SALVADORA PERSICA : A POTENTIAL SOURCE FOR TREATMENT OF HYPERCHOLESTEROLEMIA

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Abstract

Various plant extracts are widely used as traditional drugs, because of their tremendous medicinal potential and multifaceted biological functions. However, there are no detailed botanical studies on this plant parts to help in the proper identification. It is essential that any crude plant extract used for medicinal purpose needs to be subjected to botanical inspection to avoid side effects in the body. The morphological, anatomical and phytochemical analysis is the best solution for identification of such crude plant parts. Traditionally, it is believed that *Salvadora persica* has capacity to reduce blood cholesterol level. To confirm this, phytosterols content was measured from juvenile to mature stage leaf of *Salvadora persica* to know at which stage maximum phytosterols accumulate in leaf. Finally, characterization of phytosterols was done from mature leaf ethanolic extract using TLC and GC-MS analysis. It was concluded from the above analysis that leaves of *Salvadora persica* are essential source of β-sitosterol and can be utilized repeatedly for maintenance of blood cholesterol level.

Key words : Hypercholesterolemia, β-sitosterol, *Salvadora persica*

Introduction

Natural products are the main sources of bioactive molecules and have played a major role in discovery of lead compounds for the development of drugs for treatment of human diseases (Newman & Cragg 2007). Humans exploit natural plant products as a source of drugs, flavouring agents, fragrance and for a wide range of other applications. The use of plant parts as medicine is well known in rural areas of many developing countries. They believe that use of traditional medicines obtained from plants is relatively safer than synthetic alternatives (Brindha & Saraswathy 2002). The current scenario exhibits the demand for plant drugs throughout the world. The quality, efficacy and safety of plant drugs can be ensured by finding reliable characteristics through botanical identification of plant material. This would ensure the reliability and repeatability of pharmacological and clinical research to understand their bioactivity and to enhance the product quality control.

Salvadora persica is a well known medicinal plant which belongs to the family Salvadoraceae. It is commonly known as the Meswak tree, because the roots

and twings of this tree have been used for oral hygiene and dental care. It is found in shrub savannah, from northwestern India to Africa. It is an evergreen shrub, 4-6 m tall with a short trunk, white bark and smooth green leaves (Ahmad H., Rajagopal K. 2013). Aerial parts of this tree are used for the preparation of a number of medicinally important products such as abrasives, antiseptics, astringent, detergents, enzyme inhibitors and fluorides (Kumar *et al*. 2012). Roots are used for treatment of gonorrhea and relieve the pain due to spleen troubles. Bark is used for treatment of fevers. Leaves are used for treatment of asthma, cough and piles. Carminative and diuretically properties of fruits are used for treatment of rheumatisms (Khatak *et al*. 2010). The plant has a capacity to treat these diseases due to the presence of biologically active compounds such as salvadoricine, salvadourea, trimethyl amine, di-benzyl thiourea, rutin, thioglucoside, chlorine, potash, sulphur etc (Anonymous 1972, Malik et al 1987). Through traditional knowledge, It is believed that Salvadora persica has capacity to reduce blood cholesterol level. However, it is essential that before its repeated use for medicinal purpose, it should be subjected to botanical inspection to avoid side effects in the body. Since, Phytosterols are

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added to foods because of their capacity to reduce the absorption of cholesterol in the gut and thereby it lowers the blood cholesterol level of the human body (Hayes *et al.* 2002, Vorster *et al.* 2003). They are widely used in the cosmetics field as active ingredients in creams and lipsticks. After their absorption from the diet they are transferred from the plasma to the skin, playing an important role in the constitution of skin surface lipids (Piironen *et al.* 2000, Ostlund 2002, Quilez *et al.* 2003, Puglia and Bonina 2008, Rawal *et al.* 2015). Therefore, an attempt has been done to know the presence of phytosterols in leaves of *Salvadora persica* through biochemical analysis.

Materials and Methods

1. Sample preparation

Leaves of *Salvadora persica* were collected from the plant grown in botanical garden of Saurashtra University. Length and width of each leaf was measured. On the basis of their size leaves were divided in to eight stages (depending on their developmental stages) (fig: 1). All the leaf samples were washed thoroughly with distilled water; blot dried and was kept for drying in oven at 40°C for two days. Dried leaf samples were crushed to powder and were stored in vacuum tight storage vial for future analysis.

2. Anatomical analysis

Transverse sections (T.S.) were taken from each developmental stage of leaf with the help of sharp blade. Anatomical features of T.S. of leaf tissue were observed after staining with saffaranine and toluene blue stain, under light microscope (Carl Zeiss). Images were documented using Axiovion 4 software.

3. Isolation of phytosterols

2.5 g of leaf sample was weighed in a 200 ml of wide mouth bottle containing 100 ml of 50% acetic acid (prepared in ethanol). It was mixed properly and then, the extract was incubated in water bath for 10 minutes at 70°C. After incubation, the plant extract was filtered using Whatmann filter paper. Then, concentrated ammonium hydroxide solution was added dropwise to the filtered plant extract until the precipitation was observed. The whole solution was allowed to settle down and the precipitate was collected by centrifugation at 5000 xg. Further, precipitates were washed with dilute ammonium hydroxide solution. The precipitated residues were dried and then dissolved in ethanol.

4. Estimation of total phytosterols

Leaf extract (1ml) was mixed with 1ml of ferric chloride (0.05% $FeCl_3.6H_2O$ in acetic acid). It was incubated for 10 min. Then, it was centrifuged at 5000 xg for 5min. Clear supernatant was collected and it was labeled as unknown. For, standard calibration curve 1ml of cholesterol standard was mixed with 1ml of physiological saline. It was incubated for 10 min. and then centrifuged at 5000 xg for 5 min. Clear supernatant was collected and aliquots of it were diluted using 0.05% $FeCl_3.6H_2O$ in range 10 mg to 100 mg. 0.05 % $FeCl_3.6H_2O$ solution was taken as blank. 10 µl of sulphuric acid was added to all the tubes. They were allowed to incubate for 20 to 30 min. The optical density of unknown and standard was measured at 560nm in spectrophotometer.

5. Characterization of phytosterols

Characterization of phytosterols was done using thin layer chromatography. TLC plates were prepared using 20 × 20cm glass plate. Slurry of Silica gel G 254 was spreaded on the glass plate to form thin layer. Plate was kept on hotplate at 60°C for 10 minutes. Samples of isolated fatty acids were loaded on silica plate using glass capillary. The fatty acids were separated on silica plate using solvent system methanol: Water (1:1). Further, Chromatogram was observed under UV light at 366 nm. The spots observed in TLC plate were separately collected in microcentrifuge tube and residues were dissolved in their respective solvents. Centrifugation was carried out to pellet out silica gel. Supernatent was collected and it was used for GC-MS analysis. GC-MS analysis was carried out in software GC-MS solution version 2.53 SU3 which was associated with analytical instrument for data acquisition and processing. An RTX-5MS (30 m × 0.25 mm id, film thickness 0.25 ìm) fused-silica capillary column was used for analysis. Operating conditions included, initial oven temperature 70°C for 2 min then to 200°C at 25°C per min. and held for 5 min, and at last temperature of 300°C for 10 min at 3°C per min., splitless mode, injector and detector temperatures was 250°C and 300°C; carrier gas was Helium, 1.0 mL/min., injection volume was 2 µL. Mass spectrometer conditions was the voltage ionization 70 eV, ion source temperature 230°C and MS transfer line at 250°C. Qualitative data were obtained with comparison of spectra registered in a NIST library (NIST08).

Results and Discussion

In the present studies, leaves of Salvadora persica were divided into eight stages depending on the size of leaf. Microscopically, anatomical changes during growth in leaf tissue were observed by staining the transverse section (T.S.) of each leaf stage with toluene blue and saffranine. T.S. of *Salvadora persica* leaf showed that
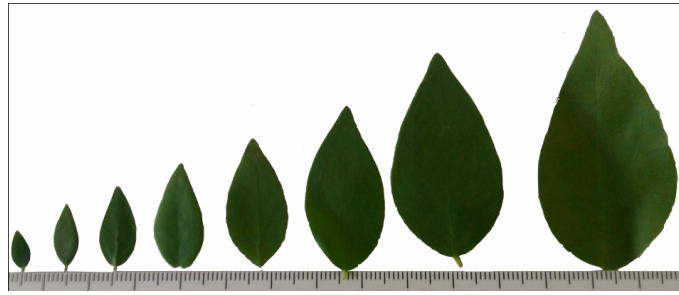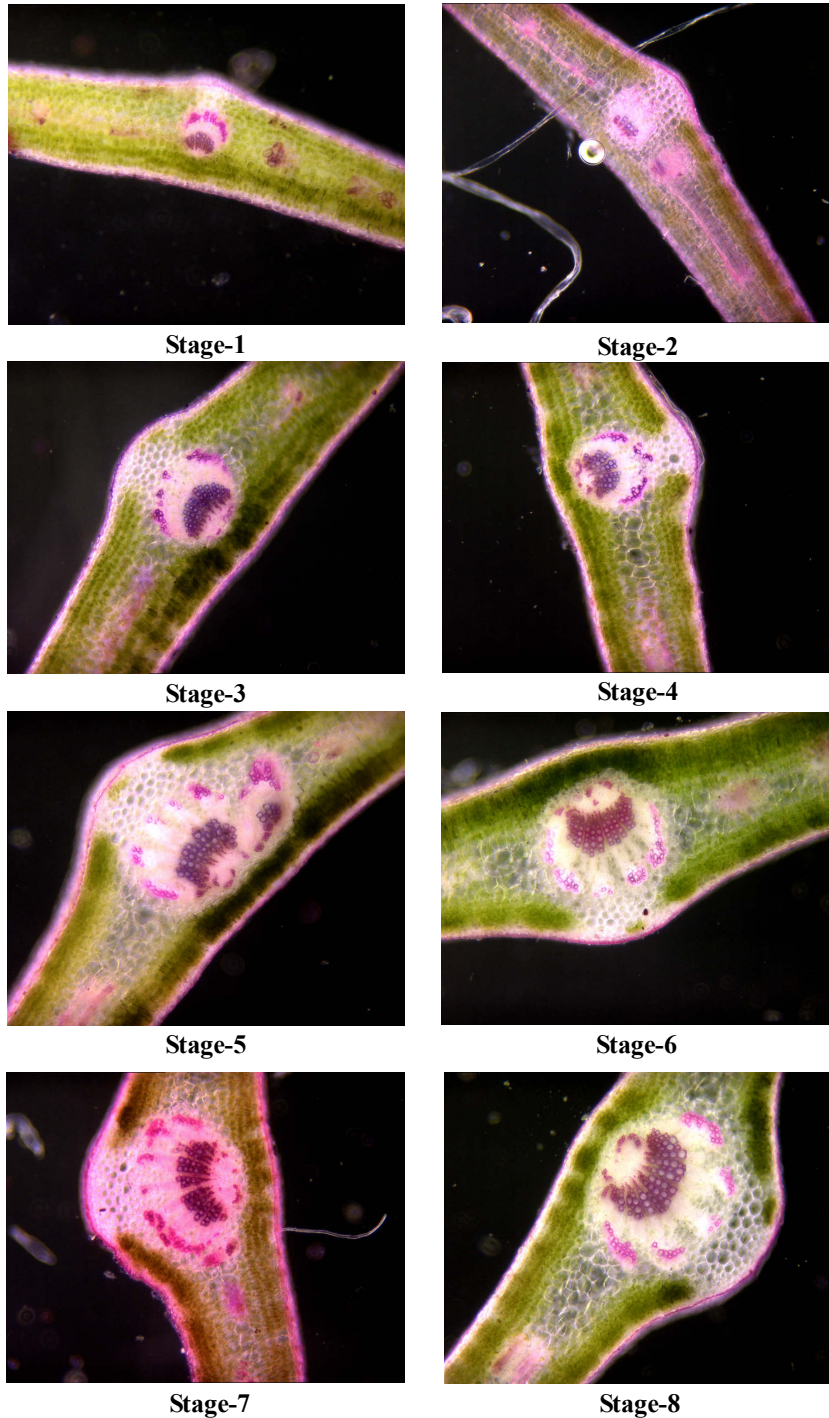
Fig.1: shows size of *Salvadora persica* leaf

Stage-1

Stage-2

Stage-3

Stage-4

Stage-5

Stage-6

Stage-7

Stage-8

Fig. 2: Shows T. S. of *Salvadora persica* leaf midrib stained with Toluene blue
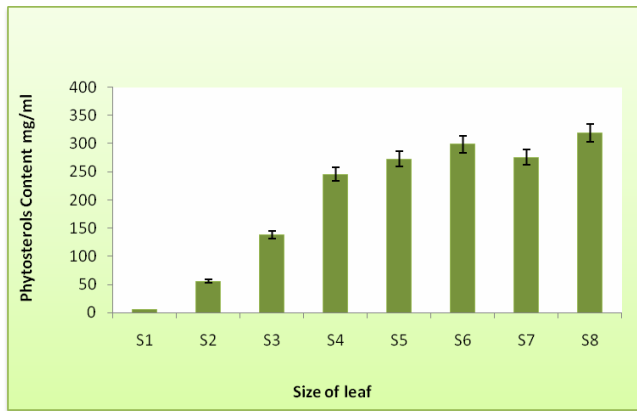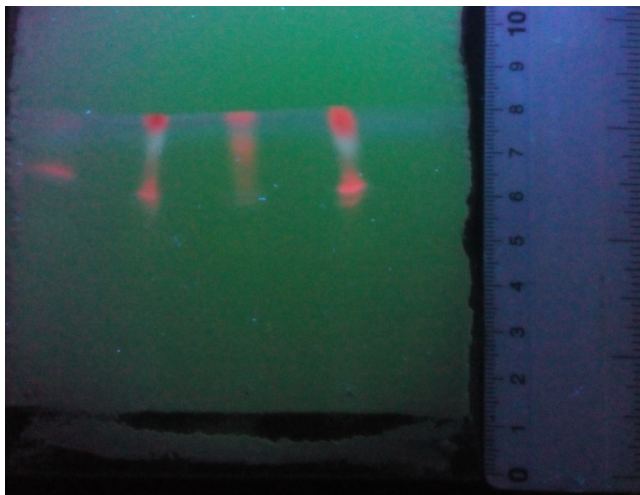
Fig.3: shows changes in phytosterol content during growth of *Salvadora persica* leaf

a) Ethanol extract
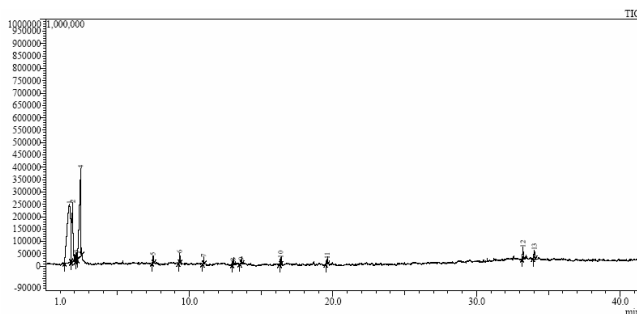**Fig.4 :** Shows TLC spots nder UV light (366nm)

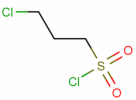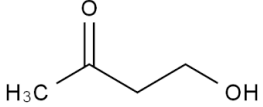**Fig. 5 :** Shows GC chromatogram of ethanolic *Salvadora persica* leaf extract

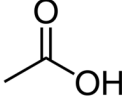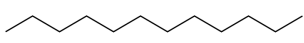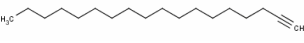upper epidermis was made of parenchyma cells forming outermost unilayer. The outer wall of cells were covered with thin cuticles. The stomata and chloroplast were absent. Lower epidermis was also made of unilayer parenchyma cells whose outer wall was covered with thin cuticles. Stomata and trichomes are present in this layer. Chloroplasts are present in guard cells of stomata. Lower epidermis helps in exchange of gases. Mesophyll is the tissue in between the upper and lower epidermis. It

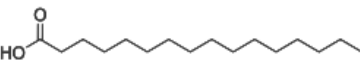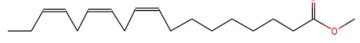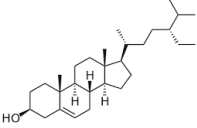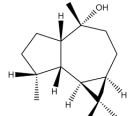was made up of three regions: (i) Palisade tissue is made of elongated oval shaped parenchyma cells. They are arranged parallel to each other at 90°C without intercellular spaces. They appear green in color due to the presence of chloroplast in them. They perform the function of photosynthesis. They are arranged just below the upper and lower epidermis. (ii) Spongy parenchyma tissue is made up of spherical parenchyma cells arranged irregularly just beneath the palisade parenchyma cells. They have intercellular space between them. They also contain chloroplast and perform the function of photosynthesis. (iii) Vascular tissue is arranged in scattered patches in the layer of spongy parenchyma. Vascular bundle of mid-rib is larger than other vascular bundles scattered in spongy parenchyma layer. Each vascular bundle is surrounded by a sheath of parenchyma cells called bundle sheath. Within the vascular bundle xylem and phloem are arranged in such a manner that vascular bundle is open collateral type. The xylem is towards upper epidermis and phloem is towards lower epidermis. The steller arrangement of vascular bundle is of ectophloic solenostele type (fig: 2).

After that total phytosterol content estimated from all the eight stages of leaf suggested that there was gradual increase in phytosterol content from stage 1 to stage 8 (6 mg to 318 mg). Hence, from the above analysis it was concluded that mature leaf contained higher amount of phytosterols than that of juvenile leaves (fig: 3). Mature leaves were further used for characterization of phytosterols. Residues of phytosterols isolated from mature leaf sample (stage 8) of *Salvadora persica* were dissolved in various solvents. Further they were characterized using TLC and GC-MS analysis. On TLC plate 3 spots were observed, under UV light (366 nm) (fig: 4). All three spots were scraped and content was dissolved in ethanol. Ethanolic solutions were then subjected to GC-MS analysis. The GC-MS analysis revealed that the ethanolic extract of *Salvadora persica* consists of compounds such as 1-Chloro-3-propanesulfonyl chloride, 2-Butanone, 4-hybroxy-, Acetic acid, Dodecane, Hexadecane, Tetradecane, 1-Octadecyne, Hexadecanoic acid, 9,12,15-Octadecatrienoic acid,methyl ester, (Z,Z,Z)- , Beta.-sitosterol and Ledol (fig: 5, table: 1).

From the above analysis, it was concluded that mature leaves of *Salvadora persica* is a potential source of β-sitosterol. Hence, it can be utilized as an important source of phytosterol for health benefits. It has an important immune modulatory and anti-inflammatory activity (Bouic *et al.* 1996, Bouic and Lamprecht 1999). It has chemical structure similar to that of cholesterol. It is a white, waxy powders with a characteristic odour, hydrophobic in nature and soluble in alcohol (Oja e*t al.* 2009). It reduces

Table1: Shows compounds identified by MS analysis from ethanolic extract of *Salvadora persica* leaves.

No.	Peak (compound)	Structure	R.Time	Area	Area%
1.	1-Chloro-3-propanesulfonyl chloride		1.640	4109909	45.75
2.	2-Butanone, 4-hybroxy-		1.875	1619921	18.03
3.	Acetic acid		2.422	2406751	26.79
4.	Dodecane		7.450	69895	0.78
5.	Hexadecane		9.316	71969	0.80
6.	Tetradecane		13.030	41660	0.46
7.	1-Octadecyne		13.597	32863	0.37
8.	Hexadecanoic acid		16.350	74783	0.37
9.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-		19.596	72770	0.81
10.	Beta.-sitosterol		33.222	165892	1.85
11.	Ledol		34.007	125798	1.40

cholesterol in blood and is used in treating hypercholesterolemia. It inhibits cholesterol absorption in the intestine (Kishore and Roy 2011). So, It can be utilized as an important nutrient in the diet. It is a good biomarker due to its biological activity. It is used as an antioxidant and antidiabetic agent. It is highly effective in the treatment of prostate enlargement, to boost the function of T cells and primes the immune system to function and operate more efficiently (Hadadare and Salunkhe, 2013). Human liver microsome studies had shown that â-sitosterol inhibits the cholesterol absorption (Rahman *et al*. 1994). It has shown antifertility, anti-inflammatory and antipyretic activity (Tapiero *et al*. 2003). It has proved to be chemopreventive in the colon cancer and breast cancer cell line by inhibiting the cancer cell proliferation (Von Holtz et al 1998, Awad *et al*. 1998,

Choi *et al*. 2003). It also plays important role in the treatment of HIV, by maintaining the CD4 lymphocyte count and regulating the immune system of the body. It is also observed in the in vitro toxicological studies that chronic administration of β- sitosterol is safe and non-toxic to human body (Sheng and Chen 2009). Hence, presence of β-Sitosterol in leaves of *Salvadora persica* makes it a potential source for treatment of hypercholesterolemia.

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