC:

As the mobile phase, a mixture of formic acid, gallic acid, and ethyl acetate (25: 40: 25: 10) was utilized. The Rf values for the formulation and the marker were both found to be 0.48, indicating the fact that the formulation includes a significant amount of gallic acid.

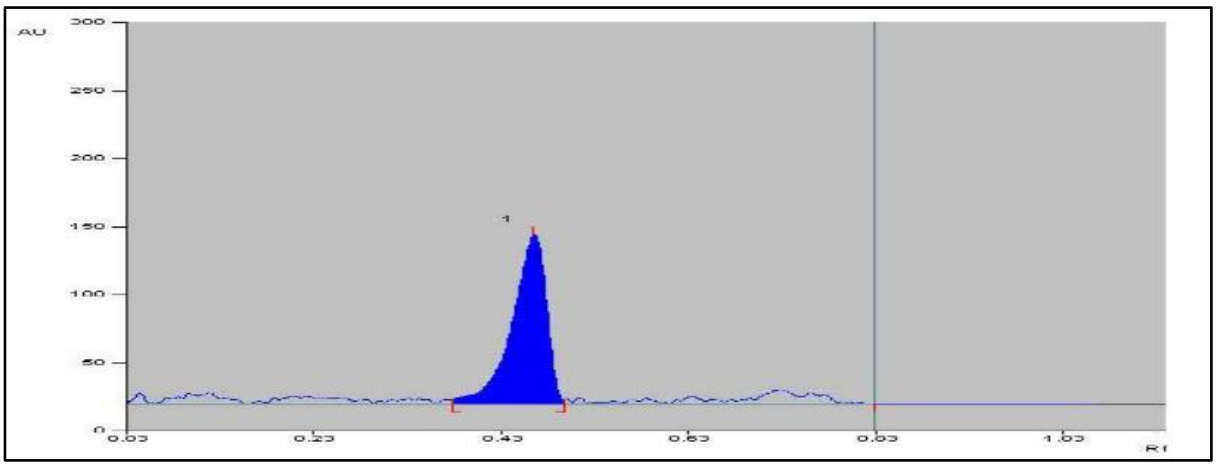

Fig 1: Chromatogramof Gallic acid

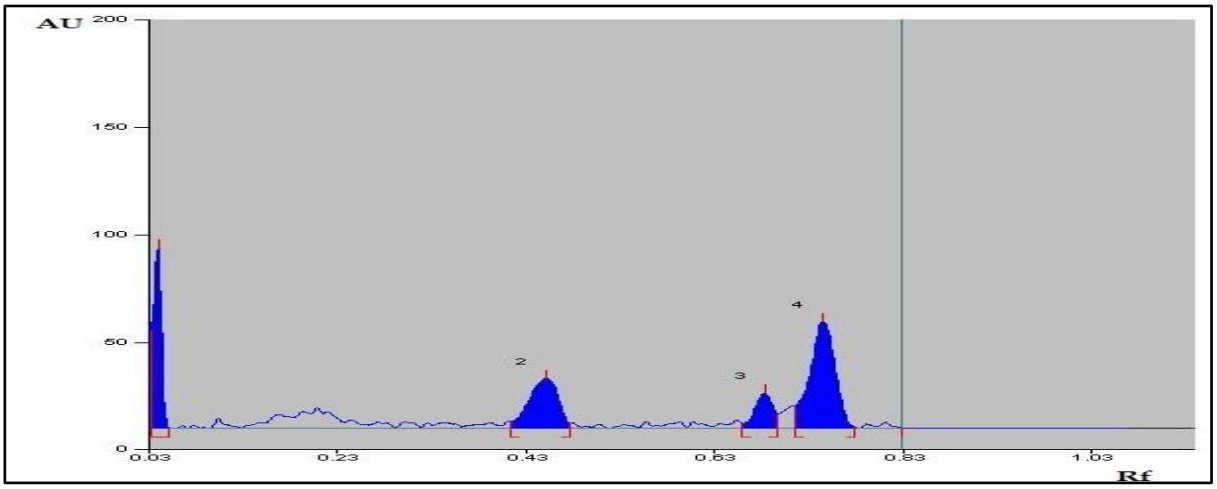

Fig. 2:Chromatogram of Polyherbal Formulation

FTIR:

FTIR spectrum was used to validate the presence of gallic acid in polyherbal juice.

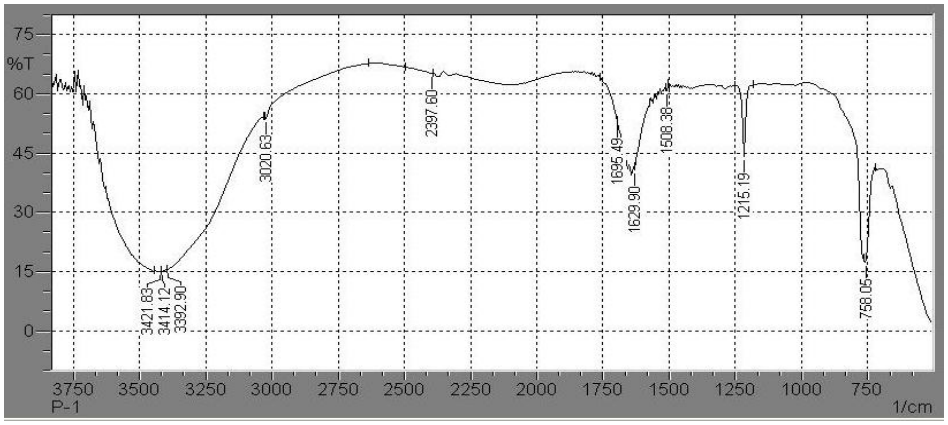

Fig 3: FTIR Spectra of polyherbal formulation

Following findings are made based on the interpretation of gallic acid's FT-IR spectrum-

A carboxylic acid's O-H stretch is 3414, alkenes' C-H stretch is 3020 (an out-of-plane bend), an aromatic ring's C=C stretch is 1629, an aromatic ring's C-H stretch is 758, and a carboxylic acid's C=O stretch is 1695. Transmittance was plotted against wavelength for the infrared spectrum.

Particle Size Analysis:

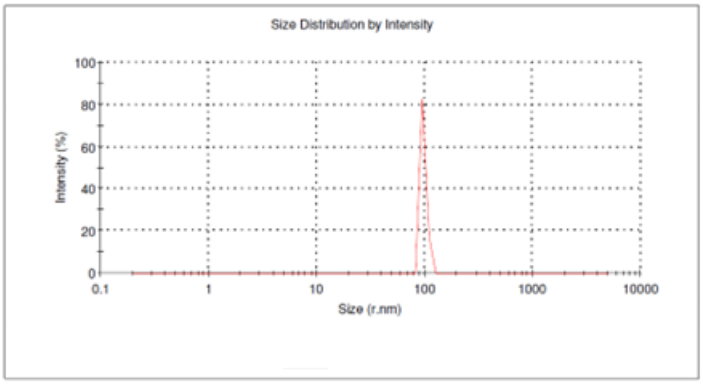

Fig 4: Particle Size Determination of formulation

The PDI with the formulation was found to be 0.950, while the particle size was found to be 1140 nm. The particle size determines the contact area that can interact with the solvent used to create the plant derivative.

Heavy Metal Determination:

ICP-AES was used to analyse a polyherbal formulation and estimate the levels of heavy metals. Heavy metals were not present in significant concentrations within World Health Organisation (WHO) guidelines(less than 0.01 ppm).

In-vivo hyperglycaemic Study:

Effects on blood glucose

The blood glucose levels that were measured in experimental and control rats at days 0, 7, 14, 21, and 28 following injections are shown in Table 3. Diabetic rats treated with alloxan exhibited blood glucose levels that were noticeably higher than control rats (p 0.01) in comparing. Once daily for 28 days, the oral administration of Vildagliptin & Metformin (10 mg/kg), Polyherbal formulation (1.5 ml/kg), Marketed formulation A (1.5 ml/kg), and Marketed formulation B (1.5 ml/kg) dosage significantly lowered blood glucose levels (p 0.01) when compared to the diabetic control group.

The mean blood glucose level reduced more in the Polyherbal treated group than it did in either of the commercial formulations, though. The stated effects could be caused by raising rat body glucose utilisation, increasing liver glycogenesis, inhibiting gastrointestinal glucose absorption, or encouraging insulin release from pancreatic cells. The polyherbal formulation also exhibited significant antioxidant activity, which may aid in the recovery of enzymatic functions, the regeneration and repair of pancreatic islets, and the reduction of liver and kidney damage. The claimed anti-diabetic efficiency may be due to the diverse varieties of medicinally beneficial components from various plants and their related modes of action. Therefore, polyherbal formulations may be useful in the treatment of metabolic disorders.

Effects on body weight, food and water intake:

The body weight the results for diabetic rats are shown in Table 4. The body weight of diabetic animals was considerably lower (p 0.01) than that of control rats on days 14, 21, and 28. Diabetic rats given Vildagliptin and Metformin (10 mg/kg) displayed substantially (p0.01) increased body weight on days 7, 14, 21, and 28. Diabetic mice were administered the Polyherbal formulation (1.5 ml/kg) on days 21 and 28, and they displayed a significant (p0.01) increase in body weight. On Day 28, the body weight of diabetic rats receiving Marketed Formulation A (1.5 ml/kg) treatment increased significantly (p0.01). On Days 14, 21, and 28, there was a substantial (p0.01) increase in body weight in diabetic animals receiving Marketed Formulation B (1.5 ml/kg).

Tables 4 and 5 display how food and water intake affected diabetic animals. Animals with diabetes generally ate and drank more than animals in good health. In all treatment groups, there was a general decrease in food and beverage consumption. Comparing treated rats to diabetic rats, it is clear that the polyherbal formulation-treated rats are consuming and utilizing glucose at levels that are normal. This is demonstrated by improvements in body weight, food

consumption, and water intake. These observed benefits could be explained by an increase in the liver's antioxidant enzymes SOD, CAT, and GSH as well as a significant drop in LPO after treatment.

Table 2: The effect of multiherbal juices on diabetics who had been induced by alloxan's blood glucose levels

Groups (n=5)	Treatment	Blood glucose concentration (mg/dl)	
		Day-0	Day-28
A	Normal	85.10 ±10.44	90.20 ±7.75
B	Control	270.05 ±13.66#	260.20 ±22.62#
C	Standard (Met.10 mg/kg)	275.50 ±9.86	125.80 ±9.07*
D	Polyherbal formulation	280.80 ±12.07	150.55 ±16.09*
E	Marketed formulation A	285.85 ±7.41	215.80 ±9.58*
F	Marketed formulation B	275.88 ±12.66	181.58 ±8.81*

Mean values ± S.D. n =5, # p < 0.01 vs. Normal; * p < 0.01 vs. diabetic control.

Table 3: The effect of multiherbal juices on the body weight of rats

Groups (n=5)	Treatment	Body weight (gm)				
		Day-1	Day-7	Day-14	Day-21	Day-28
A	Normal	168.4 ±8.87	172.0 ±6.97	179.4 ±7.59	184.0 ±4.20	189.0 ±6.76
B	Control	162.0 ±13.06	154.8 ±14.58	144.2# ±18.61	139.6# ±18.50	134# ±16.91
C	Standard (Met. 10 mg/kg)	180.2 ±4.03	177.0* ±3.34	181.6* ±6.72	187.4* ±4.33	188* ±2.44
D	Polyherbal formulation	168.6 ±10.35	168.6 ±10.78	164.4 ±12.73	168.8* ±12.37	173* ±11.76
E	Marketed formulation A	159.8 ±12.87	156.4 ±13.75	150.8 ±13.27	154.2 ±11.90	160.4* ±11.45
F	Marketed formulation B	176.2 ±8.52	172 ±9.92	172.8* ±9.83	176.4* ±10.01	179* ±9.38

The readings are mean values with standard deviation (SD), n = 5, p 0.01 vs. normal, and p 0.01 vs. diabetes control.

Table 4: Study on Food intake

Groups (n=5)	Treatment	AVERAGE FOOD INTAKE (gm)				
		Day-0	Day-7	Day-14	Day-21	Day-28
A	Normal	7.7	9.5	8.7	12.5	9.8
B	Control	8.5	11.2	16.8	18.5	16.6
C	Standard (Met. 10 mg/kg)	8.8	12.8	14.8	12.8	12.6
D	Polyherbal formulation	7.6	10.2	12.8	14.8	13.3

Groups (n=5)	Treatment	AVERAGE FOOD INTAKE (gm)				
		Day-0	Day-7	Day-14	Day-21	Day-28
E	Marketed formulation A	7.0	12.8	14.6	13.6	14.6
F	Marketed formulation B	9.7	8.8	10.4	12.6	14.8

Table 5: Study on Water intake

Groups (n=5)	Treatment	WATER INTAKE (ml)				
		Day-0	Day-7	Day-14	Day-21	Day-28
A	Normal	16.6	15.8	18.2	17.2	18.0
B	Control	28.0	22.4	26.6	20.0	26.4
C	Standard (Met. 10 mg/kg)	20.8	19.4	20.8	16.6	15.0
D	Polyherbal formulation	15.8	16.4	18.4	17.8	16.4
E	Marketed formulation A	18.6	16.4	18.8	17.8	18.0
F	Marketed formulation B	16.6	16.0	18.4	14.8	14.2

5. CONCLUSION

The polyherbal mixture exhibits impressive antioxidant and antidiabetic effects, according to recent investigations. The discovered polyherbal mixture may have phenolic and flavonoid components, which is why there is an anti-oxidant effect. The commercial formulations A and B and the polyherbal formulation were contrasted, and it was shown that the latter had more antioxidant and antidiabetic potential. In a study on animals, it was discovered that the polyherbal formulation may have helped diabetic rats gain weight (p-0.01) and maintain normal food and water intake. Polyherbal formulation has the potential to be beneficial in the treatment of diabetes because of its high antidiabetic and antioxidant effects.

6. REFERENCES

[1] LayeeqShaizi, Harisha C. R, Thakkar. A. B. A Detailed Comparative Pharmacognostical Evaluation of Wild and Cultivated varities of *Phyllanthus emblica*Linn fruits. Uni J Pharm 2013; 2: 71-76.

[2] Nirmal Kumar and Anil Kumar Singh. Phalatrikadikvatha. An Ayurvedic Hepatoprotective Drug. Int J Res Pharm Chem2013;3:123-136

[3] LayeeqShaizi, Harisha C. R, Thakkar. A. B. A Detailed Comparative Pharmacognostical Evaluation of Wild and Cultivated varities of *Phyllanthus emblica*Linn fruits. Uni J Pharm 2013; 2: 71-76.

[4] Disha Arora, Richa Shri, Sourav Sharma and Ashish Suttee. Phytochemical and Microscopical Investigations on *Emblica officinalis* Gaertn. Int JPharmacognPhytochem Res.2012; 4: 1-4

[5] Ranjita Saryam, ChandrablanSeniya, Shagufta Khan. Physico-chemical and Preliminary Phytochemical Screening of *Hemidesmusindicus.*J Chem and Pharm Res 2012; 4: 4695-4697.

[6] Manjulika Yadav, Sanjukta Chatterji, Sharad Kumar Gupta and Geeta Watal. Preliminary Phytochemical Screening of six medicinal plants used in Traditional Medicine. Int J Pharm Pharm Sci2014; 6:145-153

[7] Maithani Jyoti, Agrawal Kshitij, Sharma Vivek, Saini Prem. Preparation and Standardization of a Polyherbal Formulation. J Adv Sci Res. 2012; 3: 84-85.

[8] Amita Pandey, Shalini Tripathi. Concept of Standardization, Extraction and Pre-phytochemical Screening Stratergies for herbal drugs. J PharmacognoPhytochem. 2014; 2: 115-119.

[9] Ganesh N Sharma, Susheel K Dubey, Nitin Sati, Jyotsana Sanadya. Phytochemical screening and Estimation of Total Phenolic Content in *Aegle marmebs* seeds. Int J Pharm Clinical Res. 2011; 3:27-29

[10] Singleton VL, Rossi JA. Colorimetry of Total phenolics with phosphomolybdic- phosphotungstic acid reagents. Am J Enol. Vittie. 1965; 16:144-158.

[11] Kalita Pallab, Barman Tapan K, Pal Tapas K, Kalita Ramen. Estimation of Total Flavonoid Content and Antioxidant activities of Methanolic whole plant extract of Biophytumsensitivum Linn. J Drug Deliv Therap 2013; 3: 33-37.
[12] Adeolu A Adedapo, Florence O Jimoh, Anthony J Afolayan and Patrick J Masika. Antioxidant properties of the Methanol Extracts of the Leaves and stems of Celtis africana. Rec Nat Prod 2009; 3: 23-31.
[13] R. Vijayalakshmi and R. Ravindhran. HPTLC Method for Quantitative determination of Gallic acid in ethanolic root extracts of Diospyrus ferrea Bakh and AervalanataJuss. Ex Schultes- A Potent Indian Medicinal Plants. Asian J Pharm Clinical Res 2012; 5 :170-174
[14] M S Kondawar, K G Kamble, D S Mali. Quantitative Estimation of Gallic acid and Ascorbic acid in a marketed herbal medicine: Kivi Fruitchurna by HPTLC. Int J Pharm Tech Res. 2011; 3:1593-1599.
[15] Entessar H A, Al-Mosawe and Iman I Al Saadi. The Extraction and Purification of Gallic Acid from the Pomegranate Rind. Al-MustanisinyahJ. Sci 2012; 23 :184-192
[16] Pulok K Mukharjee, Sujay Rai, Sauvik Bhattacharyya, Pratip Kumar Debnath, Tubin Kanti Biswas, Utpalendu Jana, Srikanta Pandit, Bishnu Pada Saha and Pradip K Paul. Clinical Study of Kivi Fruit- A well-known phyto medicine from India. Iran J Pharmacol Therap.2006; 5: 51-54.
[17] R. Mohankumar, K Ilango, K Ananth Kumar, N Kasthuri Bai, G P Dubey and Aruna Agrawal. Estimation and Validation of Gallic acid in Polyherbal Formulation by HPTLC. Int J Pharm Pharm Sci. 2013; 5:203-206
[18] Lakshmi. T, Rajendran R. Antony Silverster. Evaluation of Heavy metals in ethanolic extract of Accacia catechu as indicator of pollution by Atomic Absorption Spectrophotometric Analysis. J Pharm Biol Sci. 2014; 4:129-134
[19] Ankur Rohilla and Shahjad Ali. Alloxan Induced Diabetes: Mechanisms and Effects. Int J Res Pharm Biomed Sci. 2012; 3:819-823
[20] Rajaram K. Antioxidant and Anti-diabetic activity of Tectona grandis Linn. In alloxan induced albino rats. Indian J Pharm Clin Res 2013; 6:923-927
[21] Julio Campos-Florian, Jessica Bardales-Valdivia, Lilliana Caruajulca-Guevara and Deisy Cueva-Lianos. Anti diabetic effect of *Coffea Arabica* in alloxan induced diabetic rats. Emir J. Food
[22] Snehal Salunke, Vishal Pande, Prakash Kendre, Somnath Vibhute. "Effect of Standardized Polyherbal Formulations on Blood Glucose Body Weight Food and Water Consumption of Rats", Pharmaceutical Sciences, 2015.

DOI: https://doi.org/10.15379/ijmst.v10i4.2400