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Formulation and evaluation of ayurvedic anti-diabetic drug

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Abstract
Herbal formulations are the need of the day for better acceptance of Herbalmedicine. And herbal formulations have growing demand in the world market and the plants have been reported in the literature having good anti-diabetic, anti-microbial, anti-oxidant and anti-inflammatory activity. Diabetes mellitus (DM), insulin-dependent DM (IDDM) and non-insulin dependent DM (NIDDM) is a common and serious metabolic disorder throughout the world. Traditional plant treatments have been used throughout the world for the therapy of diabetes mellitus. Among many medications and polyherbal plants, several herbs have been known to cure and control diabetes; additionally they have no side effects. In this study vati was formulated based on the anti-diabetic potential of herbals and its evaluation. Vati is a herbal formulation containing Giloy (*Tinospora Cordifolia*), Neem (*Azadirachta Indica*), Chirayata (*Swertia Chirata*), Gurmar (*Gymnema Sylvestre*), Ashwagandha (*Withania Somnifera*), Gokshura (*Tribulus Terrestris*), Haritaki Chhoti (*Terminalia Chebula*), Bahera (*Terminalia Bellirica*), Amla (*Emblica Officinalis*), Bilva (*Aegle Marmelos*), Kachur (*Curcuma Zedoaria*), Vasaka (*Adhatoda Vasica*), Haldi (*Curcuma Longa*) Kutki (*Picrorhiza Kurroa*), Jamun (*Syzygium Cumini*) Shuddha Shilajit (Asphaltum), Karela (*Momordica Charantia*), Methi (Trigonella foenum-graecum), Malabar tree (*Pterocarpus Marsupium*) widely used in treatment of diabetes. The present study was taken up to scientifically evaluate the various physicochemical parameters to standardize the formulation. To evaluate various physicochemical parameters including ash values, moisture content, extractive values,chemical test, microscopy thin layer chromatography (TLC) for standardization of VatiIngredients were identified. Physicochemical standards were loss on drying, pH, ash values, extractive values documented. Qualitative chemical tests designated the presence of alkaloid, glycoside, tannins, and steroids.

Keywords: herbal vati, *adhatoda vasica*, *Curcuma Longa*, physicochemical test

1. Introduction
Diabetes is a lifelong (chronic) disease and it is a group of metabolic disorders characterized by high levels of sugar in blood (hyperglycemia). [1] More than 230 million people worldwide are affected, and it is expected to reach 350 million by 2025. Globally the affected people are unaware of the disease and only half receive adequate treatment [2]. It is caused due to deficiency of insulin or resistance to insulin or both. Insulin is secreted by β-cells of pancreas to control blood sugar level [1]. Blurry visions, excess thirst, fatigue, frequent urination, hunger and weight loss are some of the symptoms commonly seen in diabetic patients [3].

1.1. Types of Diabetes
Diabetes results in the impairment of the body's ability to use food because either the pancreas does not make insulin or the body cannot use insulin properly. Hypoglycemia (low blood glucose) is most commonly seen in diabetic patients, when the body gets too much insulin, too little food, a delayed meal, or more than the usual amount of exercise. When the body gets too little insulin, too much food, or too little exercise, it results in hyperglycemia (high blood glucose) [4, 5, 6].

1.1.1 Type-1 diabetes (T1D)
The type 1 diabetes mellitus (T1DM) is a multifactorial autoimmune disease characterized by chronic hyperglycemia and by the development of specific vascular alterations. Autoimmune destruction of β-cell by T-cells is responsible for T1DM which results in severe insulin depletion. It is also known as juvenile diabetes [1, 9].

1.1.2. Type-2 diabetes (T2D)

Type 2 diabetes mellitus (T2DM) is a chronic disease characterized by insulin resistance, which leads to hyperglycemia. The key features of type 2 diabetes is insulin resistance associated with obesity due to the release of free fatty acids (FFA) and the release of inflammatory cytokines from the expanded adipose tissue mass. The decreased ability of insulin to regulate glucose metabolism is known as insulin resistance. Intracellular lipid accumulation occurs due to increased import of FFA into non-adipose tissues [9].

1.1. Factors Causing Diabetes

T1DM is mainly triggered by environmental factors. The main factors that contribute to the development of insulin resistance (T2DM) include obesity, physical inactivity, and smoking [12]. The prevalence of diabetes mellitus is increasing due to urbanisation, westernisation and their associated lifestyle changes (nutritional habits, lack of adequate dietary intake and low physical activity) accompanied by obesity, and low socioeconomic level [13,14]. Body weight is one of the most important modifiable risk factors in T2DM. Obesity is an independent risk factor for dyslipidaemia, hypertension and cardiovascular disease and increases the risk of cardiovascular complications and mortality in patients with T2DM [10].

Mutations in insulin gene and insulin receptors also contribute to type 2 diabetes. Sphingosine-1-phosphate (S1P) is an important bioactive phospholipid with a wide range of cellular functions. In individuals with T2D, S1PR2 was shown to be down-regulated in platelets. S1PR2 variant Val→Ala at position 286 associated significantly with the incidence of diabetes. Novel Val→Ala polymorphism at position 286 in the NPXXY motif of S1PR2 is significantly associated with incidence and age at onset of diabetes in the LURIC study cohort [15, 16, 17]. HNF4α has also been associated with the regulation of glucose transport and metabolism [18]. Disruptions in this gene can lead to (MODY), an autosomal dominant, non-insulin dependent form of diabetes known as maturity onset diabetes of the young (MODY) [19, 20].

1.3. Mechanism of Action of Herbal Anti-diabetics

The antidiabetic activity of herbs depends upon variety of mechanisms. The mechanism of action of herbal anti-diabetic could be grouped as-

- Adrenomimeticis, pancreatic beta cell potassium channel blocking, cAMP (2nd
- messenger) stimulation
- Inhibition in renal glucose reabsorption
- Stimulation of insulin secretion from beta cells of islets or/and inhibition of insulin
- degradative processes
- Reduction in insulin resistance
- Providing certain necessary elements like calcium, zinc, magnesium, manganese and
- copper for the beta-cells
- Regenerating and/or repairing pancreatic beta cells
- Increasing the size and number of cells in the islets of Langerhans
- Stimulation of insulin secretion
- Stimulation of glycogenesis and hepatic glycolysis
- Protective effect on the destruction of the beta cells
- Improvement in digestion along with reduction in blood sugar and urea[38,39]

1.4. Diagnostic Methods of Diabetes

Diabetes mellitus is diagnosed by demonstrating any one of the following methods:

Fasting plasma glucose level ≥ 7.0 mmol/L (126 mg/dL)
Plasma glucose ≥ 11.1 mmol/L (200 mg/dL)
Glycated hemoglobin (Hb A1C) ≥ 6.5%
Oral glucose tolerance test (OGTT)

People with fasting glucose levels from 100 to 125 mg/dL are considered to have impaired fasting glucose also called as pre-diabetes. Fasting plasma glucose is mostly preferred because of its low cost and is very easy to operate. Diabetes should be confirmed with a second test on a different day. HbA1c is an indicator of the average blood glucose concentration over the preceding three months and has been proposed to be a useful alternative test to screen for type 2diabetes as it overcomes many of the obstacles associated with the OGTT [47, 48]. Usual anthropometric parameters used to measure obesity are BMI (Body Mass Index), WC (Waist Circumference) & WHR (Waist Hip Ratio). The most commonly used criteria to diagnose obesity are National Cholesterol Education Program (NCEP), ATP III criteria. According to sensitivity, Pandya et al. [5]. Suggested WC as a better indicator than BMI for diabetes status.

2. Material and Methods

Table 1: Formulations

INGRIDENTS:
Gurmar Patti (*Gymnema sylvestre*),
Jamum guthli (*Syzygium cumini*)
Karela (*Momordicacharantia*)
Giloy (*Tinospora cordifolia*)
Khadir Churan (*Acacia catechu*)
Haldi (*Curcuma Longa*)
Amla (*Emblica officinalis*)
Malabar tree (*Pterocarpus marsupium*)
Tejpata (*Cinnamomum tamala*)
Gularpatti churna (*Ficus glomerata*)
Kutki (*Picrorhiza kurroa*)
Chitrakmool (*Plumbago zeylanica*)
Methi (*Trigonella foenum-graecum*)
Sudh Silajit (*Asphaltum*)
Tribanga Bhasma
Neem Patta (*Azadirachta indica*)
Sura (Alcohol)
Gurmar Patti (*Gymnema sylvestre*)

2.1. How they acts

Giloy (*Tinospora cordifolia*): It has been reported to mediate its anti-diabetic potential through mitigating oxidative stress (OS), promoting insulin secretion and also by inhibiting gluconeogenesis and glycogenolysis, thereby regulating blood glucose. Alkaloids, tannins, cardiac glycosides, flavonoids, saponins, and steroids as the major phytoconstituents of *Tinospora cordifolia* have been reported to play an anti-diabetic role. [53]

Saptarangi (*salacia chinensis*): Recent preclinical and clinical studies have demonstrated that Salacia roots modulate multiple targets: peroxisome proliferator activated receptor-alpha-mediated lipogenic gene transcription, angiotensin II/angiotensin II type 1 receptor, alpha-glucosidase, aldose reductase, and pancreatic lipase. These

multi target actions may mainly contribute to Salacia root-induced improvement of type 2 diabetes and obesity-associated hyperglycemia, dyslipidemia, and related cardiovascular complications seen in humans and rodents.

Neem (*Azadirachta indica*): *Azadirachta indica* is known to possess hypolipidemic, hypoglycemic, immunostimulant and hepatoprotective properties. While nimbocinone, nimolinone, kulactone, nimocinolides, isonimocinolide, nimbin, salanin, azadirachtin, flavonoids, myricetin, meldenindiol, vilasinin, margosinolide, isomargosinolide, desacetyldi hydronimbic acid have been isolated from *A. indica* leaves having medicinal properties, *Bougainvillea spectabilis* leaves contain d-pinitol (3-*O*-methylchiroinositol) and is claimed to exert insulin-like effect.

Chirayita *(Swertia chirayita)*: The *in vitro* anti-diabetic activity was determined by assaying the inhibitory activity of the enzyme α-amylase which involves in the breakdown of starch to produce glucose.

Karela (Momordicacharantia): Momordicacharantia can repair damaged β-cells thereby stimulating insulin levels and also improve sensitivity/signalling of insulin. Bitter gourd is also reported to inhibit absorption of glucose by inhibiting glucosidase and suppressing the activity of disaccharidases in the intestine.

Malabar tree (*Pterocarpus marsupium*): The blood sugar-lowering activity has been endorsed to be due to the presence of tannates in the extract of the plant. Antihyperlipidemic activity is contributed probably to the marsupin, pterosupin, and liquiritigenin present in the plant. (−) Epicatechin has been shown to have insulinogenic property by enhancing insulin release and conversion of proinsulin to insulin. (−).

Methi (*Trigonella foenum-graecum*): The antihyperglycemic effect has been correlated with decline in somatostatin and high plasma glucagon levels The antihyperglycemic effect of fenugreek has been hypothesized to be due to the amino acid 4-hydroxyisoleucine which acts by the enhancement of insulin sensitivity and glucose uptake in peripheral tissues.

Gurmar (*Gymnema sylvestre*): Aqueous extract of G. sylvestre has been reported to cause reversible increases in intracellular calcium and insulin secretion in mouse and human β cells with type 2 diabetes. Regeneration of the cells in the pancreas might raise the insulin levels.

Bargad (*Ficus religiosa*): Decoction prepared from the bark is used in treatment of diabetes. The plant is believed to contain several bioactive principles including tannins, saponins, polyphenolic compounds, flavonoids, and sterols. Sitosterol-d-glucoside present in the bark of *Ficus religiosa* is believed to elicit hypoglycemic activity in rabbits.

Haldi (*Curcuma Longa*): The turmeric (*Curcuma Longa* L. rhizomes) EtOH extract significantly suppressed an increase in blood glucose level in type 2 diabetic KK-A(y) mice. In an *in vitro* evaluation, the extract stimulated human adipocyte differentiation in a dose-dependent manner and showed human peroxisome proliferator-activated receptor (PPAR)-gamma ligand-binding activity in a GAL4-PPAR-gamma chimera assay.

Ashwagandha (*withania-somnifera*): Asgand along with other ingredients of a composite formulation (Transina) have been reported to decrease streptozocin (STZ)-induced hyperglycaemia in rats. This anti-hyperglycaemic effect may be due to pancreatic islet free radical scavenging activity because the hyperglycaemic activity of STZ is a consequence of decrease in pancreatic islet cell superoxide dismutase (SOD) activity leading to the accumulation of degenerative oxidative free radicals in islet-beta cells.

Harhar Choti (*Terminalia Chebula*): The present study was aimed to evaluate the anti-diabetic potential of *Terminalia chebula* (*T. chebula*) fruits on streptozotocin (STZ)-induced experimental diabetes in rats. Electron microscopic studies showed significant morphological changes in the mitochondria and endoplasmic reticulum of pancreatic *β* cells of STZ-induced diabetic rats. Also, a decrease in the number of secretory granules of *β*-cells was observed in the STZ-induced diabetic rats and these pathological abnormalities were normalized after treatment with *T. chebula* extract

Jamun (*Syzygium cumini*): The present study was carried out to isolate and identify the putative antidiabetic compound from the S. cumini [SC] seed. A compound, mycaminose was isolated from SC seed extract. The isolated compound mycaminose (50 mg/kg) and ethyl acetate [EA] and methanol [ME] extracted compounds of S. cumini seed (200 and 400 mg/kg) was undertaken to evaluate the anti-diabetic activity against streptozotocin (STZ)-induced diabetic rates.

2.2. Standardization: "the process of making something conform to a standard"

LOD: Loss on drying is the loss in weight in% w/w determined by means of the procedure given below. It determines the amounts of volatile matter of any kind (including water) that can be driven off under the condition specified (Dessicator or hot air oven).If the sample in the form of large crystals, and then reduce the size by quickly crushing to a powder. About 1.5 gm. of powdered drug was weighed accurately in a tared porcelein dish which was previously dried at 105 °C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated [53-63].

Determination of total Ash value: Ash values are helpful in determining the quality and purity of crude drug, accurately weighed about 3 gm of air dried powdered drug was taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug.

Determination of acid insoluble ash value: The ash obtained as directed under total ash was boiled with 25 ml

of 2N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Determination of water soluble ash value: The total ash obtained was boiled with 25 ml. of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450□C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

Extractive values: Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Determination of alcohol soluble extractive value: 5gms of the air-dried coarse powder of the plant material was macerated with 100 ml of90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105.C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air dried drug.

Determination of water soluble extractive value: Weigh accurately the 5 gm of coarsely powdered drug and macerate it with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105□C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

Preparation of extracts: 1 Kilogram of powdered drug was packed in soxhlet apparatus and extracted with different polarity of solvent.

Petroleum ether extract: 1 Kilogram of powdered drug was packed in soxhlet apparatus and extracted with petroleum ether (60-80 °C) until the extraction was completed which was confirmed by the colour of the siphoned liquid. The extract was filtered while hot, and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure.

Ethanol extract: The marc left after petroleum ether extraction was dried in hot air-oven below 50 °C and packed well in soxhlet apparatus and extracted with ethanol (90%) until the completion of the extraction. The extract was filtered while hot, and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. The extracts were weighed and their percentage value was recorded and also the physical appearance and color was evaluated and recorded and thereafter, were stored in refrigerator for further experimental work. The marc left after petroleum ether extraction was dried in hot air-oven below 50□C and packed well in soxhlet apparatus and extracted with ethanol (90%) until the completion of the extraction. The extract was filtered while hot, and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. The extracts were weighed and their percentage value was recorded and also the physical appearance and color was evaluated and recorded and thereafter, were stored in refrigerator for further experimental work.

2.3. Qualitative chemical tests

Qualitative chemical tests were performed to determine the presence of alkaloids, Carbohydrates, cardiac glycosides, polyphenols, saponins, tannins and terpenoids.

Test for alkaloids:

- **Dragendorff's test:** To 1 ml of the extract, add 1 ml of dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.
- **Mayer's test:** To 1 ml of the extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.
- **Hager's test:** To 1 ml of the extract, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), yellow colored precipitate indicates the presence of alkaloids.
- **Wagner's test:** To 1 ml of the extract, add 2 ml of warner's reagent (Iodine in potassium

Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids (O'Brien *et al*, 1964).

Test for saponins:

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

Test for Glycosides:

- **Legal test:** Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

- **Baljet test:** To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

- **Keller-Killiani test:** 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelein dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the

junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

- **Borntrager's test:** Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides (Brain and Turner, 1975).

Test for carbohydrates and sugars

- **Molisch's test:** To 2ml of the extract, add 1ml of o-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

- **Fehling's test:** To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

- **Benedict's test:** To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

Test for tannins and phenolic compounds:

- Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.
- To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.
- The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins (Harbone JB, 2005).

Test for flavonoids

- The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.
- Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, disappears on addition of an acid indicates the presence of flavonoids.
- Shinoda's test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

Test for steroids

- **Lumberman-Burchard test:** 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

- **Salkowski test:** Dissolve the extract in chloroform and add equal volume of conc. $H_2 SO_4$. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract (Esau K, 1964).

2.4. Chromatography

Some materials appear homogenous, but are actually a combination of substances. For example, green plants contain a mixture of different pigments. In addition, the black ink in the pens that are used in this experiment is a mixture of different colored materials. In many instances, we can separate these materials by dissolving them in an appropriate liquid and allowing them to move through an absorbent matrix, like paper. The word chromatography means "color writing" which is a way that a chemist can test liquid mixtures. While studying the coloring materials in plant life, a Russian botanist invented chromatography in 1903. The retention factor, RF, is defined as

RF = distance the solute (D1) moves divided by the distance traveled by the solvent front (D2)

RF = D1 / D2

Where

D1 = distance that color traveled, measured from center of the band of color to the point where the food color was applied

D2 = total distance that solvent traveled

There are four main types of chromatography. These are Liquid Chromatography, Gas Chromatography, Thin-Layer Chromatography and Paper Chromatography. Liquid Chromatography is used in the world to test water samples to look for pollution in lakes and rivers. It is used to analyze metal ions and organic compounds in solutions. Liquid chromatography uses liquids which may incorporate hydrophilic, insoluble molecules. It uses a strip of paper as the stationary phase. Capillary action is used to pull the solvents up through the paper and separate the solutes.[44].

3. Result and Discussion

Table 2: Morphological evaluation

S.No	Formulation	Appearance	Colour	Taste	Odour
1	XYZ	Tablet	Dark brown	Slightly bitter	Fragrantly bitter
2	XYZ	Liquid	Dark brown	Acrid	Fragrantly bitter

Microscopical evaluation

The powdered drug observed under microscope, showed clumps of rounded to squares' thin-walled parenchyma cells. Several pieces of epidermal cells were noted in surface view. Groups of polygonal cells, belonging to cork region were also observed in surface view. Fragment of the powder show upper epidermis followed by palisade and spongy parenchyma tissue and spherical aggregation of calcium oxalate. A few groups of small, angular and thin-walled cells belonging to phloem tissue were also found to be present. It also exhibited groups of conducting cells, e.g. xylem fibers, vessels mainly bearing spiral thickenings. Fragments of fibers and vessels bearing pitted and spiral thickenings were also noted, along with group of vascular elements. Epidermal fragments with wavy and straight wall bear many trichomes. The entire as well as broken trichomes are mostly two celled, the basal cell smaller and apical elongated pointed cells.

Table 3: Physical evaluation

S No.	Experimental studies	Observation % w/w
1	Total cash value	12%
2	Water soluble ash value	3.7%
3	Acid soluble ash value	1.76%
4	Water soluble extractive value	18.66%
5	Alcohol soluble extractive value	11.16%
7	Moisture content	5.21

Chemical evaluation

Chemical analysis of ethanolic extract of Divya Madhunashni Vati and Hemofit-DC revealed the presence of alkaloid, glycoside, sterols, phenols, tannins, flavanoids, terpenes, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, and phytosterols.

Table 4: Chemical evaluation

S. No	Constituent	Test	Ethanolic Extract
1	Alkaloids	Mayer's Test(cream colour ppt)	+ve
		Dragendroff's Test (orange red ppt)	+ve
		Hager's Test (yellow ppt)	+ve
		Wagner's Test (reddish brown ppt)	+ve
2	Carbohydrates	Fehling's Test(yellow or red ppt)	-ve
		Molisch's test(reddish violet ring)	+ve
		Barfoed's Test (green ppt)	+ve
3	Triterpenoids	Liebermann Burchardt's test (red ppt)	+ve
		Salkowaski'stest(greenish yellow ppt)	-ve
4	Saponin	Foam test(persistent froth)	-ve
5	Phenols	Test with FeCl3(brown to green ppt)	+ve
6	Tannins	Lead acetate (yellow white ppt)	+ve
7	Flavonoids	Shinoda test	+ve

Table 5: TLC Profile

S.No	Chloroform extract Toulene : ethyl acetate(8:2)		Pet ether extract Toulene:ethyl acetate (8:2)		Acetone extract Toulene : ethyl acetate (8:2)	
	RF Value	UV	RF Value	UV	RF Value	UV
1	0.19	_	0.287	_	0.17	_
2	0.26	_	0.387	Blue (S)	0.21	_
3	0.36	Orange ((L)	0.5	_	0.25	Orange (L)
4	0.43	Orange (S)	0.55	_	0.29	_
5	0.50	Green (L, S)	0.587	_	0.34	Orange (L)
6	0.76	_	0.7	Blue (S)	0.42	_
7		_		_	0.56	_
8		_		_	0.76	_
9		_		_	_	Green (L)

4. Conclusion

As a part of standardization procedure, both samples (Madhunashnivati) and (Hemofit DC) were tested for relevant physio-chemical parameters.

Result for quantitative analysis for physicochemical parameters such as the water-soluble, alcohol soluble extractive values, moisture content, bulk density, pH, water-soluble ash, acid-Insoluble ash, loss on drying and organoleptic characteristics were calculated & found to be within the acceptable values.

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