Journal of Medical Pharmaceutical And Allied Sciences
RESEARCH ARTICLE

www.jmpas.com
ISSN 2320-7418

STUDY OF ANTIHYPERLIPIDEMIC EFFECT OF HYDROALCOHOLIC EXTRACT OF *SOLANUM VIRGINIANUM* WHOLE PLANT IN HIGH FAT DIET FED RATS

Suraj Sahu[1*], Narendra Patel[1], Hemant Kumar Sharma[1], Prabhakar Budholiya[1], Prabhat Kumar Jain[2]

1. Sri Satya Sai University of Technology and Medical Sciences, Sehore, Madhya Pradesh, India
2. Scan Research Laboratories, Bhopal, Madhya Pradesh India

Correspondence
Suraj Sahu*
College of Pharmacy, Sri Satya Sai University of Technology and medical Science, Sehore, Madhya Pradesh, India.
✉ **surajsahu251095@gmail.com**

Keywords
Hyperlipidemia, Solanum virginianum, phytochemical profile, Cholesterol, Triglycerides High density lipoprotein cholesterol

Received
04/09/2019
Reviewed
08/09/2019
Revised/ Accepted
15/09/2019

ABSTRACT

Elevated serum cholesterol levels leading to atherosclerosis can cause coronary heart disease (CHD).Hyperlipidemia is a major risk factor for the development of coronary heart disease and is the most common cause of mortality and morbidity worldwide. Currently available synthetic drug of hyperlipidemia are associated with a number of side effects. In recent times, a large volume of work aimed at the efficacy of herbal products, as they are safe and effective alternatives to synthetic drugs. The aim of the present study is to investigate the phytochemical profile and anti-hyperlipidemic activity of whole plant of hydro alcoholic extract *Solanum virginianum* in high-fat diet induced hyperlipidemic rats at a dose of 100 and 200mg/kg. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolic and flavonoids were determined by the well-known test protocol available in the literature. The activity was assessed by estimation of serum lipid profile viz. total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) of control and drug-treated animals. Phytochemical analysis revealed the presence of phenols, proteins and amino acids, carbohydrates, saponins and diterpenes. The total phenolic content of whole plant hydro alcoholic extract was (1.158mg/100mg), followed by flavonoids (0.745mg/100mg). The extract exhibited a dose dependent anti-hyperlipidemic activity and at dose level 200mg/kg p.o. the extract showed a significant decrease in the levels of serum TC, TG and HDL-C. The present study demonstrated that the extract exhibits a potent lipid lowering activity in diet induced hyperlipidemia which account for some of the medical claims attributed to this plant.

INTRODUCTION

The medicinal properties of plants are generally desired by the presence of secondary metabolites. Among them, phenolic compounds possess multiple biological activities [1,2]. Hyperlipidemia is a heterogeneous group of disorders characterized by elevation of plasma concentrations of the various lipids and lipoprotein fractions, which is the key risk factor for cardiovascular disorders (CVD). These lipids include cholesterol, cholesterol esters, phospholipids, and triglycerides.

Lipids are transported in the blood as large 'lipoproteins' and has been reported as the most common cause of death in developed as well as developing nations. Hyperlipidemia associated lipid disorders are considered to cause the atherosclerotic cardiovascular disease. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular disease like atherosclerosis or cerebrovascular disease. Currently available drugs have been associated with number of side effects. Currently the use of complementary/alternative medicines and especially the consumption of phytochemicals have been rapidly increased worldwide. As herbal medicines are less damaging than synthethic drugs and they have better compatibility thus improving patients tolerance even on long term use [3-7]. With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential. In this regard, we have decided to explore the *Solanum virginianum*.

The genus *Solanum* comprises of herbs or shrubs, rarely small trees, and sometimes subscandent or climbing unarmed or prickly. The leaves are entire, lobed, or pinnatifid. Flowers are in lateral or terminal cymes. Fruit is a globose or elongated berry [8]. *Solanum virginianum* L. (*Solanum surattense* Burm. f.; *Solanum xanthocarpum* Schrad. & H. Wendl.) is a diffuse and very prickly undershrub belonging to the family Solanaceae. It is found growing commonly in various regions of the world on sandy soils and is distributed throughout India.

It is commonly called as yellow-berried nightshade in English, kantakari in Sanskrit, and nelagulla in Kannada. It is one of the members of dashamula of Ayurveda. The prickles are straight, compressed, and yellowish. The leaves are up to 10×6 cm, ovate or elliptic with acute apex, and pinnatified half-way down. Petioles have long pickles, and base is very unequal. Flowers are in few-flowered cymes. Calyx is prickly with ovate or lanceolate lobes. Corolla is violet in color and approximately 2 cm in diameter. Berry is globose, 2 cm in diameter, and yellow or white with green blotches [8-11].

A wide range of phytochemicals such as alkaloids, phenolics, flavonoids, sterols, saponins, glycosides, fatty acids, tannins, and amino acids have been identified from different parts of the plant. The plant is extensively used in various systems of medicine including Ayurveda. The plant is used traditionally to treat asthma, chest pain, leucoderma, scorpion bite and sterility in women. Roots are much used in medicine. The oil from seeds is used to treat arthritis. The ash from dried fruits is used to relieve toothache.

The plant is shown to exhibit various bioactivities such as antimicrobial, anthelmintic, antioxidant, hemolytic, anti-inflammatory, antidiabetic, cytotoxic, phytotoxic, hepato protective and immuno

stimulatory activities [12-19]. In the present study, we investigated Anti-hyperlipidemic activity of hydroalcoholic extract from whole plant of *S. virginianum* against high fat diet induced hyperlipedemia in rats.

MATERIALS AND METHODS

Plant materials

Whole plant of *Solanum virginianum* was collected from rural area of Bhopal (M.P), India in the months of January, 2019. The collected plant material were washed, chopped; shade dried and was pulverized with mechanical pulverizer for size reduction. It was then passed through mesh 40 and the fine powder was collected and used for the experiment and preparation of extract.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). Cholesterol, Cholic acid was obtained from LOBA Chemie Pvt Ltd, Mumbai. Orlistat purchased from local market Bhopal. All the chemicals used in this study were of analytical grade.

Extraction

Dried powdered whole plant of *S. virginianum* has been extracted with hydroalcoholic solvent(20:80) using maceration process for 48 hours, filtered and dried using vacuum evaporator at 40^0C and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [20].

Qualitative phytochemical analysis of plant extract

The *S. virginianum* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate [21, 22]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso *et al* [23]. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al* [23]. 1 ml of 2% $AlCl_3$ Methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

Animals

Wistar albino male rats (180-250 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55-65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-

free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute toxicity studies

Acute oral toxicity was conducted according to the method of Organization for Economic Co-operation and Development (OECD) [24]. Animals were kept fasting providing only water, hydroalcoholic extract of leaves of *S. virginianum* (50,100,150,200,300 mg/kg/day) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-hyperlipidemic effect.

Induction of hyperlipidemia

Rats with an average body weight were made hyperlipidemic by giving high-fat diet (HFD) for 15 days. The HFD contained Cholesterol (2%), Cholic acid (1%), Dalda (20%), and Coconut oil (6%) as major constituents. Hyperlipidemia was confirmed by measuring the levels of serum lipids and lipoproteins in the rats.

Experimental designs

Group –I: Normal (vehicle alone)
Group –II: Hyperlipidemic rats treated with vehicle alone
Group -III: Hyperlipidemic rats treated with hydroalcoholic extract of *S. virginianum* (100mg/kg, p.o.)
Group –IV: Hyperlipidemic rats treated with hydroalcoholic extract of *S. virginianum* (200mg/kg, p.o.)
Group –V: Hyperlipidemic rats treated with Orlistat (60 mg/kg/day p.o.)

After treatment for fourteen days with the test drug and on 15th day the rats are kept fasting and the blood was collected by retro orbital sinus puncture, under mild ether anesthesia. The collected samples were centrifuged for 10 minutes at 2000 RPM. and serum samples so collected were used for various biochemical tests. Serum Triglycerides (TG), total cholesterol (TC) and HDL-cholesterol (HDL-C) were estimated by using commercial kits as per the manufacturer instructions [25-27].

Statistical analysis

All the results were expressed as mean ± standard deviation. Statistical analysis was carried out by using one way ANOVA followed by Dunnett's test. Significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Dried and powdered whole plant of *S. virginianum* was subjected to maceration extraction with hydroalcoholic solvent and yielded 8.3 % w/w. Phenols, proteins and amino acids, carbohydrates, saponins and diterpenes were identified in preliminary phytochemical tests.

Table 1 Phytochemical screening of hydroalcoholic extract of *S. virginianum*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Dragendroff's test Hager's test	 -ve -ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve -ve
4.	**Phenolics** $Fecl_3$	+ve
5.	**Proteins and amino acids** Xanthoproteic test	+ve
6.	**Carbohydrates** Fehling's test	 +ve
7.	**Saponins** Foam test	 +ve
8.	**Diterpenes** Copper acetate test	 +ve

The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of hydroalcoholic extract of *S. virginianum* showed the content values of 1.158. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of hydroalcoholic extract of *S. virginianum* showed the content values of 0.745. Results are provided in Table 2 & Fig. 1, 2.

Table 2 mean body weight change

Group	Drug	Dose	Body weight (gm)	
			Onset of study	End of study
I	Normal	Normal saline	180.10±7.50	200.00±7.50
II	Control	HFD	190.05±8.50	252.10±8.50
V	*S. virginianum*	100 mg/kg p.o.	200.00±7.00	195.00±7.00
VI	*S. virginianum*	200 mg/kg p.o.	200.05±8.00	190.00±8.00
IV	Orlistat	60 mg/kg p.o.	200.00±8.00	180.50±8.00

Table 3 Total phenolic and total flavonoid content of *S. virginianum*

S. No.	Extracts	Total Phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	Hydroalcoholic	1.158	0.745

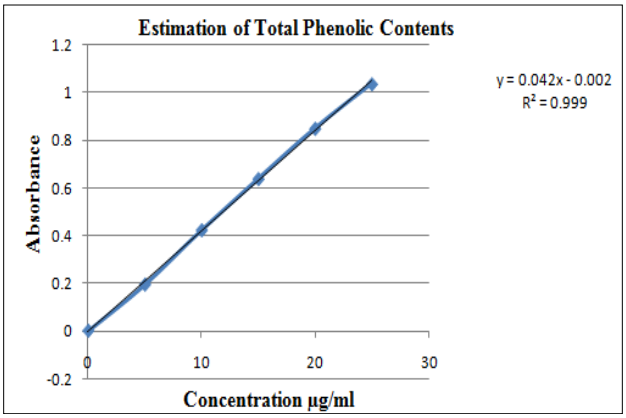

Fig. 1 Graph of estimation of total phenolic content

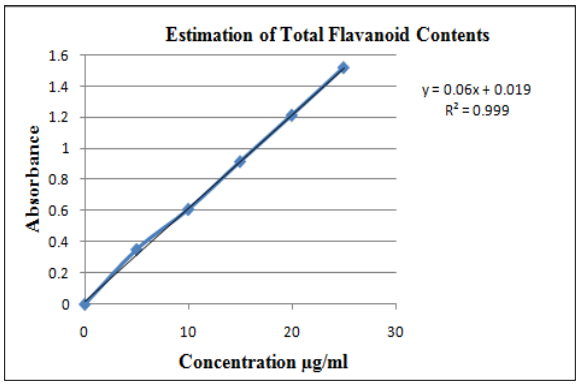

Fig. 2 Graph of estimation of total flavonoids content

Anti-hyperlipidemic effect of the hydroalcoholic extract Solanum virginianum on the high fat diet induced rats, the mean body weight as shown in Table 3 & Fig. 3. The activity levels of serum total cholesterol (TC), triglycerides (TG) and serum high density lipoprotein (HDL) were observed in normal and experimental animals. In group II animals, the activity levels of serum total cholesterol (TC) and triglycerides (TG) were significantly elevated when compared to that of normal groups. On the other hand the serum level of serum high density lipoproteins (HDL) were significantly depleted in the HFD fed rat. In group III, IV and V animals, the activity levels of serum total cholesterol (TC) and triglycerides (TG) were significantly decreased when compared to that of normal groups (Table 4& Fig. 4-6). Also HDL level was significantly increased in the same groups.

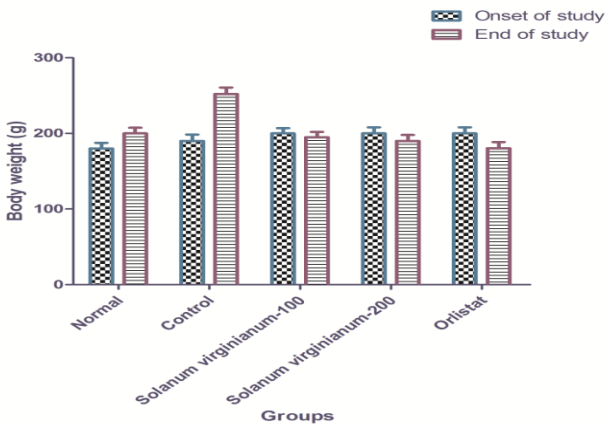

Fig. 3 Effect of the hydroalcoholic extract of *S. virginianum* on body weight in HFD induced rat

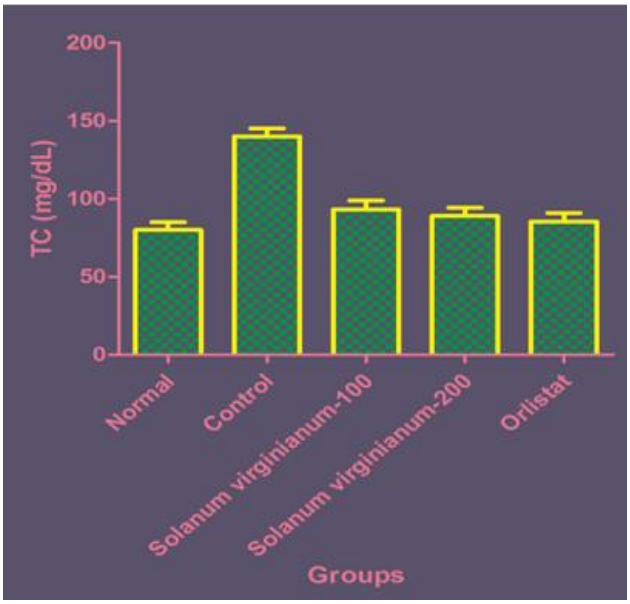

Fig. 4 Effect of the hydroalcoholic extract of *S. virginianum* on Total cholesterol levels (mg/dL) in HFD induced rat

Values are expressed as the mean ± SEM of six observations. *** $P<0.001$ vs. control treatment (One-way ANOVA followed by Dunnett's test)

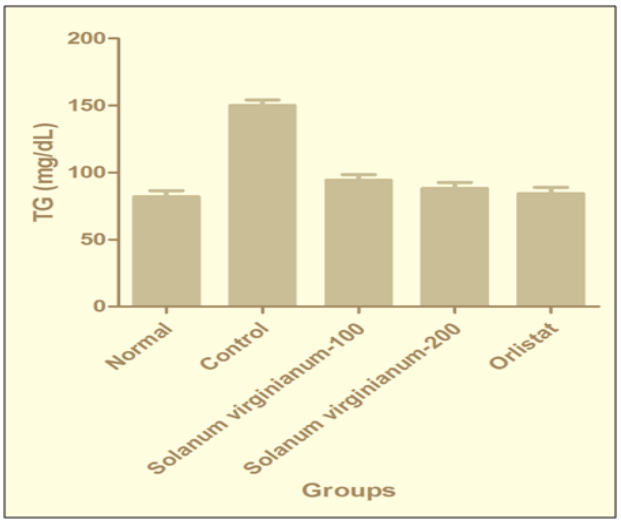

Fig. 5 Effect of the hydroalcoholic extract of *S. virginianum* on Triglycerides levels (mg/dL) in HFD induced rat

Table 4 Effect of the hydroalcoholic extract of *S. virginianum* on serum lipid profile levels (mg/dL) in HFD induced rat

Treatment	Dose	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	High density lipoproteins (mg/dL)
Normal	Normal saline	80.00 ± 5.00	82.00 ± 4.50	36.00 ± 4.00
Control	HFD	140.10 ± 5.00	150.0 ± 4.22	25.00 ± 4.60
S. virginianum	100 mg/kg p.o.	93.20 ± 5.70**	94.30 ± 4.10**	30.40 ± 4.50**
S. virginianum	200 mg/kg p.o.	89.10 ± 5.10***	88.10 ± 4.50***	31.50 ± 4.60***
Orlistat	60 mg/kg p.o.	85.40 ± 5.60***	84.20 ± 4.70***	34.10 ± 4.50***

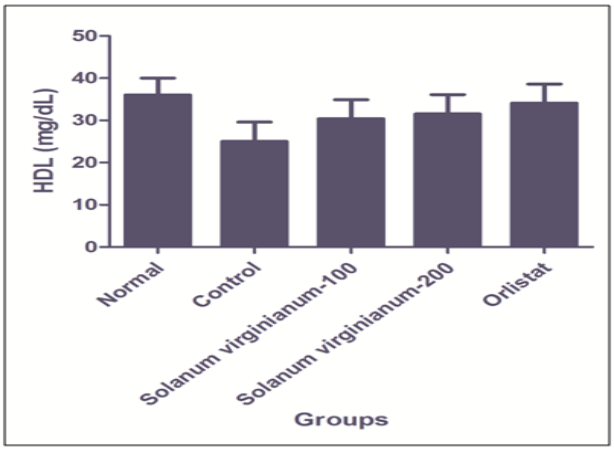

Fig.6 Effect of the hydroalcoholic extract of *S. virginianum* on High density lipoproteins levels (mg/dL) in HFD induced rat

Solanum virginianum a well-known traditional medicinal plants possesses diverse biological activities and pharmacological function including reducing blood glucose and serum lipids. It has long been used to treat diabetes mellitus and related hyperlipidemia. Hypercholesterolemia, a high cholesterol diet and oxidative stress increase serum levels resulting in increased risk for development of atherosclerosis. Cholesterol is synthesized in all animal tissue. It is important to relate to its role in the stabilization of membrane structures because of its rigid planar structure. It also as a precursor for the synthesis of steroid hormones. In the present study, feeding rats with diets rich in cholesterol resulted in increased TC and TG levels. This model was

used to study the potential of hypolipidemic effect of hydroalcoholic extract of whole plant of *S. virginianum* that contained significant amounts of antioxidants properties. From this study, we found that daily oral administration hydroalcoholic extract of *S. virginianum* shows significantly reduced total cholesterol levels in plasma after 15 days of administration. This result agrees with literature where depleted level of HFD fed hyperlipedemia.

HDL is directly anti-androgenic and it is believed to remove cholesterol from the developing lesions. The intense interest in this area results in part from the generally low toxicity of antioxidants and the hope that treatment with antioxidants might be additive with cholesterol lowering regimes. In the present study serum TG levels were significantly elevated in HFD rat. The excess of fat diet increased the TG level which is one of the causes of hardening of arteries.

CONCLUSION

In conclusion, it could be said that the hydroalcoholic extract of *S. virginianum* exhibited a significant hypolipidemic activity. Administration of HFD produced a highly significant increase in weight mesenteric fat pads. A reduction in the raised weight in the fat pads as observed in the groups of animals treated with hydroalcoholic extract of *S. virginianum* may be attributed to increased thermogenesis and decreased lipogenesis.

ACKNOWLEDGMENTS

The authors would like to thank the Mr. Prabhat Kumar Jain, Geeta Parkhe and All supporting staff of Scan Research Laboratories, Bhopal (M.P.) who helped in the experiments during research work.

REFERENCES

1. Middleton E, Jr Kandaswami, C Theoharides T C, 2000. The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease and Cancer. Pharmacology Review, 52, 673-751.
2. Nichols J A, Katiyar S K, 2010. Skin Photo protection by Natural Polyphenols: Anti-Inflammatory, Antioxidant and DNA Repair Mechanisms. Archive Dermatology Research, 302, 71-83.
3. Zeljko Reiner, Zdenko Sonicki, Eugenia Tedeschi Reiner, 2010. Public perceptions of cardiovascular risk factors in croatia: the percro survey Preventive Medicine Volume 51, Issue-6, Pages 494–496
4. Simons L A, 2002. Additive effect of plant sterol-ester margarine and cerivastatin in lowering low density lipoprotein cholesterol in primary hyper cholesterolemia. Am J Cardiol 90, 737.
5. Yokozawa T, Ishida A, Cho E J Nakagawa T, 2003. The effects of *Coptidis rhizoma* extract on hyper cholesterolemic animal model. Phyto medicine Volume 10, Issue 1, 17–22
6. Mukundh N, Balasubramanian P, Muralidharan, G Balamurugan, 2008. Anti Hyperlipidemic Activity of *Pedalium murex* (Linn.) Fruits on High Fat Diet Fed Rats, international journal of pharmacology, vol-4, issue-4, 310-313
7. Sumitra Singh, Surendra Kr. Sharma and Rajinder Mann, 2012. Anti hyperlipidemic activity of suaeda maritima (l.) Dumortier aerial parts in hyper cholesterolemic rats. Journal of Pharmacy Research, 5(3), 1400-1402.
8. Bhat GK, 2014. Flora of South Kanara. Mangalore: Akriti Prints; 2014. p. 723.

9. Rita P, Animesh DK, 2011. An updated overview on *Solanum xanthocarpum* Schrad and Wendl. Int J Res Ayurveda Pharm; 2(3):730-5.
10. Rane MH, Sahu NK, Ajgoankar SS, Teli NC, Verma DR, 2014. A holistic approach on review of *Solanum virginianum*. L. Res Rev J Pharm Pharm Sci; 3(3):1-4.
11. Reddy NM, Reddy RN, 2014. *Solanum xanthocarpum* chemical constituents and medicinal properties: A review. Sch Acad J Pharm; 3(2):146-9.
12. Kayani S, Ahmad M, Zafar M, Sultana S, Khan MP, Ashraf MA, 2014. Ethnobotanical uses of medicinal plants for respiratory disorders among the inhabitants of Gallies - Abbottabad, Northern Pakistan. J Ethnopharmacol; 156:47-60.
13. Kumar PR, 2014. Ethno medicinal plants used for oral health care in India. Int J Herb Med 2014; 2(1):81-7.
14. Rana S, Prakash V, Sagar A, 2016. Antibacterial activity of *Solanum xanthocarpum* leaf extract. Int J Curr Microbiol Appl Sci; 5(4):323-8.
15. Pardhi P, Jain AP, Ganeshpurkar A, Rai G. 2010. Anti-microbial, anti-oxidant and anthelmintic activity of crude extract of *Solanum xanthocarpum*. Pharmacogn J; 2(11):400-4.
16. Dinanath PD, Gurumeet WC, 2013. Antibacterial, antioxidant and anti-inflammatory studies of leaves and roots of *Solanum xanthocarpum*. Unique J Ayurvedic Herb Med ;(3):59-63.
17. Shah MA, Khan H, Muhammad N, Khan F, Shahnaz, Muhammad A, 2013. Cytotoxic, antioxidant and phyototoxic effect of *Solanum surattense* Burm f fruit extracts. Int J Pharmacogn Phytochem; 28(2):1154-8.
18. . Kumar SR, Hariprasanth RJ, Siddharth PM, Gobinath M, Rajukutty C, 2016. Evaluation of the antioxidant, antimicrobial, antidiabetic and hemolytic activity of organically grown *Solanum nigrum* and *Solanum xanthocarpum*. Int J Curr Pharm Rev Res; 7(5):296-9.
19. Rajakumari M, Selvi KK, 2016. Effect of fruit of *Solanum xanthocarpum* as immuno stimulant on fish *Channa striatus*. Int J Sci Technol; 4(12):76-87.
20. Mukherjee PK, 2007. Quality control of herbal drugs. 2nd Ed. Business Horizons.
21. Khandelwal KR, 2005. Practical pharmacognosy technique and experiments. 23rd Ed. Nirali Prakashan.
22. Kokate CK, 1994. Practical pharmacognosy. 4th Ed. Vallabh Prakashan.
23. Olufunmiso OO, Afolayan AJ, 2011. Phenolic content and antioxidant property of the bark extract of *Ziziphus mucronata* willd. Subsp. mucronata willd, BMC Complement *Alternative Medicine,* 11:130.
24. Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications; 1996.
25. CC Allain, LS Poon, CSG Chan, W Richmond, PC Fu, 2011. Clin Chem., 20, 470.
26. NJ Jacobs, PJ Vandemark, 1960. Arch. Biochem. Biophys, 88, 250.
27. M Werner, DG Gabrielson, J Eastman, Clin. Chem., 1981, 27, 268.