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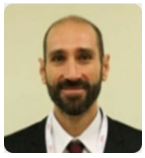

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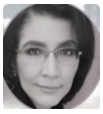

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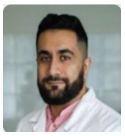

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Contents

Bone Marrow Derived Mesenchymal Stem Cells –A Boon for the Treatment of Complications in Diabetes Mellitus

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ABSTRACT

Mammal Bone marrow is an invaluable source of Mesenchymal stem cells. Bone Marrow derived Mesenchymal Stem Cells (BM-MSCs) are multipotent, self-renewing cells found in almost all postnatal organs / tissues and are used in the treatment of various disease conditions. Diabetes mellitus is a metabolic syndrome characterized by increased levels of blood glucose leading to various complications like Diabetic Foot, Diabetic Neuropathy, Diabetic Retinopathy, Diabetic Cardiomyopathy and Diabetic Nephropathy. BM-MSCs are able to differentiate into many cell types, and to proliferate ex vivo. These attributes makes them a potential therapeutic tool for cell replacement therapy in diabetes and other diseases. The present review discusses the isolation and culturing of BM-MSCs along with their potential as new therapeutic agent in the treatment of Diabetes related complications and its limitations.

Key Words: *Bone marrow, Mesenchymal stem cells, Diabetes Mellitus, Diabetic Foot Ulcer, Diabetic Poly Neuropathy, Diabetic Retinopathy*

INTRODUCTION

Diabetes mellitus is a metabolic syndrome characterized by increased levels of blood glucose. Diabetes mellitus patient suffering from defective insulin secretion rely on lifelong substitution with exogenous administration of insulin. Whole pancreas and purified pancreatic islet transplantation have offered the potential for independence from insulin injections. Transplantation of islet is not possible every time because of lack of availability. Hence, the scientists have found out the renewable source of isletreplacement tissues. Mesenchymal stem cells, also known as multipotent Mesenchymal stromal cells showed tremendous potential for this therapy as they are self-renewing cells and are found in almost all postnatal organs and tissues1. BM-MSCs are responsible for conveying some chemical molecules like stromal antigen 1, erythrocytes (glycophorin A), CD44, CD90, CD166 (vascular cell adhesion molecule), CD54/CD102 (intracellular adhesion molecule), and CD49 (very late antigen), CD105 (SH2), CD73 (SH3/4) 2, 3. Conversely MSCs lack the expression of surface markers characteristic for hematopoietic cells (CD14, CD45, and CD11a/lymphocyte function-associated antigen 1 (LFA-1)), and platelet and endothelial cell markers (CD31)4

The main functional characteristics of MSCs are their immunomodulatory ability, capacity for self-renewal, and differentiation into tissues of mesodermal origin 5, 6.Therapeutic effects and use of MSCs would be primarily based on their release of trophic and immunomodulatory factors7, 8. MSCs can alter the secretion profile of dendritic cells (DCs) resulting in increased production of anti-inflammatory cytokine interleukin (IL)-10 and decreased production of interferon-gamma (IFN-γ) and IL-125–7. BM-MSCs can inhibit T-cell proliferation by engagement of the inhibitory molecule programmed death 1(PD-1) to its ligands PD-L1and through interacting with DCs 6, 7. BM-MSCs can increase the number of CD4+CD25+FoxP3+ Tregulatory cells that suppress the immune response. Susceptibility to diabetes induction and development may be related to the activity of T-regulatory cells and expansion of Th17 cells 8,9. BM-MSCs are able to render T cells anergic by blocking differentiation of monocytes to DCs or by inhibiting DC maturation5 render Through production of soluble factors, BM-MSCs can inhibit

DC maturation5. Through production of soluble factors, BM-MSCs can inhibit proliferation and IgG secretion of B cells7.It has been reported that MSCs can be isolated in relatively high numbers from culture of bone marrow10. Previous studies have shown that BM-MSCs are able to differentiate into several cell types, including cardiomyocytes, vascular endothelial cells, neurons, hepatocytes, epithelial cells, and adipocytes, making them a potentially important source for the treatment of debilitating human diseases. Such multipotent differentiation characteristics coupled to their capacity for self-renewal and capability for the regulation of immune responses, described BM-MSCs as potentially new therapeutic agents for treatment of the complications of diabetes mellitus (DM)11.

ISOLATION OF HUMAN BONE MARROW CELLS

The Bone marrow is collected first from human and then mechanically disrupted to obtain a single cell suspension .The marrow is then diluted with α-MEM (Minimum Essential Medium eagle and subsequently the diluted samples are overlaid with density gradient solution and centrifuged . Following centrifugation, cells are removed from the plasma/Ficoll-Hypaque interface, and suspended in 5 ml of α-MEM (GIBCO) supplemented with 10% human umbilical cord blood serum (UCBS), 100 U/ml penicillin, and 100 U/ml streptomycin (GIBCO). Cells are then plated at a density of 2 x 105/cm2 in 6 well culture plates, and incubated at 37°C in a humidified atmosphere containing 5% CO2. After 72 hours, the non-adherent cells are discarded and the adherent cells are cultured for approximately 10 days. Fresh medium is replaced twice a week and the cultures are maintained for 18-20 days. Upon reaching near confluence (90%), cells are detached with TPVG (0.25% trypsin, 1 mM/l EDTA) for 3-5 minutes at 37°C. After centrifugation, cells are re-suspended with fresh medium and replated. The resulting cultures are morphologically heterogeneous, containing cells ranging from narrow spindle-shaped cells to large polygonal cells and, in confluent cultures, some slightly cuboidal cells12.

CULTURING

The majority of modern culture techniques still take a CFU-f (Fibroblast colony-forming units) approach, where raw unpurified bone marrow or ficoll-purified bone marrow Mononuclear cell are plated directly into cell culture plates or flasks. Mesenchymal stem cells, but not red blood cells or haematopoetic progenitors, are adherent to tissue culture plastic within 24 to 48 hours. However, it has been reported that Nonadherent cell population of human marrow culture is also complementary source of mesenchymal stem cells (MSCs)13. Other flow cytometry-based methods allow the sorting of bone marrow cells for specific surface markers, such as STRO-114. STRO-1+ cells are generally more homogenous, and have higher rates of adherence and higher rates of proliferation, but the exact differences between STRO-1 + cells and MSCs are not clear15

ROLEOF BM-MSCs IN VARIOUS DIABETIC COMPLICATIONS

BM-MSCs in Diabetic Foot Ulcer

Diabetic foot ulcers (DFU), chronic, non-healing wounds on the feet of diabetic patients, present a serious challenge to global health. DFUs have a huge impact on our health care system, not only in terms of economic cost, but also from a psychosocial perspective, associated with significant morbidities, decrease in quality of life, prolonged hospitalization and importantly, often result in the amputation loss of lower extremity. MSCs derived from bone marrow or adipose tissue, pre-conditioned to optimize

reparative properties, will promote vascularization of the wound and improve healing16.
Autologous skin fibroblasts on biodegradable collagen membrane combine with BM-MSCs used for the treatment of Diabetic ulcer. The bone marrow aspirate of the patient with diabetic foot was applied directly to the wound and injected into the edges of the wound, finally covered with prepared autologous biograft. The patient received two additional treatments with cultured MSC on day 7 and 17.The wound showed a steady overall decrease in wound size and an increase in the vascularity of the dermis and in the dermal thickness of the wound bed after 29 days of combined treatment. After treatment we can conclude that Closing and healing of the non-healing diabetic ulcer was achieved by using the above therapy17

BM-MSCs in Diabetic Polyneuropathy and Retinopathy

Diabetic polyneuropathy (DPN) is one of the most frequent and troublesome complications of diabetes mellitus as it are responsible for damage to nerve fibers18. It also produces Spontaneous pain, hyperalgesia, and diminished sensation. The reasons behind spreading of DPN are neural cell degeneration and decreased nerve blood flow18. Studies have shown that angiogenic cytokines like basic fibroblast growth factor (bFGF) and VEGF could be useful for the treatment of DPN29,19,20. It was shown in diabetic rats that MSCs, because of their ability to secrete bFGF and VEGF29, could be used as a new and effective therapeutic agent for the treatment of DPN19, 20. MSCs were isolated from bone marrow of adult rats and transplanted into hind limb skeletal muscles of rats with an 8-week duration of streptozotocin (STZ)-induced diabetes or age-matched normal rats by unilateral intramuscular injection. Four weeks after transplantation, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) productions in transplanted sites occur; it leads to increase in neovascularization responsible for neural cell regeneration which finally shows improvisation in diabetic polyneuropathy (Fig. 2) 20. However, by releasing paracrine factors and through differentiation into photoreceptor and glial-like cells in the retina, transplanted MSCs improved the integrity of the blood-retinal barrier, thus ameliorating diabetic retinopathy in STZ diabetic rats 21

BM-MSCs in Cardiomyopathy

Ventricular dysfunction in patients with Diabetes mellitus in the absence of coronary artery disease, valvular heart disease, or hypertension is defined as diabetic cardiomyopathy (DCM)22. BM-MSCs have the capacity to differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells. In brief, BMMSCs showing tendency to reduce cardiac fibroblast proliferation and expression of collagen I and III and they are able to promote matrix metalloproteinase secretion by cardiac fibroblasts, leading to reduced cardiac ventricular fibrosis23,24. These effects may at least partially be mediated via the release of antifibrotic factors such as hepatocyte growth factor 25. Chronic hyperglycemia is responsible for myocardial remodeling and is a central feature in the progression of DCM. An additional feature that contributes to the pathogenesis of DCM is the activity of matrix metalloproteinase (MMP)-2 and MMP926,27. The diabetic myocardium is characterized by decreased activity of MMP-2, leading to increased collagen accumulation, and increased activity of the proapoptotic factor MMP-9, which is responsible for apoptosis of endothelial cells, reduction of capillary density, and poor myocardial perfusion26,27. Microcirculatory defects, necrosis and apoptosis of cardiomyocytes, and interstitial fibrosis are the main pathological characteristics of DCM 23, 27.
BM-MSCs can also induce myogenesis and angiogenesis by releasing different angiogenic, mitogenic, and antiapoptotic factors including vascular endothelial growth factor (VEGF), insulin-like growth

factor-1 (IGF-1), adrenomedullin (AM), and hepatocyte growth factor (HGF). This was proved with the help of rat model of DCM in which intra venous administration of BM-MSCs improved cardiac function of treated animals. MSC that was transplanted get differentiated into cardiomyocytes and improved myogenesis and angiogenesis. This phenomenon increases myocardial arteriolar density and decreases collagen volume resulting in attenuation of cardiac remodeling and improved myocardial function28.

BM-MSCs improve myocardial perfusion and myocardium regeneration..Improvement in cardiac function following MSC therapy may also be attributed to the release of MSC-derived paracrine factors capable of cardioprotection. These factors include secreted frizzled-related protein 2, Bcl-2, heat shock protein 20, hypoxia-regulated heme- oxygenase-1, hypoxic Akt-regulated stem cell factor, VEGF, HGF, AM, and stromal-derived factor29. A growing body of evidence strongly suggests that these factors affect remodeling, regeneration, and neovascularization leading to the improvement of myocardium contractility and viability, ameliorating consequences of infarction29-32. Double-blind, placebo-controlled trials showed that i.v. autologous MSCs transplantation increased left ventricular ejection fraction, reduced episodes of ventricular tachycardia, and led to reverse remodeling in postinfarction patients reducing the mortality rate in patients with ischemic stroke 30,31.

BM-MSCs in Diabetic Nephropathy

Diabetic Nephropathy is a comprehensive disease with metabolic disturbance which is caused by long-term unstable blood sugar levels in patients body.BM- MSCs administration can prevent and treat diabetic nephropathy, which is the most common complication of Diabetes mellitus, and is defined as progressive kidney disease caused by angiopathy of the capillaries supplying the kidney glomeruli33. BM-MSCs have been used for the treatment of diabetic nephropathy in nonobese diabetic/severely compromised immunodeficient (NOD/SCID) and C57 black 6 (C57/BL6) mice, which succumb to DM after application of multiple low doses of STZ. About 30–60 days after STZ injection, kidneys of treated mice showed the presence of abnormal glomeruli characterized by increased deposits of ECM protein in the mesangium, hyalinosis, and increased number of macrophages in the glomeruli 33,34.Result obtained from above mice transplanted with human MSCs (hMSCs) and C57Bl/6 mice that received murine MSCs indicate that injected MSCs was engrafted in damaged kidneys was get differentiate into renal cells so, we can conclude that above treatment is effective in diabetic nephropathy33,34. Additionally, the small percentage of hMSCs in the transplanted kidneys differentiated into endothelial cells as evidenced by de novo expression of CD3134. The result of systemic administration of MSCs in diabetic mice was improvement of kidney function and regeneration of glomerular structure33,34 as MSCs are able to reconstitute necrotic segments of diabetic kidneys35. However, it is not clear whether MSCs can propagate after engraftment in the kidney. One month after MSC treatment, only a few hMSCs were detected in kidneys, suggesting that they were unable to proliferate34 so an alternative scenario for improvement of kidney function could be the ability of MSCs to scavenge cytotoxic molecules or to promote neovascularization29-32. In addition, successful MSC treatment of diabetic nephropathy could be explained by MSCs competence to differentiate into insulinproducing beta cells followed by decrease of glycemia and glycosuria, factors important for damaging renal cells33. This indicate that MSC transplantation prevents the pathological changes in the glomeruli and enhances their regeneration resulting in improved kidney function in diabetic animals. For “in vivo” tracking of BrdU-marked MSCs, immunostaining for BrdU (Bromodeoxyuridine) was performed in the heart, liver, spleen, pancreas, lung and kidney of recipient rats. BrdU positive cells were detected in the heart (into which the MSCs were infused), pancreas and kidney of recipient rats, while no positive cells were found in other organs. In the pancreas, BrdU positive cells were mainly located in the interstitium. And BrdU-

-marked MSC were mainly located in renal interstitium instead of glomerulus and renal tubles 36.

DRAWBACK OF BM-MSCsTHERAPY

Spontaneous differentiation frequency of MSCs in the host tissue is extremely rare and hence, the therapeutic efficacy of MSCs depends on its ability to control in-vivo differentiation into target cells. poor engraftment and limited differentiation under in vivo conditions is another major drawback of this therapy 37.The potential of MSCs to differentiate into unwanted Mesenchymal lineages 38, leads to impair their therapeutic activity. Apart from this, few limitations like malignant transformation and cytogenetic aberrations of MSCs.

CONCLUSION

BM-MSCs is the most trusted stem cells because BM-MSCs shows differentiation capacity, pluripotency, immunomodulatory ability and self-renewability. These functional properties makes them suitable for treatment of complications of Diabetes mellitus like Diabetic cardiomyopathy, Diabetic nephropathy, Diabetic neuropathy and retinopathy and foot ulcer. In case of embryonic stem cells ethical issue arises but BM-MSCs are free such issue. These cells also showing immunosuppressive effect. A disadvantage of this therapy includes unwanted Mesenchymal lineages differentiation, risk of malignant formation, uncontrolled differentiation, the need of pure culture of BM-MSCs. If we overcome these problems then definitely this therapy would be boon for the treatment of many life threatening diseases in human being.

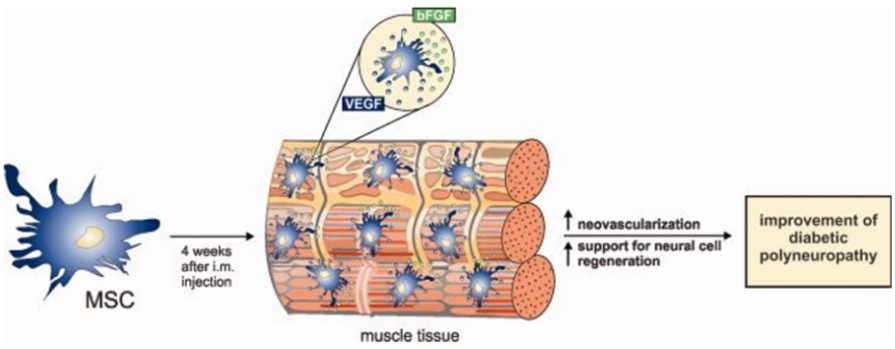

Figure 1: Effects of MSCs treatment on diabetic polyneuropathy.

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Comparison of Antibacterial Properties of Solvent Extracts of Different Parts of Jatropha curcas (Linn)

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ABSTRACT

Recent reports have suggested the urgent need for the development of alternative antibacterial substances of natural origin for the treatment of infections because of the growing cases of bacterial resistance to many available synthetic antibiotics. Jatropha curcas (J. curcas) belongs to the family Euphorbiaceae and has been widely reported for its medicinal values. It is used in traditional folklore medicine to cure various ailments such as skin infections, gonorrhoea, and jaundice and fever. Almost every part of J. curcas has been reported to have medicinal importance. In the present study, the aqueous, ethanol and pet-ether extracts of the root, stem bark and leaves of J. curcas were evaluated in vitro for antibacterial activities using Salmonella typhi (S.typhi) and Escherichia Coli (E.coli) as test organisms. The results were compared in order to identify the extract with the strongest growth inhibition properties. Preliminary phytochemical screening of the extracts revealed the presence of bioactive compounds with demonstrated antimicrobial properties. The ethanol extracts showed superior antibacterial activities compared with both the aqueous and pet-ether extracts. The root, stem bark and leaf ethanol extracts demonstrated comparable antibacterial activities against S. typhi and E. coli. Thus the root, stem bark and leaf of J. curcas are potential candidates in the search for potent antibacterial agents from medicinal plants.

Key Words: Anti-bacterial activity, Jatropha curcas, MIC, MBC, Phytochemical screening, Euphorbiaceae

INTRODUCTION

Herbal medicine, also called botanical medicine or phytomedicine refers to the use of a plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes. Long practiced outside of conventional medicine, herbal medical practices has increasingly become more significant in primary health care as improvements in analysis and quality control along with advances in chemical research demonstrates valuable they are in the treatment and prevention of diseases. Historically, medicinal plants have provided a source for novel drug compounds, and plant derived medicines have made large contributions to health needs of many societies. Thus medicinal plants have become the base for the development of a medicine or a natural blue print for the development of new drugs.

Several reports have suggested the urgent need for the development of alternative antibacterial substances of natural origin for the treatment of infections because of the growing cases of bacterial resistance to many available synthetic antibiotics. Thus, plants with medicinal potentials can be evaluated for the purpose of identifying those that may be potent against infectious organisms and hence useful in treating ailments caused by human pathogens.

Many of these plants contain large varieties of chemical substances referred to as secondary metabolites which have significant biological effects on humans.

Jatropha curcas belongs to the family Euphorbiaceae and has been widely reported for its medicinal values. It is used in traditional folklore medicine to cure various ailments such as skin infections,

gonorrhoea, and jaundice and fever1,2. J. curcas Linn is commonly called physic nut, purging nut or pig nut. Previous studies have reported that the plant exhibits bioactive activities for fever, mouth infections, guinea worm sores and joint rheumatism3,4. Fabrenro-Beyoku5 investigated and reported the anti-parasitic activity of the sap and crushed leaves of J. curcas. The water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity6. Sanni et al.7 reported antibacterial, antitumor and antiinsect activities of this plant. Several other works have also shown that many other Jatropha species possess antimicrobial activity8,9.The root methanolic extracts of the plant was shown to exhibit antidiarrhoeal activity in mice10. In addition, Mujumdar and Misar11 revealed that the root extract from J. curcas showed anti-inflammatory activity on local inflammatory induced in albino rat. Naengchommong et al.23 reported the isolation of two lathyranes from J. curcus while Aiyelaagbe et al.13 isolated diterpenoids from the same plant and also reported its antibacterial activity on some bacterial isolates. The sap from J. curcas is employed for treating sores, cleaning teeth and toothache14. The anti-microbial activities of the crude extract of the bark has also been reported15.

In Ghana, various communities use different parts of J. curcas to cure various ailments. In some parts of northern Ghana, the decoction of the roots is used for the treatment of gonorrhoea, diarrhoea and rheumatism. The latex of the plant is also used to arrest bleeding, toothache, and wound healing. The leaf decoction is also used to treat typhoid fever and diarrhoea.

Almost every part of J. curcas has medicinal importance. Despite the extensive reports on the medicinal potentials of J. curcas, the efficacies of the extracts with potent anti-bacterial activities have not been compared. The overarching aim of the present study is to determine the phytochemical constituents present in the aqueous, ethanol and petroleum ether extracts of the root, stem bark and leaf of Jatropha curcas. The growth inhibitory activities of the various extracts on Salmonella typhi and Escherichia Coli have been evaluated and compared in order to determine the extract with the greatest anti-bacterial potential.

MATERIALS AND METHODS

Plant Materials

Fresh leaves, stem bark, and roots were collected in the month of November in Gognia, a suburb of Navrongo and were identified by Dr. Walter Kpikpi of the Department of Applied Biology, Faculty of Applied Sciences, University For Development Studies. The samples were air dried. The stem bark and the roots were pulverized in a mill. The leaves were also made into powder. The samples were stored in an airtight container for further use.

Preparation of Extracts

Exactly 200g each of the pulverized stem bark and roots were cold extracted in ethanol (95%), distilled water and petroleum ether separately for five days with occasional shaking16. Exactly 250g each of the powdered leaves were also extracted separately in cold using ethanol (95%), distilled water and petroleum ether for five days with occasional shaking. The extracts were then separately filtered through Whatman's No. 1 filter paper and the filtrates were concentrated to dryness in vacuo using a rotary evaporator to remove the solvents.

Phytochemical Screening

The tests were carried out using standard procedures in accordance with Trease and Evans17 and

Harborne16 with little modification.

Test for alkaloids

About 1ml of the plant extract was stirred with few drops of 1% HCl on a steam bath. The solution obtained was filtered and 1ml of the filtrate was treated with 1 drop of Mayer's reagent. Turbidity of the extract filtrate on the addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract

Test for saponins

About 2ml of the extract was measured into a test tube and shaken vigorously. Test becomes positive if characteristic honeycomb froth persists for at least 30 minutes. Test for cynogenic glycosides About 2ml of the extract was measured into a test tube and 1ml of chloroform added to it. A piece of picric acid paper was then inserted into the test tube just above the extract and folded over the rim of the tube. The test tubes was then stoppered and warmed at about 35ºC in a water bath for about 30 minutes. A change in colour of the yellow picric paper to various shades of red indicates the presence of cynogenic glycosides.

Test for reducing sugars

About 0.5ml each of Fehling's solutions A and B were measured into a test tube. About 0.5ml of the extract was added to the solution and heated in a water bath. A brick-red precipitate denotes the presence of reducing sugars. Test for polyuronides About 2ml of acetone was measured into a test tube. About 2ml of the filtered extract was added to the acetone in the test tube. Positive test is observed if the solution precipitates.

Test for phenolics

About 2ml of the extract was measured into a test tube and three drops of ferric chloride solution added. A positive test is confirmed if the solution turns blue, blue-black, green or blue-green and precipitates. Test for flavonoids About 3 drops of dilute NaOH was added to 1ml of the extract. An intense yellow colour was produced in the plant extract which becomes colourless on addition of few drops of dilute HCl indicates the presence of flavonoids.

Test for tannins

About 0.5ml of the plant extract was heated in a steam bath for about 5 minutes. About 2 drops of 5% FeCl3 was then added. Presence of greenish precipitate indicated the presence of tannins. Test for anthracenosides About 5ml of the extract was transferred into a separating funnel and about 3 drops of diethyl ether added to it and shaken gently and allowed to stand for about 10 minutes. About 2ml of the diethyl ether portion was measured into a test tube. 2ml of Ammonium hydroxide was added and shaken gently. A cherish-red colour of the alkaline solution indicates the presence of anthracenosides.

Test for triterpenes and phytosterols

About 5ml of the diethyl ether portion (same procedure from the test for anthracenosides) was evaporated to dryness. The residue was dissolved in 0.5ml acetic anhydride and then in chloroform

(0.5ml).The solution was transferred to a dry test tube and 2ml of concentrated sulphuric acid added to it and shaken gently. A brownish-red colour was observed for the presence of triterpenes and a green colour was observed for the presence of phytosterols.If triterpenes and phytosterols are absent, no colour will be observed as compared with the control or reference.

Bacteriological Analysis

Preparation of Muller-Hinton agar About 19g of Muller-Hilton agar was weighed into a 500ml conical flask. 500ml of distilled water was added and agitated. The content was heated to boil on a hot plate with a magnetic stirrer until the powder dissolves completely in the water. The media was then poured into a media bottle, corked and was autoclaved at 121oC for 15 minutes. The media was allowed to cool and stored in the refrigerator until use.

Bacteria culture

Salmonella typhi and Escherichia coli were first sub-cultured in a nutrient agar and incubated at 370C for 24 hours at the microbiology laboratory.

Antimicrobial susceptibility test

The agar well diffusion method was used. The Muller-Hinton agar media was poured in the sterilized petri dishes and allowed to solidify. About 0.1ml each of the Salmonella typhi and Escherichia coli was spread uniformly over the surface of the Muller-Hinton media with a sterile glass rod spreader. A hole was bored by 5mm cork borer in the middle of each inoculated agar plate. About 0.1ml of each extract (10mg/ml in DMSO) was pipetted into the respective holes. Ciprofloxacilin which was used as a control. Triplicates of plates were allowed to stand for about 1 hour to allow the extracts to diffuse into the media. The plates were then incubated upside down at 370C for 24 hours. The diameters (mm) of the zones of inhibition were measured from underneath the plates using a pair of dividers and a ruler and their means were also recorded.

Determination of Minimum Inhibitory Concentrations (MIC)

Various concentrations of extracts ranging between 2.0 and 10.0 mg/ml were introduced into different test tubes; each tube was inoculated with an overnight culture of S. typhi and E. coli diluted to give a concentration of 106 cells per ml. The tubes were incubated at 37°C for 24 h. The least concentration of extract that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the minimum inhibitory concentration (MIC) in each case18.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC of the plant extracts was determined by a modification of the method of Spencer and Spencer19. Samples were taken from plates with no visible growth in the MIC assay and sub-cultured on freshly prepared nutrient agar plates, and later incubated at 37°C for 48 h. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

Statistical Analysis

Data collected in the study are expressed as the mean ± standard error of mean (S.E.M.)

RESULTS AND DISCUSSION

Phytochemical Screening

The extracts showed variations in the type of phytochemicals present. Phytochemical analysis revealed the presence of reducing sugar in all the extracts. Polyuronides, Cynogenic glycosides and Anthracenosides were however absent in all extracts. (Table 1). Ethanol extract of leaves, aqueous extracts of root and leaf showed the presence of saponins. These phytochemicals are biologically active and therefore may aid the antibacterial activity of J. curcas. Alkaloids were found to be present in all ethanol and aqueous extracts. Alkaloids were also found to be present in the leaf pet-ether extract. Alkaloids have been reported extensively for their anticancer activities20. Phenolics were found only in the stem bark ethanol extract. The presence of these phenolic compounds may have contributed to the antibacterial properties of the stem bark ethanol extract and thus the usefulness of this plant in herbal medicament. Flavonoids were present only in the leaf aqueous extract. Phytosterols were also present only in the stem bark ethanol extract. Phytosterols have cholesterol reducing properties and may act in cancer prevention21. Triterpenes were found in the root and leaf ethanol extracts as well as in the root aqueous extract. Triterpenes have demonstrated antibacterial activities22. The presence of this phytochemical thus supports the antibacterial potentials of J. curcas. Tannins are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery23. Therefore its presence may have a significant effect in the inhibition of the test organisms as demonstrated by ethanol and pet-ether extracts. The absence of many of the phytochemicals in both aqueous and pet-ether extracts may be responsible for the little antibacterial activity shown by these extracts. The activity of the aqueous extracts against the bacterial strains investigated in this study is consistent with previous works which show that aqueous extracts of the plant generally showed little or no antibacterial activities24,25,26,27.

Antibacterial Activity

Root extracts of the various solvents exhibited antibacterial activity on the test organisms with the leaf ethanol extract demonstrating the highest activity (Zone of inhibition = 12.20±0.10mm against S. typhi). (Table 2). The result is consistent with the type phytochemicals present in the root ethanol extract. All the stem bark extracts, with the exception of the stem bark pet-ether extract, showed some activity on both test organisms. The stem bark ethanol extract again demonstrated the highest antibacterial activity in this category. (Zone of inhibition = 12.00±0.60 against E. coli). The leaf ethanol extract showed significant antibacterial activity on both test organisms. (Zone of inhibition = 13.00±0.50mm against S. typhi; 12.80±0.30mm against E. coli). The result reveals that the ethanol extracts of the plant have greater antibacterial potential than the aqueous and pet-ether extracts. It further reveals that the root, stem bark and leaf ethanol extracts have comparable antibacterial activities on the test organisms The MIC of the ethanol extracts ranged between 4.00 and 6.00 mg/ml. The MIC of the pet-ether extracts ranged between 8.00 and 10.00 mg/ml (Table 3). The extract with the least MIC is the most potent. The MIC of the aqueous extracts was not determined due to the low antibacterial activity demonstrated by the aqueous extracts. This supports results from previous works. The MBC of the extracts were consistent with the MIC and increased with the MIC. The MBC of the ethanol extracts ranged between 8 and 10mg/ml while those of the pet-ether extracts ranged between 18 and 22mg/ml (Table 4).

CONCLUSION

In conclusion, the aqueous, ethanol and pet-ether extracts of the root, stem bark and leaf of J. curcas showed varying inhibitory activities against Salmonella typhi and Escherichia coli. The phytochemicals present in the extracts play significant roles in inhibiting the test bacteria. The ethanol extracts showed superior antibacterial activities compared with both the aqueous and pet-ether extracts. The root, stem bark and leaf ethanol extracts demonstrated comparable antibacterial activities against S. typhi and E. coli. This suggests that the root, stem bark and leaf of J. curcas may contain similar bioactive compounds and thus, are all potential candidates in the search for potent antibacterial agents from medicinal plants

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Table 1: Results of phytochemical screening of extracts of *J. Curcas*

Phytochemical Constituents	Ethanol Extracts			Aqueous Extracts			Petroleum Ether Extracts		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Saponins	-	-	++	+++	-	++	-	-	-
Reducing sugars	+++	+++	+++	+++	+++	+++	++	+++	++
Phenolics	-	++	-	-	-	-	-	-	-
Polyuronides	-	-	-	-	-	-	-	-	-
Alkaloids	+++	+	+++	+	-	+	-	-	++
Cynogenic glycosides	-	-	-	-	-	-	-	-	-
Anthracenosides	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	++	-	-	-
Triterpenes	+++	-	++	++	-	-	-	-	-
Phytosterols	-	++	-	-	-	-	-	-	-
Tannins	+++	+++	++	-	-	-	+	++	-

KEY: +++ = Abundant; ++ = Moderately abundant; + = Present; - =Absent

Table 2: Antibacterial profile of extracts of *J. Curcas*

Test organism	Zone of inhibition (mm) (Mean±SEM)									
	Aqueous Extracts			Ethanol Extracts			Petroleum ether Extracts			Control
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	
S. typhi	4.00 ±0.00	3.50 ±0.30	2.60 ±0.40	12.20 ±0.10	10.60 ±0.60	13.00 ±0.50	5.60 ±0.00	7.10 ±0.50	0.00 ±0.00	15.50 ±0.90
E. coli	4.00 ±0.20	7.80 ±0.40	5.90 ±0.00	12.10 ±0.00	12.00 ±0.60	12.80 ±0.30	5.00 ±0.50	0.00 ±0.00	0.00 ±0.00	13.40 ±0.20

Table 3: Minimum Inhibitory Concentrations (MIC) of extracts of *J. curcas*

Test organism	Minimum Inhibitory Concentrations (mg/ml)									
	Aqueous Extracts			Ethanol Extracts			Petroleum ether Extracts			Control
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	
S. typhi	nd	nd	nd	4.00	4.00	4.00	10.00	8.00	nd	2.00
E. coli	nd	nd	nd	4.00	6.00	4.00	nd	nd	nd	2.00

Nd = Not determined

Table 4: Minimum Bactericidal Concentrations (MBC) of extracts of *J. curcas*

Test organism	Minimum Bactericidal Concentrations (mg/ml)								
	Aqueous Extracts			Ethanol Extracts			Petroleum ether Extracts		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
S. typhi	NA	NA	NA	8.00	8.00	8.00	22.00	18.00	NA
E. coli	NA	NA	NA	8.00	10.00	6.00	NA	NA	NA

NA = Not Applicable

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Phytochemical Screening and GC-MS Studies on the Ethanolic Extract of Cayratia pedata

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A B S T R A C T

Cayratia pedata is an indigenous herb belonging to the family Vitaceae. The leaves of Cayratia pedata are used as astringent and refringent. The phytoconstituents present in this medicinal plant have been studied to possesses diuretic activities. The present research was designed to investigate the ethanolic extract of the medicinal plant Cayratia pedata, which contains alkaloids, steroids, carbohydrates, terpenoids, tannin, phenolic compounds and flavonoids and all of them were confirmed through phytochemical screening and GC-MS analyses.

***Key Words**:GC-MS, Phytochemical screening, Diuretic activity, Cayratia pedata, Vitaceae*

INTRODUCTION

Cayratia pedata1, (Tamil: Pannikkodi, Kattupirandai, Sanskrit: Suvaha, Gobhapadi, Malayalam: Velutta sori valli, Tripadi) is an indigenous herb belonging to the family Vitaceae. It is a woody climber with cylindrical stem and grown mostly in semi evergreen to evergreen forest. Traditionally, the leaves of this plant were used in the treatment of ulcers and diarrhea. The decoction of the leaves was used to check uterine and other fluxes2. The plant has also found to possesses anti-inflammatory3 and antinociceptive activities4 .The aim of the present study was to identify the phytocomponents of the plant through to GCMS analysis of the ethanolic extract of the plant leaves.

MATERIALS AND METHODS

Collection of plant materials

The leaves of the plant Cayratia pedata were collected from Kollimalaihills. They were identified and authenticated by, The Rapinet Herbarium, St. Joseph's college (Autonomous), Tiruchirappalli, Tamilnadu, India5.

Sample Preparation

The leaves of Cayratia pedata were shade dried and pulverized well. About 20g of the powdered leaves were soaked in 100 mL of ethanol. It was left for 24 hours so that alkaloids, terpenoids, and other constituents if present will get dissolved. The ethanolic extract was filtered using Whatmann (number 1) filter paper and the residue was removed.

Phytochemical Screening

Phytochemical screening of the plant leaf extract was carried out as per the methods and tests given by Harbone6 to decipher the presence or absence of various phytoconstituents.

Gas Chromatography—Mass Spetroscopy7

The ethonolic extract was subjected to GC-MS analysis on the instrument GC-MS SHIMADZU Qp2010 with Elite – DB-5M column and the GC-MS solution version 2.53 software. Initially oven temperature was maintained at 70 C for 2.0 minutes, and the temperature was gradually increased upto 300 C at 10.0/35.0 min and 4.0 µL of sample was injected for analysis .Helium gas 99.995% of purity was used as a carrier gas as well as a eluent. The flow rate of helium gas was set to 1.5 mL/min. The sample injector temperature was maintained at 260º C and the split ratio is 20 throughout the experiment periods. The ionization mass spectroscopic analysis was done with 70 eV. The mass spectra was recorded for the mass range 40-1000 m/z for about 35 minutes. Identification of components was based on comparison of their mass spectra. As the compounds separated, on elustion through the column,were detected in electronic signals. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization detector where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments were actually charged ions with a certain mass. The m/z ratio obtained was calibrated from the graph obtained which was called as the mass spectrum graph which is the fingerprint of the molecule. The identification of compounds was based on the comparisons of their mass spectra with NIST Library 2008 WILEY8, FAME.

RESULTS AND DISCUSSION

Phytochemical screening of the plant Cayratia pedata by GC-MS method

The phytochemical active compounds of Cayratia pedata were qualitatively analysed and the results are presented in Table.1 which indicates that the ethanolic extract of Cayratia pedata leaves showed the presence of phytochemical active compounds such as alkaloids, carbohydrates, steroids, tannin, phenolic compounds, flavonoids and terpenoids.

GC-MS Analysis

GC-MS analysis was carried out on the ethanolic extract of Cayratia pedata and 33 compounds were identified. The GC-MS analysis was done using the instrument GC-MS SHIMADZU QP2010 with GCMS solution version 2.53 software. The sample volume was 4.0 µL. The sample of ethanolic extract was run for 35 minutes. The Chromatogram (Figure.10) shows 7 prominent peaks in the retention time range 8.208 - 29.068. The peak at 18.080 retention time is having the peak area 49.82. This largest peak is due to the presence of Phytol (Mmass, 296). The Second less prominent peak at 29.068 retention time has the peak area 13.66 is due to the presence of Lupeol (Mmass, 426). The third less significant peak at 27.910 retention time with the peak area 7.21 is characteristic of Gamma-stigmasterol (Mmass, 414). The Fourth less prominent peak at 23.703 retention time with the peak area 6.32 denotes All-trans-Squalene (Mmass, 410). The other less prominent peaks at other retention times are given in Table 2. The total ion chromatograph (TIC) showing the peak identities of the compounds identified have been given in Figure 1.

CONCLUSION

The result of the present investigation reveals that the successive extracts of Cayratia pedata possessed significant diuretic activity which was analyzed by phytochemical screening and GC-MS analysis. The plant extract reveals the presence of alkaloids, carbohydrates, steroids, tannin, phenolic compounds, flavonoids and terpenoids. The GC-MS analysis of the ethanolic extract of Cayratia pedata reveals the presence of phytoconstituents belonging to the type-acids, esters, alcohols, ethers, etc.Thus, the medicinal plant Cayratia pedata is found to possess significant phytoconstituents. The presence of such a variety of phytochemicals may be attributed to the medicinal characteristics of this plant Cayratia pedata.

Table 1: Phytochemical screening of the leaves of *Cayratia pedata*

S.No.	Tests	*Results*
1.	Alkaloids	(+)`
2.	Amines	(-)
3.	Carbohydrates	(+)
4.	Cardiac Glycosides	(-)
5.	Steroids	(+)
6.	Saponins	(-)
7.	Fixed oils and Fats	(-)
8a.	Tannin	(+)
8b.	Phenolic compounds	(+)
9.	Proteins and Free amino acids	(-)
10.	Flavonoids	(+)
11.	Terpenoids	(+)

(+) Present (-) Absent

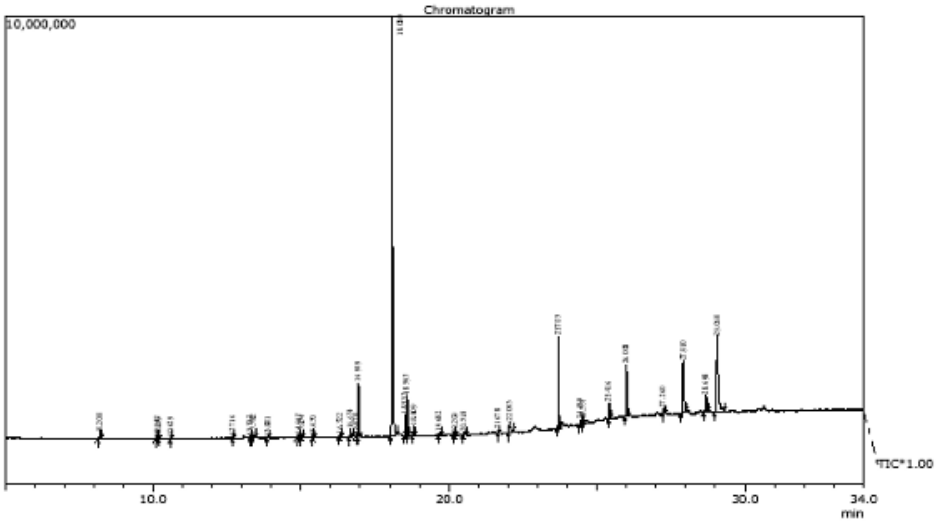

Fig.1. GC-MS Chromatogram of ethanolic extract of *Cayratia pedata.*

S.No	RT (min)	Name of the compound	Peak Area (%)
1.	8.208	L-Glutamic acid	0.57
2.	10.142	Biphenyl	0.16
3.	10.209	2-Methyl-4-heptanone	0.03
4.	10.629	2,6,10,10-Tetramethylbicyclo(7.2.0) undeca-1,6-diene	0.07
5.	12.716	Di-Isodectyl Phthalate	0.07
6.	13.302	1,2,4,5-Tetroxane,3,3,6.6-Tetraphenyl-	0.06
7.	13.382	3-Oxo-Alapha,-Ionol	0.37
8.	13.881	3-Buten-2-ol,4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)	0.08
9.	14.917	Methyl 7-hydroxy-2-methyl-3,5-octadienoate	0.33
10.	15.034	4-Hydroxy-3,5,5-Trimethyl-4-(1E)-3-oxo-1-butenyl)-2-cyclohexene-one	0.18
11.	15.430	Hexahydropseudoionone	0.08
12.	16.322	4-(2-Hydroxy-2,6,6-Trimethylcyclohexyl)-3-buten-2-one	0.25
13.	16.674	n-Hexadecanoic acid	0.76
14.	16.878	E-11- Hexadecanoic acid,Ethylester	0.13
15.	16.949	EthylHexadecanoate	3.18
16.	18.080	Phytol	49.82
17.	18.532	Ethyl(9Z,12Z)-9,12- Octadecanoate	1.21
18.	18.593	Ethyl Linolenate	2.74
19.	18.809	Ethyl Octadecanoate	0.69
20	19.682	1-Hexadecanol	0.32
21.	20.209	3,7-dimethyl-1-octyl methylphosphonofluoridate	0.09
22.	20.518	Ethyl icosanoate	0.30
23.	21.678	DEPH;1,2-Benzenedicarboxylicacid,bis(2-hylhexyl)ester	0.18
24.	22.043	2-Phenoxyl-2-phenylpropanic acid	1.65
25.	23.703	All-trans-squalene	6.32
26.	24.455	Methyl Linolenate	0.33
27.	24.537	Methyl cis-11,14,17-Icosatrienoate	0.38
28.	25.416	Gamma-Tocopherol	1.48
29.	26.008	Di-Alpha,- Tocopherol	4.58
30.	27.260	Stigmasterol	0.68
31.	27.910	Gamma-stigmasterol	7.21
32.	28.698	Lupenone	2.18
33.	29.068	Lupeol	13.66

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Phytochemistry and Pharmacological Activities of Silybum marianum: A Review

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A B S T R A C T

Silybum marianum (Milk thistle), a member of the Asteraceae family, is a tall herb with large prickly whiteveined green leaves and a reddish-purple flower that ends in sharp spines. Certain phytoconstituents were revealed such as silybin A, silybin B, isosilybin A, isosilybin B, silychristin, silydianin, apigenin 7-O-β-(2"O-α-rhamnosyl)galacturonide, kaempferol 3-O-α-rhamnoside-7-O-β-galacturonide, apigenin 7-O-βglucuronide, apigenin 7-O-β-glucoside, apigenin 7-O-β-galactoside, kaempferol-3-O-α-rhamnoside, kaempferol, taxifolin and quercetin. The plant is exclusively used as anti-diabetic, hepatoprotective, hypocholesterolaemic, anti-hypertensive, anti-inflammatory, anti-cancer, and as an anti-oxidant. Seeds of the plant are also used as an anti-spasmodic, neuroprotective, anti-viral, immunomodulant, cardioprotective, demulcent and anti-haemorrhagic. The plant is also serves as a galactagogue and used in the treatment of uterine disorders. This review paper focuses mainly on phytochemistry and pharmacological activities of the legendary plant milk thistle.

Key Words: *Silybum marianum, Silybin, Silychristin, Phytochemistry, Anti-diabetic, Hepatoprotective*

INTRODUCTION

Medicinal plants are important to the global economy. In 1980, WHO estimated the world trade at US$500 million1. Traditional medicine is an important part of African culture. More than 80% of Africans rely on plant-based medicine. Latin Americans also rely on traditional medicines for their health care needs2. In India, about 2500 plants have been reported to be used in ethno-medicine3. Silybum marianum, commonly known as 'milk thistle' belonging to family Asteraceae / Compositae is one of the oldest and thoroughly researched plants in the treatment of liver diseases4. It is being used as a general medicinal herb from as early as 4th century B.C. and first reported by Theophrastus5. Extract from the seeds of the milk thistle is being used traditionally as a herbal remedy against hepatotoxicity and acute and chronic liver diseases6 . Silymarin effects have also been indicated in various illness of different organs such as prostate, lungs, CNS, kidneys, pancreas, and skin7.

PLANT PROFILE

Milk thistle (Silybum marianum), is an annual or biennial native to the Mediterranean regions of Europe, North Africa and the Middle East and in some parts of USA8. In India, it is commonly found in Jammu and Kashmir 5. It grows to a height of three to ten feet with an erect stem that bears large, alternating, pricklyedged leaves. The common name, milk thistle, is derived from the "milky white" veins on the leaves, which, when broken open, yield a milky sap. Each stem bears a single, large, purple flower ending in sharp spines. The fruit portion of the plant is glossy brown or grey with spots. The plant grows at an altitude of 1800-2400m in rocky or sandy soil. The plant cherish with flowers in monsoon

season from June to August9.

Taxonomical Classification[10]

Domain	:	Eukaryota
Kingdom	:	Plantae
Subkingdom	:	Viridaeplantae
Phylum	:	Tracheophyta
Subphylum	:	Euphyllophytina
Infraphylum	:	Radiatopses
Class	:	Magnoliopsida
Subclass	:	Asteridae
Superorder	:	Asteranae
Order	:	Asterales
Family	:	Asteraceae
Genus	:	Sylibum
Species	:	Marianum
Botanical name	:	*Silybum marianum*

Vernacular Names[11]

Dutch : Mariendistel, Vrouwendistel
English : Holy thistle, Lady's thistle, Milk thistle
French : Artichautsauvage, Chardon marie
German : Feedistel, Mariendistel, Silberdistel
Greek : Silybon
Italian : Cardodel latte, Cardomariano
Malta : Blessed thistle
Romanian: Armurariu
Russian : Ostropestro
Spanish : Cardolechal, Cardolechero
Swedish : Sempertin

Morphology

Roots: Usually taproots, sometimes fibrous.
Stems: 20-150 cm high, rarely shorter, glabrous or slightly downy, erect and branched in the upper part.
Leaves: Alternate, large, white veined, glabrous with strongly spiny margins.
Inflorescences: These are large and round capitula, solitary at the apex of the stem or its branches, surrounded by thorny bracts.
Florets: Florets are hermaphrodite, tubular in shape with a red-purple corolla.
Fruits: Hard skinned achenes 6 to 8 mm long, generally brownish in color with a white silk like pappus at the apex 10, 12.

Microscopy

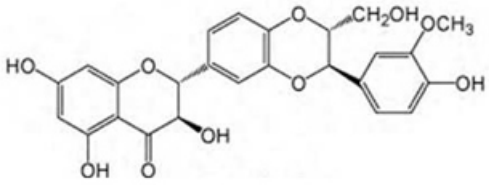
Pericarp epidermis a colourless palisade layer of cells (about 75 mm long and 8 mm wide) with a strongly thickened outside wall, which reduces the lumen in that part of the cell to a slit; sub-epidermal layer composed of colourless, thin-walled, parenchyma cells or groups of parenchyma cells alternating with a variable number of pigmented cells; innermost layer mostly collapsed and containing cigar-shaped or monoclinic prismatic crystals of calcium oxalate. Testa epidermis consists of large, lemon-yellow, palisadelike, elongated cells (about 150 mm long) with striated walls and narrow lumen widening slightly at the ends; sub-epidermal layers have lignified and pitted cells13, 14.

TRADITIONAL USES

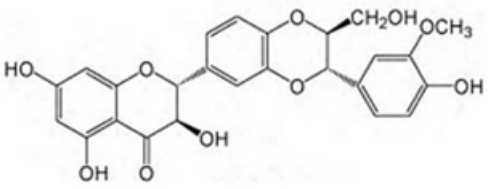
In Europe, milk thistle is used in jaundice and other biliary affections. As a diet or in infusion it is said to be a reliable galactagogue. Silymarin is often used as supportive therapy in food poisoning due to fungi.
Root: Root is eaten boiled as a pot herb.
Herb: Herb is used for intermittent fevers, dropsy & uterine troubles. A decoction of it is said to be beneficial as an external application in cancer.
Leaves: Leaves are sudorific and aperient. Young leaves serve as salad and flowering heads are consumed by diabetics.
Seeds: Seeds are pungent, demulcent and antispasmodic. They are used for the treatment of jaundice and calculi of liver and gall-bladder and are useful in controlling haemorrhages. Alcoholic extracts of the seed and to a lesser extent of the plant also, increase peristalsis of the small intestine and galenical preparations, both of the seed and oil are mild purgative. Seeds are used as a substitute for coffee15.

PHYTOCHEMISTRY

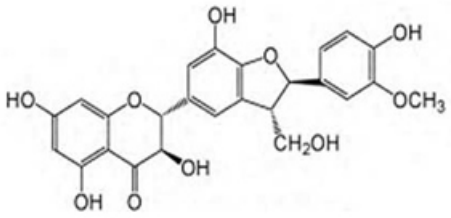
The pharmacological active ingredient present in the plant is the flavonoid complex silymarin, which is the main constituent with about 80% of the extract. Silymarin consists of a large number of flavolignans including silybin (or silybinin), isosilybin, silydianin and silychristin16, 17. Besides these taxifolin, quercetin, betaine and silybonol have also been isolated by Kren V. et al18, 19.



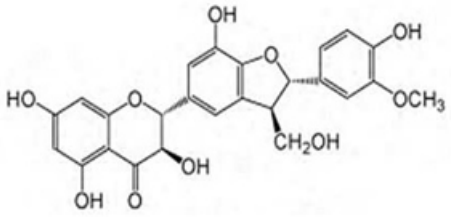
Silychristin



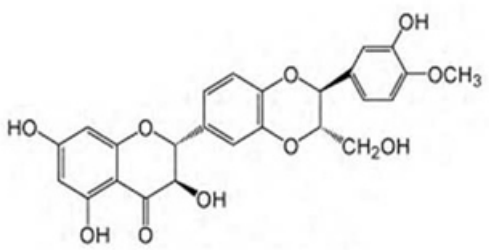
Silydianin



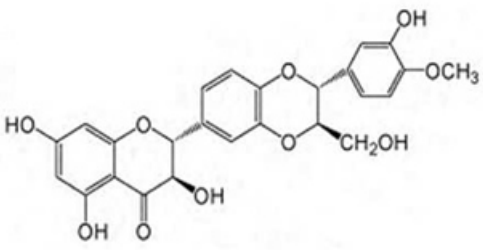
Silychristin A



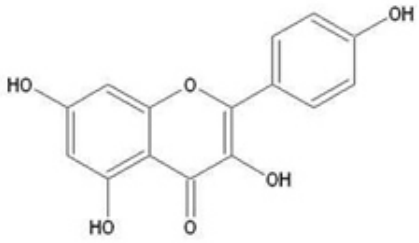
Silychristin B



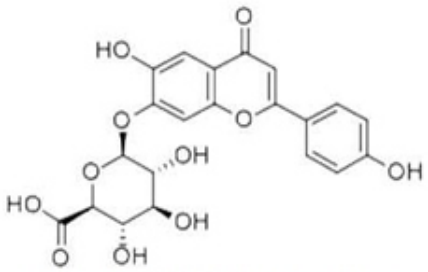
Silybin A



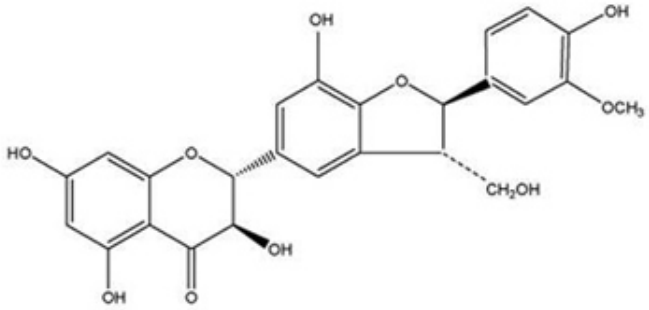
Silybin B



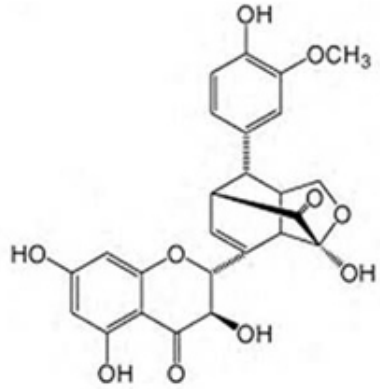
Kaempferol



Apigenin 7-O-β-glucuronide



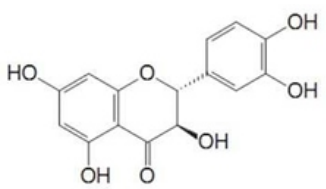
Isosilybin A



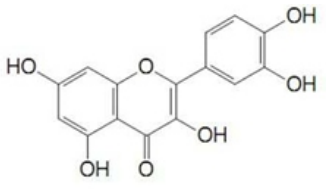
Isosilybin B

Ahmed A, et al. reported seven flavonoids from an aqueous methanol extract of the flowers of Silybum marianum viz., apigenin 7-O-β-(2″-O-α-rhamnosyl)galacturonide, kaempferol 3-O-α-rhamnoside-7-O-βgalacturonide, apigenin 7-O-β-glucuronide, apigenin 7-O-β-glucoside, apigenin-7-O-β-galactoside, kaempferol-3-O-α-rhamnoside and kaempferol20.

Barreto JFA, et al. examined the batch extraction of silymarin compounds from milk thistle seed meal in 50, 70, 85, and 100°C water as a function of time. After 210 min of extraction at 100°C, the yield of taxifolin was found to be as 1.2 mg/g of seed, a 6.2-fold increase over the results obtained in a Soxhlet extraction with ethanol on pretreated (defatted) seeds. Similarly, the yield of silychristin was reported as 5.0 mg/g of seed, a 3.8-fold increase. The yields of silybinin A and silybinin B were 1.8 and 3.3 mg/g of seed, respectively, or roughly 30% of the Soxhlet yield. The more polar compounds (taxifolin and silychristin) were preferentially extracted at 85°C, while the less polar compounds (silybinin A and B) were favored at 100°C21.



Taxifolin



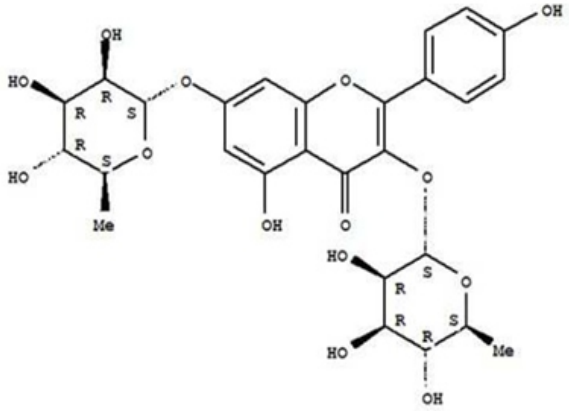
Quercetin

Bilia AR, *et al.* separated all classes of flavonoid (flavones, flavonols, flavanonols, and flavanolignans)

using simple reversed-phase HPLC method. The relative standard deviations obtained from investigation of the repeatability of the method were reported as silybin 2.33%, taxifolin 2.19%, quercetin 2.08% and isoquercitrin 2.21%22.

Parry J, et al. investigated for FA, tocopherol, and carotenoid compositions, total phenolic contents (TPC), antioxidant activities, oxidative stability index (OSI), color, and physical properties in cold-pressed milk thistle seed oils. The total mono-unsaturated fatty acid (MUFA) and poly-unsaturated fatty acid (PUFA) contents were found to be as 25.2 and 61.1 g/100g of oil, respectively. The ratio of oleic to linoleic acid was 0.4, whereas the carotenoid content was 2.30 μmol/kg. The α-, γ-, and δ-tocopherol contents were reported as 156.3 ± 0.9, 35.1 ± 0.4 and 7.0 ± 0.0 mg/kg, respectively. The oxidative stability index (OSI) and refractive index were found to be as 13.3 ± 0.3h and 1.433523.

Wallace SN, et al. evaluated the milk thistle seeds containing flavanolignan and dihydroflavanol compounds. The maximum yields of taxifolin, silychristin, silydianin, silybinin A, and silybinin B in ethanol were reported as 0.6, 4.0, 0.4, 4.0, and 7.0 mg/g of defatted seed, respectively24. Duan L, et al. extracted milk thistle seeds with hot water at 100, 120, and 140° C using the same water flow rate (0.30 mL/min) and seed meal particle size (0.4 mm). The yields of taxifolin, silychristin, silybinin A and silybinin B at 140° C were reported as 0.5, 2.4, 1.2 and 2.0mg/g seed25.



Kaempferol-3-O-α-rhamnoside

PHARMACOLOGICAL ACTIVITIES

Anti-diabetic Activity

Huseini HF, et al. had worked on the valuable effect of antioxidant nutrients on the glycemic control of diabetic patients in experimental and clinical studies. The average fasting blood glucose level in the silymarin group at the beginning of the study was reported as 156 ± 46 mg/dL, which decreased significantly ($p<0.001$) to 133 ± 39 mg/dL after 4 months of silymarin treatment. The average fasting glucose level in the placebo group at the beginning of the study was 167 ± 47 mg/dL, which increased significantly ($p<0.0001$) to 188±48 mg/dL after 4 months of placebo treatment26.

Hepatoprotective Activity

Silymarin protect sliver cells against many hepatotoxins in humans and animals. Desplaces J, et al. examined severe Amanita poisoning in 60 patients treated with infusions of 20 mg/kg of slibinin with

excellent results showing no death of the patients treated. Silymarin also offers liver protection tetracycline, d-galactosamine and thallium-induced liver damage and erythromycin estolate, amitryptiline, nortryptiline and tert-butyl hydroperoxide exposure of neonatal hepatocytes27, 28. Vogel G, et al. reported the action of silybinin for anti-hepatotoxic activity against Amanita phalloides, ethanol, paracetamol (acetaminophen) and carbon tetrachloride-induced liver injury. Silybinin produced hepatoprotective effects in acute viral hepatitis, alcohol related liver cirrhosis at doses ranging from 280 to 800 mg/day29.

Madani H, et al. treated with the polyphenolic extracts of Silybum marianum which reduced the level of serum aminotransferases activities including SGOT and SGPT and also alkaline phosphatase (ALP) activity and the level of total bilirubin, comparing with thioacetamide group. The extracts were injected to the rats, at the dose of 25 mg/kg body weight together with thioacetamide at the dose of 50 mg/kg body weight. It was reported as the liver cells around central veins showed relatively a high number of necrosis apoptosis. Some acute and chronic inflammatory cells were also seen around the necrotic cells. In the groups treated with polyphenolic extracts of the plant, central veins were congested and dilated30.

Hypocholesterolaemic Activity

Skottova N, et al. reported the hypocholesterolaemic activity of silymarin on the basis of experimental evidence showing that silybin inhibits HMG-CoA reductase activity in vitro; and silymarin improved the binding of low density lipoproteins (LDL) to rat hepatyocytes, decreased the liver cholesterol content in rabbits fed with a high-cholesterol diet, decreased the plasma-cholesterol and LDL-cholesterol levels in hyperlipaemic rats31.

Anti-hypertensive Activity

Jadhav GB, et al. evaluated the effect of silymarin (300 mg/kg and 500 mg/kg, p.o, for 4 weeks) in Deoxycorticosteroneacetate (DOCA) salt induced hypertensive rats. It was reported that silymarin (300, 500 mg/kg/day, p.o) significantly ($p<0.05$) reduced systolic blood pressure, heart rate, basal arterial blood pressure and pressor responses to nor-adrenaline, adrenaline, phenylephrine and serotonin (5-HT) in animals treated with DOCA salt as compared with DOCA-salt hypertensive rats32.

Anti-inflammatory Activity

Dehmlow C, et al. reported the effect of silybin which inhibited the synthesis of leukotriene B4 (IC50 15 µmol/l) in isolated rat Kupffer cells, but had no effect on prostaglandin E2 formation at concentrations up to 100 µmol/l. Minonzio F, assessed the anti-inflammatory activity of silybin in human polymorphonuclear leukocytes in vitro. The mechanism of anti-inflammatory activity involved the inhibition of hydrogen peroxide formation33, 34.

Anti-oxidant Activity

Haddad Y, et al. proved that the production of superoxide anion radicals and nitric oxide after treatment in the isolated rat Kupffer cells with silybin (IC50 80 µmol/l) was inhibited. Treatment with silibinin (200 mg/kg) improved liver steatosis and inflammation and decreased non-alcoholic steatohepatitis-induced lipid peroxidation, plasma insulin and plasma tumor necrosis factor-α (TNF-α). Silibinin also decreased superoxide radical ($O2 \bullet -$) release, and returned the relative liver weight as well as GSH back

to normal35.

Neuroprotective Activity

Kittur S, et al. evaluated that milk thistle enhanced nerve growth factor (NGF)-induced neurite outgrowth in PC-12 neural cells and prolonged their survival in culture. Milk thistle extract also protected cultured rat hippocampal neurons against oxidative stress-induced cell

Anti-cancer Activity

Bhatia N, et al. worked on the treatment of different prostate, breast, and cervical human carcinoma cells with silibinin resulted in a highly significant inhibition of both cell growth and DNA synthesis in a timedependent manner with large loss of cell viability only in case of cervical carcinoma cells. The higher doses (100±200 μM) of silymarin induced programmed cell death specifically in human ectocervical carcinoma A431 cells37.

Anti-viral Activity

Das SK, et al. evaluated the inhibitory action on inflammatory and cytotoxic processes induced by viral infection. It was reported that silibinin strongly inhibited growth of both HepG2 (hepatitis B virus negative; p53 intact) and Hep3B (hepatitis B virus positive; p53 matured) cells with relatively more cytotoxicity in Hep3B cells which is associated with apoptosis induction. Silymarin also showed inhibitory activity against other viruses in different cell lines38.

Immunomodulatory Activity

Meeran SM, et al. reported the ultraviolet radiation-induced immunosuppressive activity of silymarin in experimental rodents. It was found as silibinin inhibited the activation of human T-lymphocyte, human polymorpho-nuclear leucocyte. Silymarin also significantly suppressed the inflammatory mediators, expression of histocompatibility complex molecules and nerve cell damage. Long-term administration of sliymarin improved immunity by increasing T-lymphocytes, interleukins and reducing all types of immunoglobulins39, 40.

Cardioprotective Activity

Vereckei AS, et al. worked on the activity of Amiodarone as a very potent antiarrhythmic drug however, its use is limited due to direct cytotoxicity, development of lysosomal phospholipidosis, indirect immunologically mediated toxic effects and membrane destabilization41. Administration of silybin together with amiodarone decreased significantly lysosomal phospholipidosis42 and this effect was further in combination with vitamin E, as demonstrated by Agoston M, et al43. During the amiodarone treatment (rats) silymarin itself as well as in combination with vitamin E significantly decreased conjugated diene concentration44 but not attenuated the antiarrhythmic activity of amiodarone, as reported by Gyonos I, et al45.

ADVERSE EFFECTS

The main adverse effects reported are headaches, gastroenteritis and dermatological symptoms; amongst them gastrointestinal symptoms at higher dose (> 1500 mg/day) are the most common which involves mild laxative effect due to increased bile secretion and flow, reported by Kren V, et al18, 46. Allergic reactions to milk thistle have been reported47. Burgess CA, reported that silymarin with other drugs that were conjugated by uridine diphosphoglucuronosyl transferase (UGT1A6/9) led to a reduction in the clearance of certain drugs and a potential for increased toxicity48. Anaphylactic shock has been reported in a patient ingesting a tea prepared from crude drug49.

NUTRITIONAL VALUE

Fixed oil (16% to 18%), betaine, trimethylglycine and amines. The seeds contain 1.5-3% flavolignans, collectively referred to as silymarin; 20-30% fixed oil, of which approximately 60% is linoleic acid, approximately 30% is oleic acid, and approximately 9% is palmitic acid; 25-30% protein; 0.038% tocopherol; 0.63% sterols, including cholesterol, campesterol, stigmasterol, and sitosterol; and some mucilage50.

MARKETED FORMULATIONS51-53

Dosage Form	Dose	Supplier	Price	Uses
Tablets (Uncoated)	400mg	Almeta Health Labs	Rs.132.75/-	Stomach and liver tonic
Tablets (Coated)	450mg	Apex Neutraceuticals, LLC	Rs.153.5/-	Antioxidant, anti-inflammatory
Tablets (Coated) (Livergol®)	70mg	Goldaru Pharmaceutical Lab, Iran	N/A	Hepatoprotective, choleretic
Capsules (Thisilyn®)	175mg	Nature's Way, USA	US$ 27	Stomach and liver tonic
Capsules (Legalon®)	70mg	Madaus AG, Cologne, Germany	N/A	In liver toxicity, hepatic cirrhosis
Aqueous suspensions (Siliphos®)	200mg	Indena, Italy	N/A	Hepatocyte protection
Extract	175mg	Dietceutical Supplements, LLC, USA	Rs.177.5/-	Stomach and liver tonic
Powder	275mg	Dietceutical Supplements, LLC, USA	Rs.177.5/-	Stomach and liver tonic
Milk thistle complex	450mg	Dietceutical Supplements, LLC, USA	US$ 125	Stomach and liver tonic

Milk thistle extract is now marketed as silymarin and silybinin capsules and tablets with an improved bioavailability under the trade names like Livergol®, Silipide® (Siliphos®) and Legalon®54. Indena, Italy

experimented in rats that after oral administration of 200 mg/kg of silybin, the plasma levels of silybin and its conjugated metabolites were below the analytical detection limit, while, after oral administration of Silipide® (200 mg/kg as silybin) the plasma levels of silybin (free and total) were easily measurable, being well absorbed within minutes when in phytosomal form55, 56. Livergol® capsules were tested for the study of acute, viral hepatitis, in which 29 patients were treated with silymarin showing a definite therapeutic influence on the characteristic increased serum levels of bilirubin and liver enzymes

compared with a placebo group57, 58. In a study of Livergol® capsules in chronic viral hepatitis, silymarin was shown to result in dramatic improvement. Use at a high dose (420 mg of silymarin) for periods of 3-12 months resulted in a reversal of liver cell damage (biopsy), an increase in protein level in the blood, and a lowering of liver enzymes. Common symptoms of hepatitis (abdominal discomfort, decreased appetite, and fatigue) were all improved57, 59.

CONCLUSION

This article briefly reviews the phytochemistry, pharmacological, therapeutic applications, traditional knowledge and different formulations of the plant Silybum marianum. The plant had been extensively used as a medicinal and legendary plant since a long period of time. Some of its branded products in the market along with its dose and price have also been mentioned. This is an attempt to compile and document the information on different aspects of S. marianum and highlight the needs for research and development in future.

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