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Pharmacognostical and phytochemical screening of ethanolic extract of *Silybum marianum* (L).

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ABSTRACT

The fluorescence characteristics of seed powdered extracts were determined under UV radiation of long and short wavelengths and ordinary visible light. When the small-grained drug and extracts were treated with totally different reagents and determined below ultraviolet illumination UV actinic radiation normal light, they emitted various color radiations. The colour modification for the seed powder and individual extract were distinctive and consistent revealing the solvent properties to the phytoconstituents and knowledge is gift. Phytochemical presence of absence in compounds in Plants *Silybum marianum* is the rich source of all the elements essential for humans. The moister content was calculated through the loss of the drying method and values were recorded. Vitamin E, vitamin C, along with beta carotene is antioxidants.

Keywords: Fluorescence, Phytochemical, Vitamins, Hyperglycemia, Color radiations, and Inorganic substances.

1. INTRODUCTION

Silybum marianum is a widely recognized medicinal herb belongs to family Asteraceae and is originated in the Mediterranean basins, but now naturalized throughout the world. In Pakistan it grows wild in Punjab, Khyber Pakhtunkhwa and Kashmir [1]. The plant is a herbal supplement having silymarin as an active constituent for the treatment of many liver disorders. Silymarin is a mixture of flavonolignans comprised of silybin (50 to 60%), is silybin (5%), silychristin (20%) and silydianin (10%) [2]. *Silybum marianum* (Milk thistle) is described as an annual, winter annual and biennial herb. The main stem is stout, ridged and branched, and the overall plant size can range from 2 to 6 feet tall. It has large scalloped leaves that are glossy green and thick and marbled with white vein like markings and having prickly

margins. The flowers are violet to purple. After flowering, thick, white, fluffy thistledown develops and scatters the seed. The seeds are obliquely ovoid and are 6-7 mm long and 3 mm wide and are brown or black having yellowish projecting swollen ring at its tip and a canaliculated hilum at another end [3]. The silibinin affects the metabolic pathways of glycaemia, especially the glycogenolysis and gluconeogenesis. Silibinin inhibited gluconeogenesis in the fasted state, inhibited glycogenolysis and glycolysis in the fed state. The mechanisms by which silibinin exerted these activities were manifold and complex. It inhibited the pyruvate carrier and activity of glucose 6-phosphatase and reduced the effectiveness of mitochondrial energy transduction [4]. Hyperglycaemia, the key symptom of diabetes mellitus, causes reactive oxygen species (ROS) that cause lipid peroxidation and damage to the membrane. ROS plays an important role in the development of secondary complications such as cataract, neuropathy and nephropathy in diabetes mellitus. Antioxidants protect β-cells from oxidation by inhibiting the peroxidation chain reaction and thus they play an important role in diabetes. Plants containing natural antioxidants like tannins, flavonoids, vitamin C and E can preserve β-cell function and prevent diabetes-induced. The use of aldose reductase inhibitors and α-glucosidase inhibitors has been reported for the treatment of diabetic complications [5]. The ever-increasing incidence of T2D has become a severe threat to humanity. In spite of advancements recorded to this point on basic and clinical investigations into polygenic disorder (especially in African nation and area unita geographic area geographical wherever health care resources are limited) a properly effective final remedy does not exist. The existing therapeutic remedies (which embody simply four core classes of oral hypoglycaemic agents viz: biguanide, glucosidase inhibitors, sulfonylurea and pioglitazone) are grossly inadequate [6]. Among the various treatment strategies, diet therapy, pharmacotherapy, and insulin therapy are the main treatment options available to control diabetes, in addition to a wide range of glucose-lowering drugs that exert their hypoglycemic effects through various mechanisms [7]. However, these treatment choices haven't gained abundant significance as these treatment ways are ordinarily related to disadvantages, like drug resistance, facet effects, and toxicity. Hence, the supplementation of herbal-based drugs to is now a promising and reliable treatment strategy due to its safe and non-toxic nature [8]. During the last decade, *silymarin* and its major constituent silibinin have been discovered to possess anticancer effects in different in vitro and in vivo models of epithelial cancers such as skin, prostate, breast, cervix, lung, liver, bladder, kidney, and colon cancer [9].

Diabetes, conjointly called DM, may be a progressive disorder is characterized by elevated levels of aldohexose or sugar within the blood. The disease is caused by inadequate secretion of insulin, the body's poor response to insulin, or both, which severely damage the body's systems, including the heart, blood vessels, eyes, kidneys, and nerves. Diabetes type 2 (DM2) is the most common form of this disorder, representing 90% of the total affected population [10, 11]. When ketones body is present in the blood or urine, it is called ketoacidosis, hence proper treatment should be taken immediately, else it can lead to other diabetic complications [12]. Diabetes is a condition of the body in which it does not produce enough insulin or does not use it properly. Insulin is a hormone which converts sugar and other food items into energy. Hereditary reasons and the lifestyles of the people play an important role in causing diabetes. When the pancreas gland does

not produce enough insulin, the blood glucose level rises and results into the diabetic condition. This condition is termed as Diabetes Mellitus. The isolation led first to a mixture that was named silymarin and it was this flavonolignan mixture with which most of the clinical studies were carried out. One of the important issues regarding silymarin is that it may be accepted as a safe herbal product, since no health hazards or side effects are known, in conjunction with the proper administration of designed therapeutic dosages.

2. MATERIALS AND METHODS

2.1. Plant collection

Fresh, healthy, and young seeds of *Silybum marianum* were collected from Laycowin Organic, Herbal and Medicinal Products, Thanjavur (10.7821° N, 79.2756° E), Tamilnadu, India.

2.2. Preparation of Seed extract

The seed was cleaned and dried in shade for 7 days and then ground well to a fine powder. Total six different extracts ethanolic, methanolic, aqoueus, chloroform, ethyl acetate and petroleum ether were prepared by the seed powder. For preparation of different extract, about 500 g of dry powder was extracted (80%) at 70°C by continuous hot percolation using Soxhlet apparatus for 24 hrs. The different solvents extract was then filtered and kept in a hot air oven at 40°C for 24 hrs for evaporation of ethanol. After 24 hrs a dark brown residue was obtained and was kept separately in airtight containers and stored at -4° C for further use.

2.3. Preliminary Phytochemicals screening

Preliminary phytochemical screening was performed using standard procedures Khandelwal KR (2002), Kokate CK (2005) and British Pharmacopoeia Commission (2010) [13-15] with some modifications. The extracts obtained from different solvents were subjected to identification tests for the detection of different phytoconstituents through the fluorescence analysis of seed powdered and various extracts was carried out by the standard method [16], organic and inorganic elements analysis with the method of British Pharmacopoeia Commission (2010) [15] Qualitative analysis was performed to evaluate the presence of vitamins and was analyzed by Patel DK *et al* (2011) and Pearson Thomas A. (1999) [17-18] method with some modification.

2.4. Basic nutritional analysis of whole powdered seed

2.4.1. Protein estimation

The presence of total protein in the sample was estimated by Folin-lowry method. A known volume of sample was taken and diluted by adding distilled water to make 1ml of the sample. 5ml of Lowry's solution was added to sample and incubated for 10 min at room temperature. About 0.5 ml of Folin-Cocteau reagent was added to the solution mixture. The solution was incubated at room temperature for 30 minutes. The resulting solution was observed at 660nm using UV visible spectrophotometer. A standard graph was plotted against bovine serum albumin to calculate the amount of protein in the sample.

2.4.2. Estimation of total carbohydrates (Phenol sulphuric acid)

The total carbohydrate content of the sample was estimated using the phenol sulphuric acid method.

The sample was dissolved in 100 ml of distilled water to which 1ml of phenol (5% solution) was added along with 5ml of concentrated sulphuric acid (96%). The solution was mixed properly and placed in a water bath at 25-30 ^{o}C for 20 minutes. It was allowed to stand until a colour development. The absorbance was read at 490 nm using a spectrophotometer. Glucose was used as the standard for comparison.

2.4.3. Estimation of total free fatty acid (Acid value)

The total fatty acids were estimated in the sample by using the method of Sample (1.0 g) was weighed and dissolves in 50ml of ethanol in a conical flask. Two drops of phenolphthalein indicator were added and titrated to until the pink endpoint with 0.01N potassium hydroxide solution (KOH). The acid value was calculated as per the formula is given below:

$$\text{Acid value} = \frac{56.1 \times V \times C}{M}$$

Where,

56.1 - Equivalent weight of KOH,
V - Volume in ml of standard volumetric KOH solution used,
C - Extract concentration in KOH solution used (0.1 N) and,
m - Mass in grams of the test portion (0.1 g).

2.4.4. Determination of moisture content

Dry an empty dish and lid in the oven at 105 ^{o} C for 3 hrs, transferred to desiccator for cooling and the weight was recorded. About 3g of sample was placed in the dish and spread to uniformity. The dish was placed in the oven for 3 h at 105 ^{o} C. After drying, transfer the dish with part coated lid to the desiccator to chill. The weight was recorded was final report with following equation.

$$\text{Moisture} = \frac{(W1 - W2) \times 100}{W1}$$

Where,

W1 - Weight (g) of the sample before drying
W2 - Weight (g) of the sample after drying

2.4.5. Determination of ash content

The crucible with lid was placed in the furnace at 550 ^{o}C overnight to ensure the degradation of all the impurities. The crucible was cooled in the desiccator and weight was determined. About 5 g of sample was placed into the crucible and heated over low Bunsen flame with the lid half covered. When fumes are no longer produced, place crucible and lid in the furnace and heat at 550 ^{o}C for overnight. Cool down in the desiccator and record the weight difference for total ash content with following equation.

Calculation

$$\text{Ash (\%)} = \frac{W1 \times 100}{W2}$$

W1 =Weight of ash
W2 =Weight of sample

2.5. Different vitamin analysis of whole powdered seed

2.5.1. β-carotene analysis

The β-carotene analysis was carried out according to the Association of Official Analytical Chemists. About 10g of the macerated sample was mixed with 50 ml of ethanol (95%) and maintained at 70-80 ^{o}C in a water bath for 20 minutes with periodic shaking. The supernatant was decanted and allowed to cool. The ethanol concentration of the mixture was brought to 85% by adding distilled water and it was further cooled in a container of ice water for about 5 minutes. The mixture was transferred into a

separating funnel and 25 ml of petroleum ether (pet-ether) was added followed by pouring over the cooled ethanol. The bottom layer was run off into a beaker while the top layer was collected. The process was re-extracted with 10 ml pet-ether for 5-6 times until the extract became fairly yellow. The entire pet-ether was collected into conical flask and transferred into separating funnel for re-extraction with 50 ml of 80% ethanol. The absorbance of the funnel extract was measured at 436 nm using a UV spectrophotometer (model 22 UV/VIS).

2.5.2. Estimation of Vitamin C or Ascorbic acid

The vitamin C from the sample was estimated with ascorbic acid as standard. The mixture of the sample and distilled water was added to 1.5 ml of 6% TCA and allowed to stand for 5 min followed by centrifugation. To the supernatant, 0.3 g of acid-washed Norit was supplemented, agitated and filtered. To the filtrate 0.5 ml of dinitrophenylhydrazine (DNPH) was added and placed in a water bath at 37 ^{o}C for 3 hrs. After exposure the solution was placed in ice-cold water and added 2.5 ml of 85% sulphuric acid. The contents of the tubes were mixed well and allowed to stand at room temperature for 30 min. The colour developed was read at 540 nm and compared with standard preparations of ascorbic acid.

2.5.3. Estimation of Vitamin E

The level of vitamin E in the sample was estimated with standard α-tocopherol. The mixture of sample and distilled water was added to 1.5 ml of ethanol. The solution was mixed followed by centrifugation. The supernatant was evaporated at 80 ^{o}C and to the precipitate, 3.0 ml of ferric chloride were added. All the tubes were mixed well and kept in dark for 5 min and 4.0 ml of n-butanol was added. The red colour developed was read at 520 nm and compared with standard.

2.5.4. Folic acid determination

The folic acid was determined by adding 2ml of 5M hydrochloric acid to 1ml of $NaNO_2$ (2%) and cooled. To this solution, 1.5 ml of 2% sulfamic acid was added along with 2ml of 0.5% iminodibenzyl and 3ml of alcohol. The solution was placed in a boiling water bath for 5 min, cooled and diluted to H_2SO_4. The absorbance was measured at 580 nm and the calibration graphs were constructed corresponding to a standard solution.

2.5.5. Estimation of nicotinic acid

The powdered sample was taken at 1 mg/ml in ethanol and calibrated using 0.5 ml of 0.05% aqueous cyanide solution followed by 0.5 ml of saturated bromine water and kept for 1 minute with occasional shaking. Excess bromine was removed by drop wise addition of formic acid. To this, 2 ml NaOH (2mol/l) was added and the solution was placed in a hot water bath at 80 ^{o}C for 5 min. Then, the solution was cooled to room temperature. To this mixture, added 1 ml of sulphuric acid (1% w/v) and 1 ml of 1% solution nitrite and kept in an ice bath for 2 min. The mixture was allowed to stand for 10 min for colour development and absorbance was recorded at 470 nm and the calibration graphs were constructed according to a standard solution.

2.6. Mineral analysis of whole powdered seed

The sample was analysed for mineral analysis using Atomic absorption spectroscopy (AAS). The sample was frozen and freeze-dried using a lyophilizer (Sub Zero Lab Pvt. Ltd, India). About 65% nitric acid was

Table 1: Qualitative phytochemical analysis of different solvent extracted powdered seed of *Silybum marianum*

S. No	Analyzed photochemical factor	Ethanol	Methanol	Water	Chloroform	Ethyl Acetate	Petroleum Ether
1.	Tannin	++	+	+	+	+	-
2.	Phlobatannins	+	-	++	+	-	+
3.	Saponin	+++	+	++	+	-	_
4.	Flavonoids	+	++	-	+	-	++
5.	Steroids	-	-	+	++	-	+
6.	Terpenoids	++	+	+	-	++	+
7.	Triterpenoids	+	+	-	+	-	+
8.	Alkaloids	+++	++	+	+	-	++
9.	Carbohydrate	+	-	+	-	+	-
10.	Protein	++	+	++	-	+	-
11.	Anthraquinone	+	+	+	-	-	+
12.	Polyphenol	++	+	++	++	+	-
13.	Glycoside	+	+	-	+	-	+

added to obtain a dilution factor of 10, with amounts in the ranges of 0.2-0.6 g of sample and 2.0-6.0 ml of nicotinic acid. The ready samples were allowed to face long to slow mineralization. Then the samples were mineralized in a microwave oven. Trace element concentration in the mineralized samples was determined using an AAS 7000 spectrometer (Shimadzu, Japan) with flame atomization (F-AAS).

2.7. Statistical analysis

Statistical analysis was performed by one way Analysis of Variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Software Package for the Social Science (SPSS) software package version 15.00. Results were expressed as Mean ± Standard Deviation for p values <0.05 were considered significant.

3. RESULTS AND DISCUSSIONS

The results of the present study revealed the presence of various medicinal active constituents in their seed extracts. The phytochemical active compounds of *Silybum marianum* were qualitatively analyzed and their results were presented. Present study indicated the presence of phytochemicals in different solvent extract like Tannin, Phlobatannins, Saponin, Flavonoids, Terpenoids, Triterpenoids, Alkaloids, Carbohydrates, Protein, Anthraquinone, Polyphenol and Glycoside absences of Steroids. The ethanolic extract show more prominent phytochmeicals as compared to other solvent extract, hence the highest concentration ethanolic extracts were used for further studies.

Table 2: Basic nutritional components present in the whole seed from *Silybum marianum*

Sr. No.	Analyzed factor	Quantity/100g
1	Total protein	27.5g
2	Total carbohydrates	37.49g
3	Total free fatty acid	28.13g
4	Moisture content	3.67%
5	Ash content	4.75%

Table 3: Estimation of vitamins in the whole seed from Silybum marianum

Sr. No.	Analyzed factor	Quantity/100 g
1	β-carotene	7.2mg
2	Ascorbic acid	7.6mg
3	Vitamin E	3.07mg
4	Folic acid	107µg
5	Nicotinic acid	203µg

Plant derivatives with hypoglycemic properties have been used in folk medicine and traditional healing systems around the world from ancient times. Medicinal plants are wont to treat symptoms and hyperglycemic conditions are a unit of sizeable interest to ethno-botanical community because the plants contain valuable medicative properties in its totally different elements [19].

3.1. Basic nutritional components present in the whole seed from *Silybum marianum*

The carbohydrate content is relatively high more than other components. The moisture content in drugs should be at minimal level so as to discourage the growth of bacteria, yeast and fungi during the storage time. Estimation of extractive values determines quantity of the active constituents during a given amount of stuff once extracted with a specific solvent. The extractions of any crude drug with a specific solvent yield containing totally different phytoconstituents nutritional components [20].

The compositions of those phytoconstituents rely on the character of the drug and therefore the solvent used. It also gives an indication whether the crude

Table 4: Fluorescence analysis whole seed from *Silybum marianum*

Sr. No.	Analysed phytochemical factor	Visible Light	Short UV	Long UV
			254nm	365nm
1	Plant powder (pp)	Light Brown	Black	Dark Black
2	PP with water	Light Brown	Light Brown	Dark Brown
3	PP with Hexane	Light Brown	Dark Brown	Brown
4	PP with Chloroform	Light Brown	Creamish white	Yellow
5	PP with Methanol	Dark Brown	Yellow	Dark Black
6	PP with acetone	Brown	Dark Black	Brown
7	PP with IN Sodium hydroxide in water	Light Brown	Brownish - Yellow	Light Yellow
8	PP with IN Hydrochloric acid	Dark Brown	Off White	Light Brown
9	PP with sulphuric acid with an equal amount of water	Light Black	Dark Brown	Light Black
10	PP with Nitric acid diluted with an equal amount of water	Dark Yellow	Light Brown	Brown

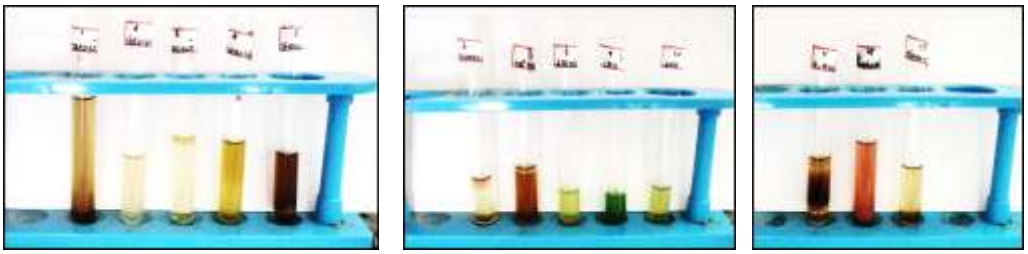

Fig 1. Qualitative phytochemical analysis of ethanolic solvent extracted powdered seed of *Silybum marianum*

drug is exhausted 3.67%. Free fatty acid 28.13g components. Ash values are used to determine quality and purity of crude drug.4.75%. The water soluble ash is employed to estimate the quantity of compound gift in medicine. The protein Lower than 27.5g. Basis nutrients present in the *Silybum marianum*.

Although there are several enzymes present within the body that scavenge free radicals, the principle micronutrient (vitamins) higher than Nicotinic acid 203µg/100g, antioxidants are lower than vitamin E 3.07mg/100g (α- tocopherol) and B-carotene 7.2mg/100g. The body cannot Folic acid 107 µg/100g and Ascorbic acid 7.6mg/100g manufacture these micronutrients so they must be supplied in the diet.

All folks need variety of advanced organic/inorganic compounds in diet to fulfill the requirement for his or her activities. The important constituents of diet are minerals. Every constituent in plant plays an important role in its growth and any deficiency of constituent may lead to abnormal and irregular developments. Magnesium (Mg) 5.7 mg. Plants *Silybum marianum* are the rich source of all the elements essential for human. Diabetes mellitus is a metabolic disorder characterized by the presence of high levels of glucose in the blood that occurs either due to insulin deficiency or malfunction [21-22]. Under normal physiological conditions the insulin activates lipoprotein lipase which acts on triglycerides and separates the fatty acids and glycerol. These fatty acids then undergo either oxidation to generate energy or re-esterification to store it in body tissues [23].

3.2. Fluorescence analysis whole seed from *Silybum marianum*

Table 5: Fluorescence analysis whole seed from *Silybum marianum*

Sr .No.	Analyzed factor	Quantity/100g
1	Calcium (Ca)	78.01mg
2	Iron (Fe)	7.2mg
3	Magnesium (Mg)	225.37mg
4	Zinc (Zn)	7.0mg
5	Copper (Cu)	09.5mg
6	Chromium (Cr)	0.7mg
7	Nickel (Ni)	3.1mg
8	Manganese (Mn)	5.7mg

The study of fluorescence is a method for evaluating components in the plant that give a definite idea of the chemical existence. Equally, extracts be in addition subjected to UV chamber and fluorescence is been observed and consistency was noted as an additional character for identification. The fluorescence analysis of the powdered drugs helps to detect the adulteration as the phytoconstituents of the sample when got mixed with the reagents exhibit characteristic fluorescence under ultraviolet light (UV). The fluorescence exhibited by the sample corresponds to the chemical constituents present in the sample. Much previous to the phytochemical screening, a irregular estimation of phytoconstituents be done by the behaviour of powder drug with different chemical reagents. The powdered drug showed different colours when it gets varied with the different reagents, as it reflects the presence of phytochemicals in accordance to colours obtained. Fluorescence behaviour analysis of phytochemical factors presence of different chemicals represented [24].

4. CONCLUSION

The *Silybum marianum* seed extract was used in the present study to prepare the different extract for estimation of phytochemical constituents. Out of which ethanol extract shows highest concentration may be a good source of phytochemicals. Vitamin E, vitamin C, along with beta carotene is antioxidants. Some studies suggest that the trio might help to *Silybum marianum* strengthen the body's immune system and play a role in cancer prevention [24]. More bioactive study on *Silybum marianum* can bring some useful and curing remedy for major diseases.

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6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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