

Chemical Composition and Hepatoprotective Activity of *Saponaria officinalis* on Paracetamol-induced Liver Toxicity in Rats

Mallikarjuna Rao Talluri¹, Veda Priya Gummadi^{2,*}, Ganga Rao Battu²

ABSTRACT

Background: The present day life style causing different illness including liver diseases and different health complications. So, there is a need to identify new chemical entities with more efficiency in the treatment of diseases and less side effects. There were many reports in recent times, about identifying new drugs from different medicinal plants and also precursors for synthesis new bioactive molecules for treating various diseases. **Objective:** The present study was carried out on root parts (rhizomes) of *S. officinalis* for phytochemical analysis and hepatoprotective activity on Paracetamol-induced liver toxicity. **Materials and Methods:** The phytochemical analysis was carried out to know biological active compounds in different extracts of *S. officinalis* using standard procedures and quantified the total alkaloid and phenolic contents. Hepatoprotective activity of the *S. officinalis* extracts were carried out by using Paracetamol-induced hepatotoxicity in rats. **Results:** The phytochemical analysis of *S. officinalis* roots' extracts showed presence of sterols, terpenoids, glycosides, carbohydrates, proteins, flavanoids, alkaloids, phenols, tannins and absence of saponins and oils. The methanolic extract showed more phenolic and alkaloid contents on their quantification. The *S. officinalis* roots extracts are found to be safe at 2000 mg/kg b. w. in acute toxicity study and showed dose dependent percentage protection on liver toxicity. Methanol extract showed more activity at 500mg/kg b. w. and is comparable with standard drug Liv 52 on altered liver biomarker enzymes AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein with percentage protection 66.67%, 60.63%, 65.93%, 64.24% and 60.98%. **Conclusion:** The present study results indicates that phytochemical constituent's diversity in *S. officinalis* and those extracts possess hepatoprotective activity. Further studies are needed and should involve the isolation of pure, biologically active compounds.

Key words: *Saponaria officinalis*, Roots, Paracetamol, Liver, Toxicity.

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INTRODUCTION

Liver is one of the most important organ in the human body, it performs fundamental metabolisms in the body those include homeostasis, carbohydrates, proteins, lipids metabolisms and mainly detoxification.¹⁻² It is important to maintain healthy liver for good health conditions. The present day life style causing different illness including liver diseases.³ The drugs using for the present day diseases getting resistant to them and may cause side effects which can lead to iatrogenic diseases. The drugs mainly inducing the vital organs damage in the body like kidney, liver etc.⁴⁻⁶ The drug-induced liver diseases are more common compared to natural liver diseases around the world.⁷⁻⁸ The liver diseases like cirrhosis, cancers, alcoholic liver diseases and non-alcoholic liver diseases are main causes for the liver failure and causing mortality around the world.⁹ The development in modern medicine developed many medication for treatment many diseases including liver diseases.¹⁰⁻¹² But, these treatments are not satisfactory and as said above on the long-term usage of drugs causing different side effects.¹³ So, there

is a need to identify new chemical entities with more efficiency in the treatment of diseases and less side effects.

Traditional medicine is mainly based herbal treatments, which is based on knowledge, skills and practices based on the theories, beliefs and experiences in different indigenous cultures to maintain health. Herbal products that contain parts of plants or other plant materials as active ingredients.¹⁴ There are very little literature availability on plant materials as active ingredients in herbal medicines i.e. Ayurveda and Unani medicines and less scientific data regarding the identity and effectiveness of these herbal products. In current times, several researchers identifying new drugs and provide significant formulations from different medicinal plants and those drugs are also precursors for synthesis new bioactive molecules for treating various diseases including liver diseases.¹⁵⁻¹⁸ However, there were ample of medicinal plants available on world without their identification and scientifically not proven about their medicinal uses.

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Saponaria officinalis is one of such medicinal plant commonly called as soapwort plant belongs to Caryophyllaceae family. The different parts of *S. officinalis* has been used in traditional medicine, roots as blood purifier, diuretic, diaphoretic; roots and leaves for scrofula and skin diseases; sap for scabies, hepatic eruptions, to increase bile flow.¹⁹ But, there were no scientific reports on hepatoprotective activity of root parts of *S. officinalis*. In this regards, the present work carried out to evaluate the phytochemical analysis and hepatoprotective activity of *S. officinalis*.

MATERIALS AND METHODS

Chemicals and Drugs

All the chemical used in the present study were analytical grade. Diagnostic kits for serum parameter analysis were purchased from Span diagnostics Ltd, Gujarat, India. Paracetamol tablets and Live 52 was purchased from local medical shop, Viskhapatnam, A.P, India.

Preparation of extracts

The plant materials *Saponaria officinalis* (Voucher number: 1221) were collected from Tirupati region, Andhra Pradesh and authenticated by the taxonomist Dr. K. Madhava Chetty, Depart of Botany, Sri Venkateswara University. Freshly collected roots of plant material were shade dried and then material was milled to obtain a coarse powder. The powdered material was successively extracted with ethyl acetate, chloroform and methanol using in a Soxhlet apparatus. Finally, collected solutions were concentrated to dryness under vacuum by using Rota-vapor and stored in desiccators.

Phytochemical analysis

Phytochemical studies were carried out for different extracts of *Saponaria officinalis* to identify the presence of different phytochemical constituents using standard procedures.²⁰⁻²³

Total phenolic content estimation

Total phenolic content was estimated using folin-ciocalteau reagent. Briefly, folin-ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765nm. The intensity of the light absorption at that wave length is proportional to the concentration of phenols. By using standard Gallic acid calibration curve, measure the concentration of phenolic content in Gallic acid total equivalents using unit's mg/gm. (GAE).^{22,24}

Total alkaloid content estimation

The plant extract (1mg/mL) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of Bromocresol green (BCG) solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 mL volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of Mean \pm S.E.M.^{22,25}

Selection of Animals

Albino rats of either sex weighing between 180-250 gm were obtained from M/s. Mahaveer Enterprises, Hyderabad, India. The animals were maintained under controlled environmental conditions (temperature of $22 \pm 1^\circ\text{C}$) with an alternating 12h light-dark cycle and relative humidity of $60 \pm 5\%$), one week before the start and also during the experimental period. They were fed up with standard laboratory diet and water *ad libitum* during experimental period. Food was withdrawn 12h before the terminating experiment and water was allowed *ad libitum*. The protocols

were approved by Institutional Animal Ethics Committee and the lab was approved by CPCSEA, Government of India (Regd. No. 516/01/A/CPCSEA).

Acute toxicity studies

The acute toxicity study was conducted for extracts of *S. officinalis* extracts as per OECD guidelines 420 and regulations.²⁶ The albino rats of single sex, were selected and divided into three groups (n=6). They were maintained for one week before the experiment, under room temperature and allowed free access to water and diet. The animals were subjected for acute toxicity study using each extract at a dose of 2000 mg/kg orally in 3 groups at regular intervals of time for 24h. During this period, animals were under examination to note different conditions like skin changes, morbidity, aggressiveness, oral secretions, sensitivity to sound and pain, respiratory movements and finally their mortality.

Evaluation of hepatoprotective activity using Paracetamol-induced hepatotoxicity

Hepatoprotective activity of the *S. officinalis* extracts were carried out by using Paracetamol-induced hepatotoxicity in rats.²⁷ The animals for the activity, selected animals were divided into twelve groups (XII; n=6). Group I was served as control treated with only Saline (2 mL/kg b.w., per orally) for one week. Group II was administered with saline solution (2 mL/kg b.w., per orally) for 7 days. The animals of group III were administered with Liv 52 (25 mg/kg per day, p. o.) for 7 days. Group IV to XII animals were administered with ethyl acetate, chloroform and methanol extracts of *S. officinalis* (125, 250 and 500 mg/kg respectively) for 7 days. On 5th day for all groups excluding group I were treated with paracetamol (200 mg/kg b. w., s.c.). On 7th day after dosage of 2 h, the groups were anaesthetized by chloroform and blood was collected from retro-orbital plexus. The collected samples were centrifuged using centrifuge at 2400rpm for 15 min and then serum was clearly separated. The collected serum was analyzed for biochemical parameters i.e. AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels using Autoanalyzer.²⁷

Statistical analysis

All the results were expressed as mean \pm SEM and analysed by using Two-way ANOVA followed by Dunnet's multiple comparison test. All groups were compared with Liv 52 group. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns= Non significance.

RESULTS

Phytochemical analysis

Qualitative phytochemical screening of *S. officinalis* extracts revealed the presence of different phytochemical constituents like steroids, terpenoids, flavonoids, alkaloids, glycosides, phenols, tannins and carbohydrates. All the extracts revealed the presence of phenols, alkaloids, steroids and glycosides, tannins and gave negative results to amino acids, quinones and oils. The ethyl acetate and methanol extracts revealed the presence of saponins, flavanoids but the chloroform extract gave negative results. The chloroform and methanol extracts revealed the presence of carbohydrates, but ethyl acetate extract gave negative results (Table 1). The quantified phenolic content of *S. officinalis* extracts was ranging from 17.52 ± 0.46 to 22.51 ± 1.05 (mg/gm). The methanol extract has more phenolic content 35.47 ± 0.42 (mg/gm) than other extracts and alkaloid content was ranging from 18.43 ± 0.74 to 22.64 ± 0.78 (mg/gm). The ethyl acetate extract has more alkaloid content 22.64 ± 0.78 (mg/gm) than other extracts (Table 2).

Table 1: Nature of phytoconstituents in different extracts of *Saponaria officinalis*.

Name of the Phytochemicals	Extracts of <i>Saponaria officinalis</i>		
	Chloroform	Ethyl Acetate	Methanol
Phytosterols	+	+	+
Terpenoids	+	+	+
Glycosides	+	+	+
Saponins	-	+	+
Flavonoids	-	+	+
Tannins	+	+	+
Carbohydrates	+	-	+
Alkaloids	+	+	+
Amino acids	-	-	-
Oils	-	-	-
Quinones	-	-	-
Phenols	+	+	+

+ = Present, - = Absent

Table 2: Total phenolic and alkaloid contents (mg/gm) of *Saponaria officinalis* extracts.

Name of the extract	Total Phenolic content (mg/gm) (Mean ± SEM)	Total alkaloidal content (mg/gm) (Mean ± SEM)
Ethyl acetate	21.59±0.17	22.64±0.78
Chloroform	17.52±0.46	18.43±0.74
Methanol	22.51±1.05	20.58±0.59

Acute toxicity studies

The *S. officinalis* root extracts were found to be safe after the toxicity test dose of 2000 mg/kg administration, because there were no sign of mortality and no behavioral changes were observed in rats.

Hepatoprotective activity

S. officinalis root extracts (ethyl acetate, chloroform and methanol) were evaluated for their hepatoprotective activity on Paracetamol-induced liver toxicity in rats at different concentrations (125 mg/kg b.w, 250 mg/kg b.w and 500 mg/kg b.w) and found concentration dependent percentage protection based on SGOT, SGPT, ALP, Total serum bilirubin and Total protein levels on end of experiment (Table 3, Figure 1, 2 and 3).

The control group which was given normal saline did not showed any significant changes but Group II which is treated with normal saline and paracetamol on 5th day showed significant changes compared to group I, The animals of group III was induced with paracetamol and was treated Liv 52 (Positive) showed the significant restoration of altered biomarker enzymes with percentage protection 94.79%, 92.97%, 96.49%, 97.57% and 90.24% of liver (AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein) levels.

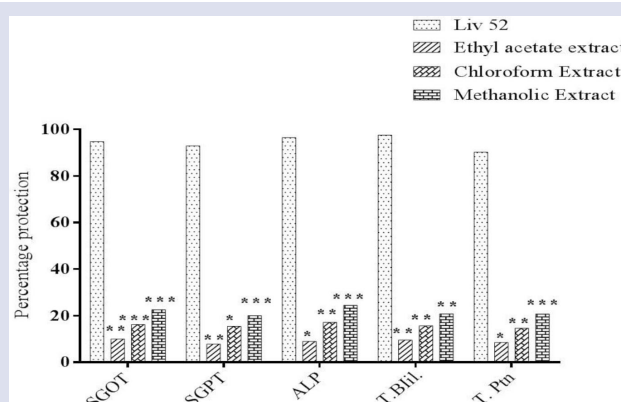
The percentage protection of ethyl acetate extract of *S. officinalis* (SOEAE) at 125mg/kg, 250mg/kg and 500 mg/kg b.w on AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels at were 9.95%, 7.81%, 9.00% 9.55% and 8.54%, 21.19%, 19.84%, 24.49%, 20.83% and 19.51%, 41.77%, 37.97%, 45.96%, 38.19% and 40.24%.

The percentage protection produced by the chloroform extract of *S. officinalis* (SOCE) on AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels were 16.19%, 15.47%, 17.18%, 15.63% and

Table 3: Enzymes Levels of groups I to XII animals due to the effect of different extracts of *S. officinalis*.

Name of the Drug	Name of Enzymes				
	AST (U/L)	ALT (U/L)	ALP (U/L)	T. bil (mg/dl)	T. ptn (gm/dl)
Control	89.17±1.11	52.33±1.73	183.50±0.85	0.25±0.01	6.93±0.06
Paracetamol	332.17±2.18	159.00±0.89	529.83±3.04	2.17±0.06	4.20±0.04
Liv 52 25mg//kg b. w.	101.83±1.72	59.83±1.11	195.67±1.56	0.29±0.01	6.67±0.08
SOEAE 125mg/kg b.w	308.00±0.82	150.67±0.76	498.67±1.36	1.98±0.05	4.43±0.08
SOEAE 250mg/kg b.w	280.67±1.94	137.83±0.87	445.00±1.46	1.77±0.06	4.73±0.04
SOEAE 500mg/kg b.w	230.67±1.09	118.50±0.96	370.67±1.09	1.43±0.06	5.30±0.09
SOCE 125mg/kg b.w	292.83±2.34	142.50±1.06	470.33±1.45	1.87±0.07	4.60±0.05
SOCE 250mg/kg b.w	254.83±2.26	125.83±1.89	409.50±3.01	1.57±0.06	5.03±0.06
SOCE 500mg/kg b.w	195.67±1.17	100.83±2.10	316.67±3.12	1.07±0.04	5.67±0.07
SOME 125 mg/kg b.w	277.17±2.51	137.67±0.49	445.00±1.44	1.77±0.06	4.77±0.06
SOME 250mg/kg b.w	234.00±2.42	116.50±1.96	375.83±1.78	1.37±0.06	5.27±0.04
SOME 500mg/kg b.w	170.17±0.91	94.33±1.78	301.50±1.67	0.93±0.07	5.87±0.04

n=6 and Mean± SD

**Figure 1: Percentage protection produced by different extracts of *S. officinalis* at a dose of 125mg/kg.**

Results were analysed by using Two-way ANOVA followed by Dunnet's multiple comparison test. All groups were compared with Liv 52 group. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns = Non significance (n=6), T.Bil= Total Bilirubin; T.Ptn= Total Protein.

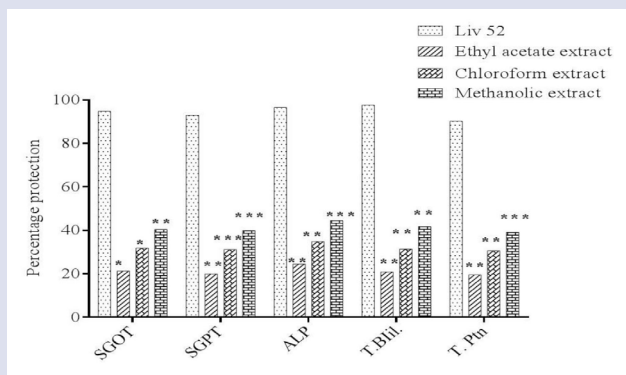


Figure 2: Percentage protection produced by different extracts of *S. officinalis* at a dose of 250mg/kg.

Results were analysed by using Two-way ANOVA followed by Dunnet's multiple comparison test. All groups were compared with Liv 52 group. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns= Non significance (n=6), T.Bil= Total Bilirubin; T.Ptn= Total Protein.

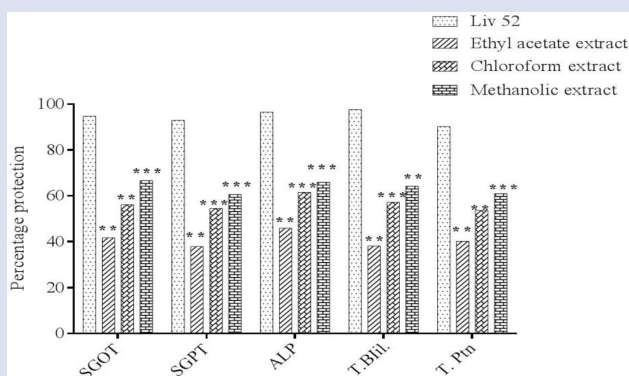


Figure 3: Percentage protection produced by different extracts of *S. officinalis* at a dose of 500mg/kg.

Results were analysed by using Two-way ANOVA followed by Dunnet's multiple comparison test. All groups were compared with Liv 52 group. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns= Non significance (N=6), T.Bil= Total Bilirubin; T.Ptn= Total Protein.

14.63%, 31.82%, 31.09%, 34.74% 31.25% and 30.49%, 56.17%, 54.53%, 61.55% 57.29% and 53.66% respectively.

The percentage protection produced by the methanol extract of *S. officinalis* (SOME) on AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein levels were 22.63%, 20.00%, 24.49%, 20.83% and 20.73%, 40.40%, 39.84%, 44.47%, 41.67% and 39.02%, 66.67%, 60.63%, 65.93%, 64.24% and 60.98% respectively.

DISCUSSION

The studies on natural products and isolation of new drugs from them is getting more attention because of currently using drugs are becoming inactive and micro-organisms becoming resistant to them.²⁸⁻³¹ As mentioned in introduction, *S. officinalis* one of the traditional medicinal plant with less scientific literature, so the present work carried out on *S. officinalis* root part for their phytochemical analysis using different

extracts and evaluation of hepatoprotective activity. Several studies have been reporting on pharmacological and biological activities of medicinal plants like anti-microbial,³²⁻³³ antioxidant and hepatoprotective,³⁴⁻³⁷ anti-inflammatory,³⁸ and cytotoxicity etc.

The phytochemical analysis of different extracts of *S. officinalis* root part showed presence of bioactive compounds in them and variation in the presence of them in different extracts. All extracts gave positive results for terpenoids, alkaloids, steroids, glycosides and phenols and gave negative results for amino acids, quinones and oils. Methanol and chloroform extracts gave positive results for saponins and flavanoids. Carbohydrates gave positive results for chloroform and methanol extracts, absent in ethyl acetate extract. There were some earlier reports about the presence of biological active compounds in *S. officinalis*, quillaic acid from rhizome (root) part,³⁹ triterpenoid saponins.⁴⁰

The extracts were analyzed for their hepatoprotective activity because recently drugs induced liver damage causing one of the major mortality problem around the world.⁴¹ In the present study, the extracts of *S. officinalis* showed concentration dependent hepatoprotective activity. As the concentration of extracts increasing the protective nature of them was also increased in restoration of the liver biomarker enzymes like SGOT, SGPT, ALP, total bilirubin and total protein. As overall the methanolic extract showed better activity compared to other two extracts (Table 3) but individually different extracts showed variation on the enzymes level protection at higher concentrations. Ethyl acetate and chloroform extracts have more percentage protection on ALP levels but methanolic extracts have more protection on AST levels (Figure 3). The protective nature of *S. officinalis* extracts was comparable with standard drug Liv 52. In our earlier studies, we have reported about the antioxidant and antibacterial activities of these extracts.⁴²⁻⁴³ As in those studies, the methanol extract showed more antioxidant and antibacterial activity as hepatoprotective activity in the present study.

In recent decades, different new drugs have been isolating from natural products.⁴⁴ The plants contains wide diversity of chemical constituents in them, but many medicinal plants are still unexplored about their medicinal uses.⁴⁵ The results of present study and previous studies indicating that *S. officinalis* roots possess different bioactive compounds with different biological activities like antioxidant, antibacterial, hepatoprotective and similar to us, antitumor effects,⁴⁶ antimethanogenic effect⁴⁷ and antimethanogenic effect⁴⁸ of *S. officinalis* was reported. However, the results obtained in present study are warrant for further studies on different medicinal plants used as food and in traditional medicine.

CONCLUSION

The present research reveal that *S. officinalis* roots contains different phytochemical constituents and those extracts possess hepatoprotective activity and in our earlier research work we found these also possess antibacterial and antioxidant activities. The liver damage will occur mainly due to the over production of free radical and *S. officinalis* roots showed free radicals inhibition. So, it may conclude that common biological active components of them were responsible for their activities either individually or synergistically. So, further work is needed to isolate and characterize the responsible bioactive molecules from these extracts as well as from different medicinal plants for development of effective and safe medicines.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

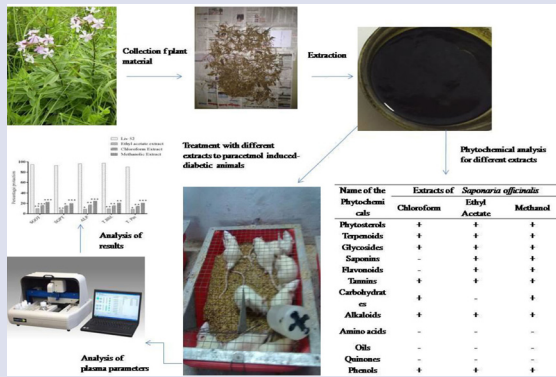
GAE: Gallic acid total equivalents; **BCG:** Bromocresol green; **OECD:** Organisation for Economic Co-operation and Development; **AST (SGOT):** Aspartate aminotransferase; **ALT (SGPT):** Alanine aminotransferase; **ALP:** Alkaline phosphatase.

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GRAPHICAL ABSTRACT



SUMMARY

Saponaria officinalis is a traditional medicinal plant belonging to the family Caryophyllaceae, growing in tropical and sub-tropical regions. There have been several reports on the medicinal use of different parts of it in traditional medicine, but there is no scientific evidence. So, phytochemical profiling of it using different extracts and their hepatoprotective activity were carried out in the present study. *S. officinalis* roots' extracts showed the presence of sterols, terpenoids, glycosides, carbohydrates, proteins, flavonoids, alkaloids, phenols, tannins and absence of saponins and oils. The methanolic extract showed more phenolic and alkaloid contents on their quantification. Methanol extract showed more activity at 500mg/kg b. w. and is comparable with standard drug Liv 52 on altered liver biomarker enzymes AST (SGOT), ALT (SGPT), ALP, total bilirubin and total protein with Percentage protection 66.67%, 60.63%, 65.93%, 64.24% and 60.98%.