GMR
The Original

PHYTOPHARMACOLOGICAL EVALUATION OF GLYCINE MAX (L.) MERR. AGAINST UROLITHIASIS: AN IN VITRO STUDY

Vandana[1], Swati Trivedi[1*], Anubhav Dubey[1], Vikram Kumar Sahu[1], Sribatsa Lanchhana Dash[1] Amit Mishra[2]

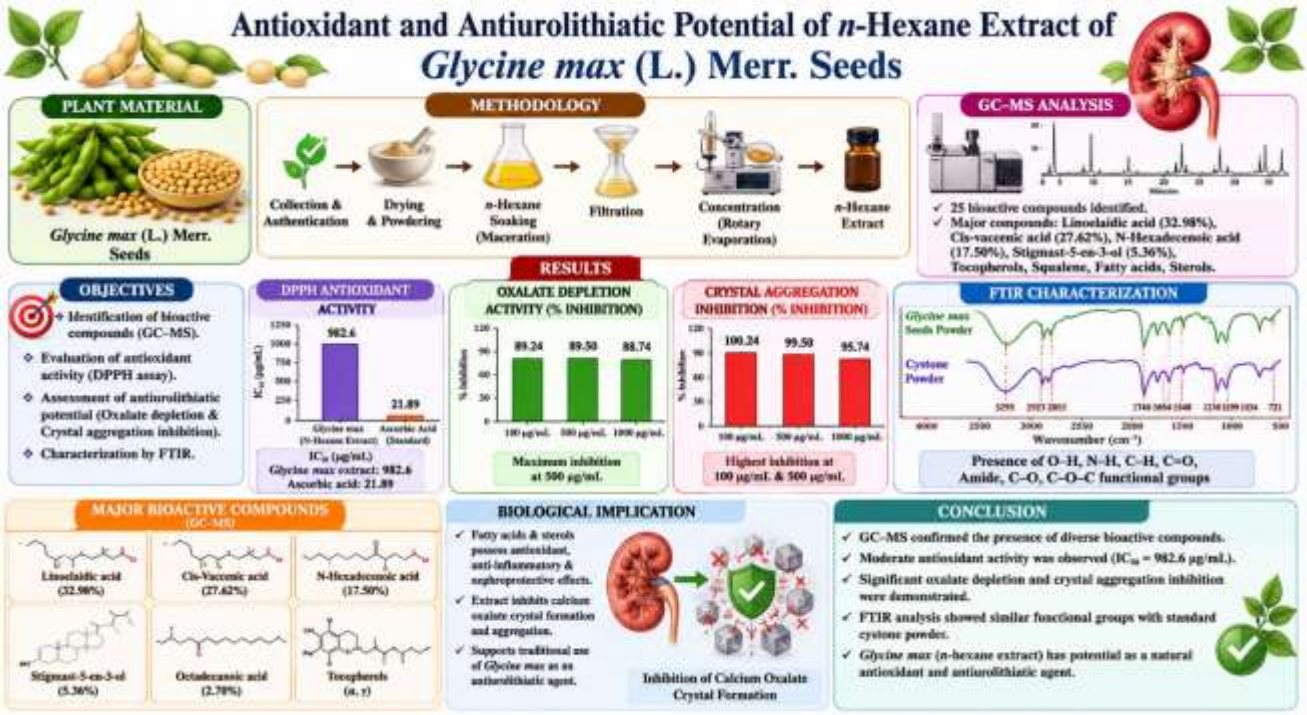
[1-]Maharana Pratap College of Pharmacy Kothi, Mandhana, Kanpur -209217, Uttar Pradesh, INDIA.
[2-] Maharana Pratap College of Pharmaceutical Sciences, Kothi, Mandhana, Kanpur -209217, Uttar Pradesh, INDIA.
*Corresponding Author: swatipharma10@gmail.com

ABSTRACT

The present study was designed to evaluate the phytochemical composition, antioxidant activity, and antiurolithiatic potential of the n-hexane extract of Glycine max (L.) Merr. seeds. The seeds were extracted using the soaking (maceration) method with n-hexane solvent and subjected to GC–MS, DPPH antioxidant assay, oxalate depletion assay, crystal aggregation inhibition assay, and FTIR characterization. GC–MS analysis revealed the presence of 25 bioactive compounds, among which linoelaidic acid (32.98%), cis-vaccenic acid (27.62%), n-hexadecenoic acid (17.50%), stigmast-5-en-3-ol (5.36%), octadecanoic acid (2.70%), ergost-5-en-3-ol (2.22%), and stigmasta-5,22-dien-3-ol (2.02%) were identified as major constituents. These compounds are known for antioxidant, anti-inflammatory, and nephroprotective activities. The antioxidant activity evaluated by DPPH assay showed moderate free radical scavenging activity with an IC50 value of 982.6 µg/mL compared to standard ascorbic acid (21.89 µg/mL). The antiurolithiatic activity demonstrated significant inhibition of calcium oxalate crystal aggregation and oxalate depletion activity at different concentrations. Maximum oxalate depletion activity was observed at 500 µg/mL, while crystal aggregation inhibition was highest at 100 µg/mL and 500 µg/mL. FTIR analysis confirmed the presence of important functional groups such as hydroxyl, amine, carbonyl, ester, and aliphatic groups, indicating the presence of phenolics, proteins, fatty acids, and other phytoconstituents. The FTIR spectra also showed similarities with standard cystone powder. The findings of the present study suggest that the n-hexane extract of Glycine max seeds possesses promising antioxidant and antiurolithiatic properties, which may help prevent calcium oxalate crystal formation and kidney stone development. Therefore, Glycine max seeds could serve as a potential natural therapeutic agent for the management of urolithiasis.

KEYWORDS: Glycine max, n-hexane extract, GC–MS analysis, FTIR spectroscopy, Antioxidant activity, Antiurolithiatic activity.

GRAPHICAL ABSTRACT



INTRODUCTION

Urinary calculi have been identified in the graves of North American Indians dating from 1500–1000 BC and in the tombs of Egyptian mummies from 4000 BC. Ancient Sanskrit texts from India, dating between 3000 and 2000 BC, reference stone production. In El-Amara, the pelvic bones of a 7000-year-old adolescent male were found to harbor the earliest known urinary tract calculi [1]. The formation of kidney stones, or nephrolithiasis, is among the most common urinary disorders. Kidney stones adversely affect individuals and society due to their prevalence, high treatment costs, and significant recurrence rate. In recent years, there has been a worldwide rise in concern regarding kidney stones, which can induce severe back pain and may lead to serious complications such as acute renal failure and acute kidney injury. Bioinformatics research will examine ferroptosis related genes to identify potential indicators of kidney stones. Kidney stones are affected by these genes [2]. Due to their efficacy, low toxicity, and absence of adverse effects, interest in herbal remedies is increasing. Pifferi et al., (1999) indicate that numerous phytotherapeutic agents have been utilized for centuries to address urolithiasis. Litholytic herbs have been employed to treat kidney stones since antiquity, predating the advent of modern therapies. Phytotherapy has proven crucial in diminishing stone recurrence, prompting significant scientific research focus on its Gilhotra and Christina, (2011). Botanical remedies have historically been employed to address urolithiasis. Before the advent of modern medicine, kidney stones were addressed with litholytic herbs [3]. Furthermore, individuals are reverting to natural remedies for safe medications due to the misuse of synthetic pharmaceuticals, which heightens the risk of adverse drug reactions. Numerous sources assert that the origins can be traced to the 1976 Canberra meeting of the World Health Organization, which promoted the concept of "traditional" medicines for developing countries [4]. Plants are a significant source of medication is essential to maintaining global health. It has been long been that medicinal herbs or plants can be significant sources of therapeutic or curative help. Recently, WHO (world health organization) estimation that 80% of people worldwide on herbal medicine for some aspect of their primary health care needs. Glycine max, commonly known as soybean, belongs to the family Fabaceae and is one of the most valuable medicinal and nutritional plants. It is an annual herbaceous plant native to East Asia and widely cultivated throughout the world. The plant grows up to 0.5–1.5 meters in height with erect, hairy stems, trifoliate leaves, purple or white flowers, and elongated pods containing round seeds. Soybean seeds are rich in proteins, isoflavones, saponins, flavonoids, phenolic compounds, lecithin, and essential fatty acids, which contribute to its therapeutic properties. Traditionally, Glycine max has been used for the management of cardiovascular disorders, diabetes, osteoporosis, inflammation, and kidney-related diseases [5]. Pharmacologically, Glycine max exhibits significant antioxidant, anti-inflammatory, nephroprotective, and antiurolithiatic activities. Urolithiasis is characterized by the formation of stones in the urinary tract due to supersaturation and crystallization of minerals such as calcium oxalate. The phytoconstituents present in soybean, particularly flavonoids and phenolic compounds, help reduce oxidative stress and inhibit crystal aggregation in renal tissues. Soybean extracts have been reported to enhance urinary output and reduce calcium oxalate deposition, thereby preventing stone formation. The antioxidant activity of isoflavones such as genistein and daidzein protects renal epithelial cells from oxidative injury associated with urolithiasis.In experimental studies, Glycine max demonstrated the ability to lower urinary oxalate, calcium, and phosphate levels while improving renal function markers. The nephroprotective effect may be attributed to its free radical scavenging activity and modulation of inflammatory mediators. Furthermore, soybean proteins may help maintain mineral balance and reduce urinary supersaturation. Due to these pharmacological actions, Glycine max is considered a promising natural therapeutic agent for the prevention and management of urolithiasis and related renal disorders [6-8].

METHODOLOGY

Plant material collection and identification

Glycine max (L.) Merr Seeds were employed in this investigation. The authors gathered the samples at Maharana Pratap College of Pharmacy's and approved the plant Central Institute of Medicinal & Aromatic Plants in Lucknow. They placed a voucher specimen in the Plant Systematics Laboratory. (No. CIMAP-HA-1002720261).

Extraction

The seeds of Glycine max (L.) Merr. were collected, cleaned properly to remove dust and foreign particles, and shade dried for several days. The dried seeds were then pulverized using a mechanical grinder to obtain coarse powder. The powdered material was stored in an airtight container until extraction. For extraction, a known quantity of seed powder was soaked in n-hexane solvent using the maceration (soaking) method. Usually, the powder and solvent were taken in a ratio sufficient to completely immerse the plant material. The mixture was kept in a closed conical flask at room temperature for 48–72 hours with intermittent shaking to ensure maximum penetration of solvent and efficient extraction of bioactive constituents, particularly lipophilic compounds such as fixed oils, fatty acids, sterols, and other non-polar phytochemicals. After completion of soaking, the mixture was filtered first through muslin cloth and then through Whatman No. 1 filter paper to separate the liquid extract from the marc (residual plant material). The filtrate obtained was concentrated under reduced pressure using a rotary evaporator or by evaporation on a water bath at low temperature to remove excess n-hexane solvent. A semi-solid crude extract was obtained after complete evaporation of the solvent. The extract was dried, weighed, and stored in an airtight amber-colored container under refrigerated conditions until further phytochemical and pharmacological evaluation [9-10].

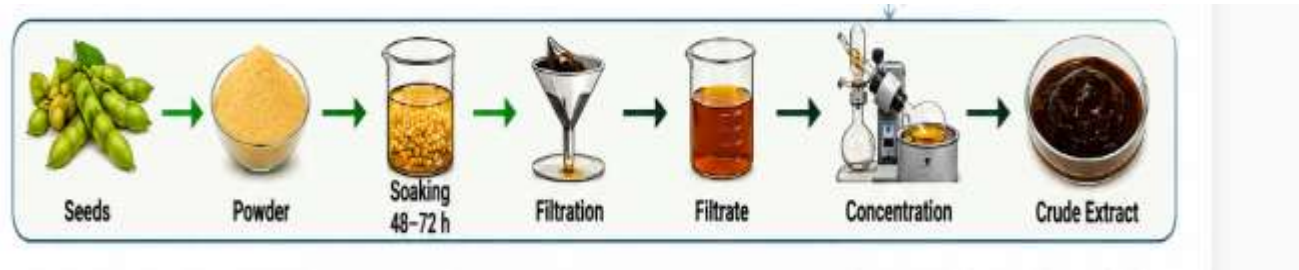


Figure-2 N-hexane extractions of Glycine max (L.) Merr Seeds

In vitro antioxidant activity

DPPH Scavenging Assay

To evaluate antioxidant activity, 10 µL of different concentrations of test samples or standard (Ascorbic Acid, SRL Cat. No. 23006) were mixed with 200 µL of 0.1 mM DPPH solution (SRL Chem, Cat. No. SR-29128) in a 96-well plate. Reactions were prepared in quadruplicate; duplicate blanks (sample + methanol without DPPH), red blanks (without DPPH), and untreated wells served as controls. Plates were incubated in the dark for 30 min, and absorbance was read at 517 nm using a microplate reader (iMark, Bio-Rad). A combination of test solution with 20 µL deionized waters served as control. Scavenging activity (%) was calculated relative to control. IC_{50} values were derived using GraphPad Prism 6 by plotting inhibition (%) vs. sample concentration [11].

Calculations:

% RSA= (ABS control-Abs Sample)/Abs control) x100

GC-MS Analysis

GC-MS analysis of **N-hexane** extracts was performed using a PerkinElmer Clarus 600 system equipped with an Rtx-5MS capillary column. Helium (99.99%) was used as the carrier gas at a constant flow rate of 1.0 mL/min. Injection volume: 1 µL, split ratio: 10:1, ionization energy: 70 eV, scan range: 40–650 m/z, scan time: 0.2 sec. Injector temperature: 260°C. Oven temp held at 50°C for 3 min, then ramped to 300°C at 10°C/min. Compounds were identified by comparing retention times and mass spectra with those in the NIST and Wiley-8 libraries [12].

FTIR Analysis

Samples were put into sample containers, and the FTIR spectrophotometer was equipped with ATR attachments. The detector used is called DTGS (deuterated triglycine sulphate). Measurements are made using wave numbers between 1000-4000 cm-1. With the aid of the software OPUS 7.2.139.1.24, the FTIR spectrum is displayed. The spectrum is further analysed to determine the different molecular bonds and functional groups present in the sample [13].

IN VITRO MODELS OF ANTI UROLITHIASIS ACTIVITY

Oxalate Depletion Assay

Varying concentration of sample were prepared in distilled water. And 4mM $CaCl_2$ solution and 4 mM NaC_2O_4 solution (0.5ml each) were added to 1 ml Tris-HCL (10mM) and NaCl(90mM) buffer (pH 7.4). To this was added 30µl of CaOx, crystals Slurry were then determined by measuring the rate of oxalate depletion from the solution at 214 nm wavelength for 600 seconds. Effect of each concentration of sample on crystal growth was then determined by addition of 1ml of sample (100µg/ml, 500µg/ml, 1000µg/ml,) to the reaction mixture and change in OD was again recorded.

Calculations

$$\% \text{ of inhibition} = [OD_{Control} - OD_{Test}/OD_{Control}] * 100$$

Crystal Nucleation Assay

10µl of sample dilutions was added in 96 well plate. 90µl of calcium chloride and sodium oxalate solution were added to the reaction mixture and read absorbance at 620nm for different time intervals [14].

Calculation

$$\% \text{ of inhibition} = [(OD_{CONTROL}/OD_{TEST})/ OD[_{CONTROL}] \times 100$$

RESULT AND DISCUSSION

Identification of components:

Identification was done using the calculated fragments, molecular mass, and molecular structure. The NIST database and the Willy8-library, which has more than 62,000 pattern entries, were used to interpret the GC-MS spectra. Together with their molecular weights and chemical structures, the components of the test materials were determined. The percentage amounts discovered in the sample were contrasted with component spectra from the Willy8 collection and the NIST library. This is done in order to determine whether this plant species has any chemicals or a combination of them that could support its traditional and commercial therapeutic purposes. It also helps determine the most effective methods for removing harmful compounds. The test materials' constituent parts were determined (Table 1, and Figure 2).

Table 1- Compound of Glycine max

Peak Report TIC-Sample- V1				
Peak	**R. Time**	**Area**	**Area %**	**Name**
1	8.361	257017	0.36	Dodecane
2	11.196	299141	0.42	Pentadecane
3	12.619	205312	0.29	Phenol, 2,4-bis(1,1-dimethylethyl)
4	13.708	169676	0.24	Hexadecane
5	17.120	344526	0.48	2-bromotetradecane
6	17.628	12587881	17.50	N-Hexadecenoic acid
7	18.889	202479	0.28	Cis-11,14-Eicosadienoic acid, methyl ester
8	19.296	23721383	32.98	Linoelaidic acid
9	19.346	19863888	27.62	Cis-Vaccenic acid
10	19.526	1939742	2.70	Octadecanoic acid
11	20.866	245203	0.34	2-((8Z,11Z)-Heptadeca-8,11-dien-1-yl)-4,5-dihydrooxazole
12	22.298	341783	0.48	1H-Indene, 1-hexadecyl-2,3-dihydro
13	22.642	320520	0.45	Bis(2-ethylhexyl) phthalate
14	23.670	236662	0.33	9,12-Octadecadienoic acid (Z,Z)
15	23.891	225967	0.31	Heneicosane
16	24.727	170661	0.24	Squalene
17	25.312	153825	0.21	Pentacosane
18	25.568	635504	0.88	Glycerol tricaprylate
19	25.723	338749	0.47	. Delta. -Tocopherol
20	26.594	220719	0.31	. Gamma.-Tocopherol
21	27.166	467270	0.65	2-(Decanoyloxy) propane-1,3-diyl dioctanoate
22	28.551	1592995	2.22	Ergost-5-en-3-ol, (3.beta.,24r)-
23	28.854	1455759	2.02	Stigmasta-5,22-dien-3-ol, (3. Beta.,22e)-
24	29.640	3851226	5.36	Stigmast-5-en-3-ol, (3. Beta.)-
25	33.690	2070265	2.88	Tris(2,4-di-tert-butylphenyl) phosphate
		71918153	100.00	

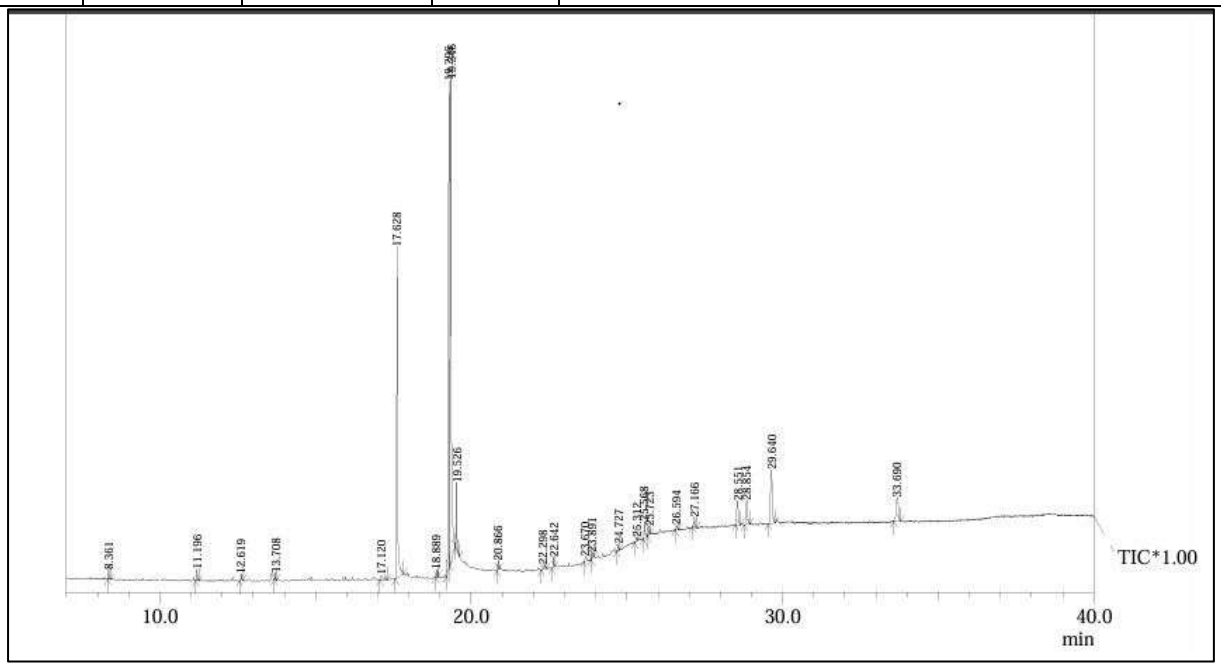


Figure-2 Glycine max chromatogram

The GCMS analysis of sample- V1 revealed the presence of many compounds. In sample- V1 a total of 25 compounds were identified, which are already mentioned in above table.

Activities of antioxidants

Oxidative stress has been linked to age-related neurodegenerative diseases in a number of studies. Numerous studies have also examined the protective effects of antioxidants in preventing or reducing neuronal death that occurs in the pathophysiology of this disorder. Indicators of a compound's antioxidant potential also include its total antioxidant activity and ability to scavenge radicals. To determine the plant extracts' ability to act as

antioxidants, activities—DPPH evaluated. The 1, 2-diphenyl-2-picryl hydroxyl radical (DPPH) was used to examine the antioxidant activity of many extracts. Table 2 presents the results.

Table-2PPH Scavenging Assay

Compounds code	**IC_{50} value (microgram/millilitre)**
Ascorbic Acids	21.89±0.02
Glycine max -VI	982.6±0.02

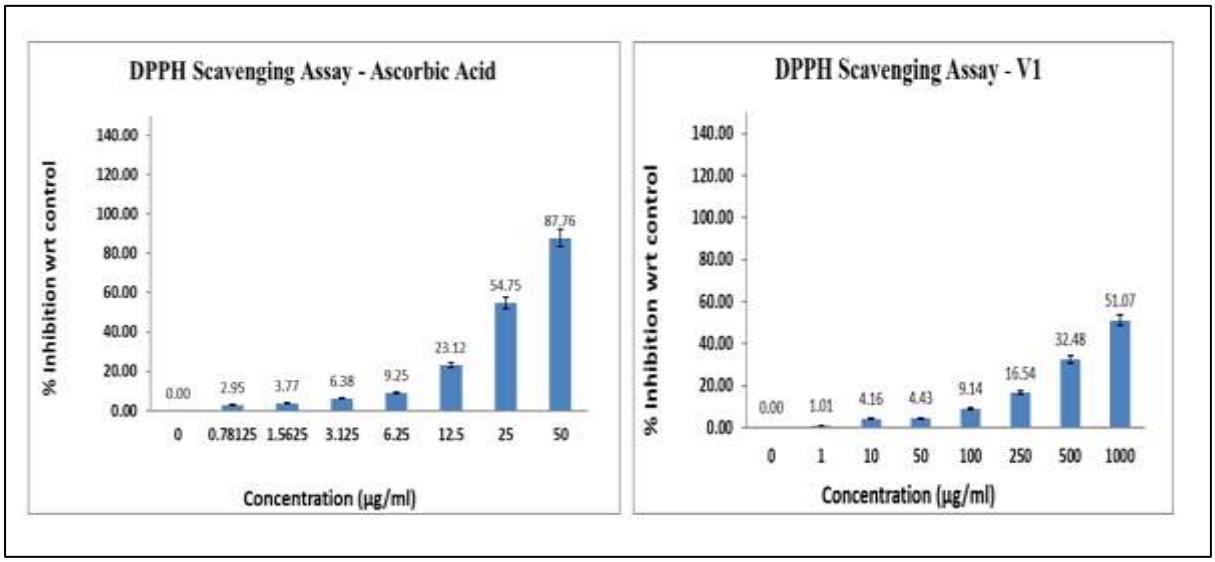


Figure-3 DPPH Scavenging Assay of Glycine max N-hexane Extract

Based on the results obtained from the experimental work, Antioxidant activity (DPPH Assay) was estimated in the sample, and 50% inhibitory concentration (IC_{50}) was mentioned in table 2. Samples – V1 was found to be less active. 982 µg of sample-P1 was found equivalent to 21.89 µg of standard Ascorbic acid.

Table 3: Effect of Glycine max N-hexane extract of seeds on aggregation of calcium oxalate crystals

Sample ID	**% Inhibition**
Control	0
100µg/ml	89.24
500µg/ml	89.50
1000µg/ml	88.74

Based on the result obtained, the Oxalate depletion assay demonstrates that the sample- V1 possesses oxalate depletion capability. Among the tested concentrations, 500 µg/ml showed maximum oxalate depletion activity, suggesting it may be the optimal concentration for effective oxalate degradation under the given experimental conditions

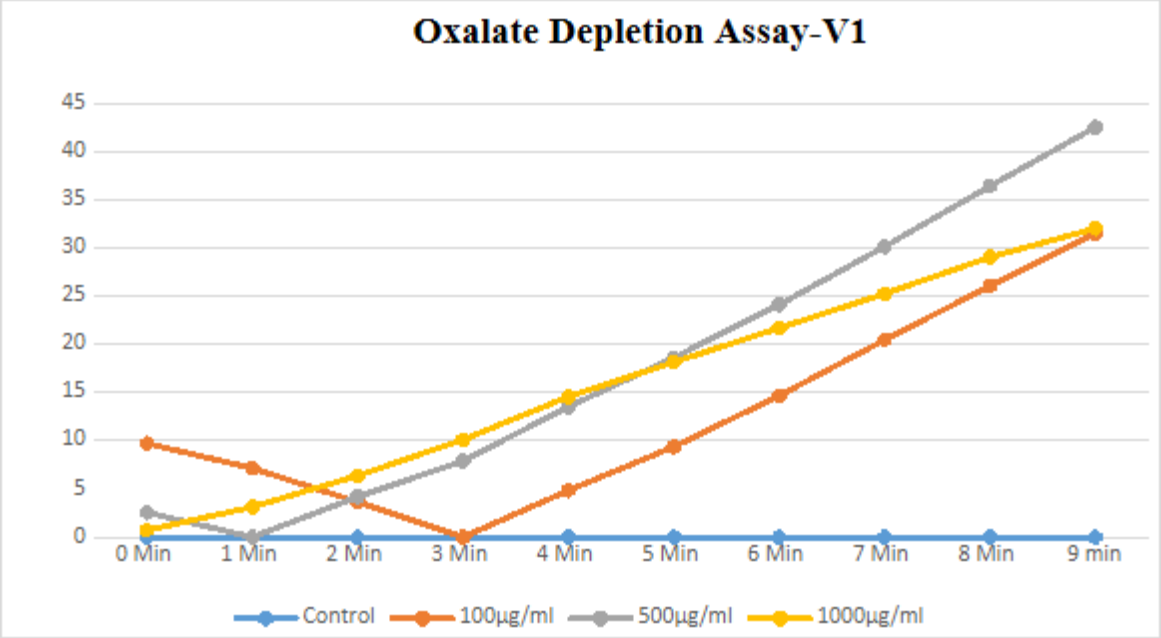


Figure-4 Displaying the graph of maximum oxalate depletion activity of Glycine max by using N-hexane Extract

Table 4: Effect of Glycine max N-hexane extract of seeds on aggregation of crystals

Sample ID	% Inhibition
Control	0
100µg/ml	100.24
500µg/ml	99.50
1000µg/ml	95.74

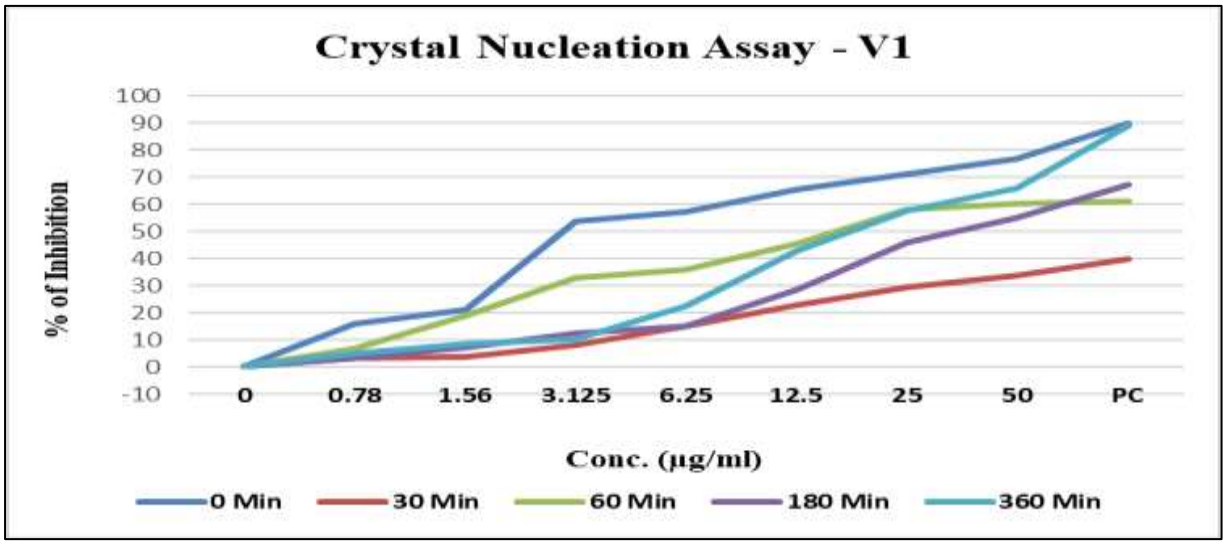


Figure-4 Displaying the graph of maximum crystal depletion activity of Glycine max by using N-hexane Extract

Characterization of Glycine max seeds by using N-hexane Extract

The FTIR analysis was carried out to examine the chemical bondings which eventually provided the information about the molecular structure of the compound. Two samples (seeds powder and standard drug powder) were provided for FTIR analysis and were compared for similarities in terms of chemical bondings and active functional groups. The FTIR spectra are shown in Fig. 5. In Fig. 5– FTIR spectrum of Glycine max seeds powder A broad band between the wavelengths 3305 to 3385 cm-1 indicates the presence of OH group of alcohol along with hydrogen bonding at wavelength 3385 cm-1. Thus, the seeds powder shows the presence of hydroxychavicol molecules, thereby giving a positive test for phenolic compounds. The peak at 2925 cm-1 is due to the presence of saturated aliphatic compounds especially having a C-H asymmetrical stretch of alkane. The band at 1740 cm-1 shows different compounds having ester linkages out of which the seeds powder has coumarin molecules that

fall around the same band of the spectra i.e., 1745 cm-1. The peak at 1608 cm-1 indicates C=C-C aromatic ring stretch. The peak at 1414 cm-1 shows nitrogen group thus confirming the presence of amino acids in the given seeds powder. The band at 1105 cm-1 and 1043 cm-1 indicates several aromatic C-H linkages with in-plane bend structures. The lowest peak at 723 cm-1 indicates aromatic C-H 1,2-disubstitution (ortho) molecules. The chemical bondings and the active functional groups are summarised in Table-5. In Fig. 5– FTIR spectrum of standard drug (cystone) powder. The spectrum generated by IR spectroscopy for the standard drug (cystone) powder shows similar peaks and broad bands to that of the seeds powder. Thereby, it confirms the presence of phenolic compounds, amino acids, alkaloids, and flavonoids in the standard drug. Since the phytoconstituents in seeds and the standard drug (cystone) are nearly the same in nature, the antiurolithiatic potential of its extracts shows similar effects on raphides (calcium oxalate) and struvite crystals seen in [Table-5]

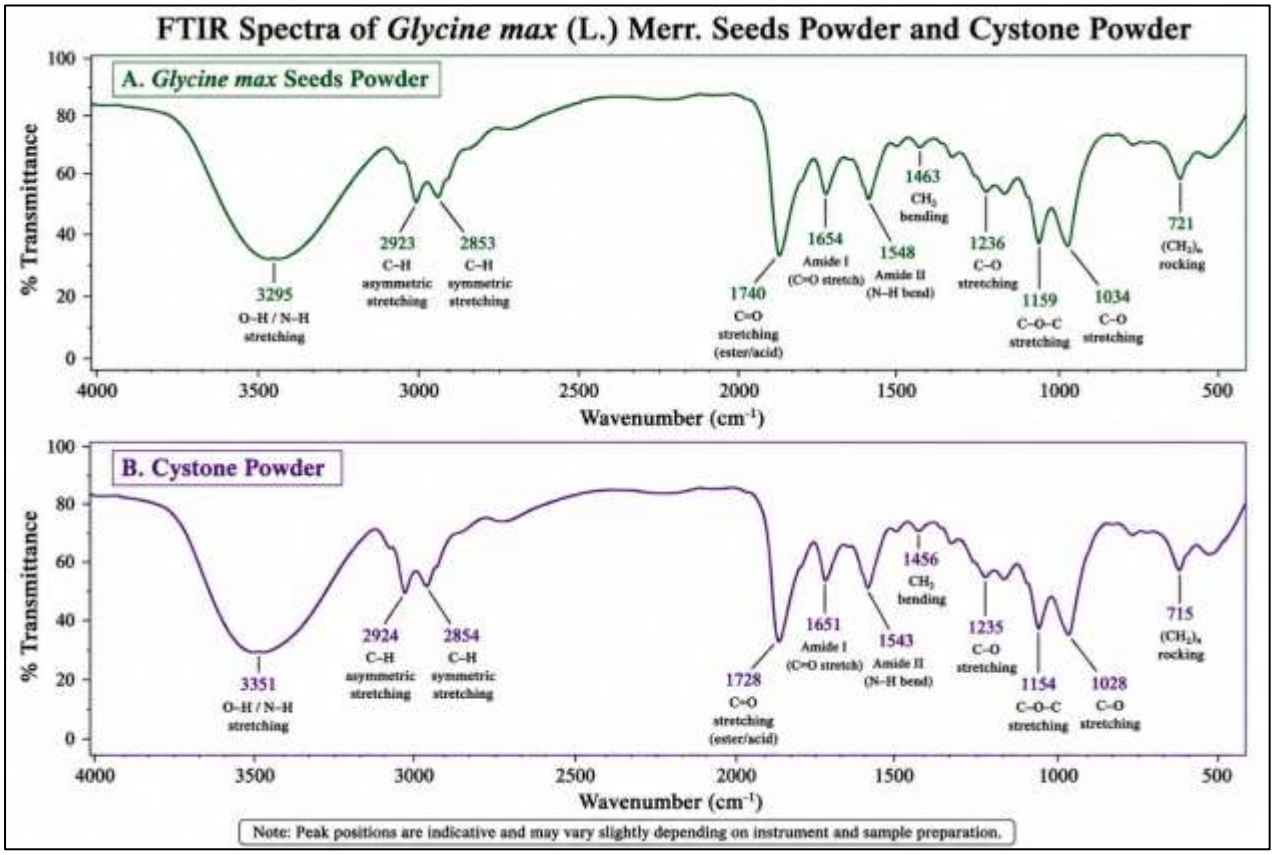


Figure-5- FTIR spectrum of Glycine max seeds powder with Cystone powder

Table-5 Wavelength Numbers and Functional Groups of Glycine max Seeds Powder (FTIR Analysis)

Sl. No.	Wavenumber (cm⁻¹)	Functional Group / Bond	Peak Assignment
1	3295	O–H / N–H	Stretching vibration
2	2923	C–H	Asymmetric stretching
3	2853	C–H	Symmetric stretching
4	1740	C=O	Carbonyl stretching (ester/acid)
5	1654	Amide I	C=O stretching vibration
6	1548	Amide II	N–H bending vibration
7	1463	CH_2	Bending vibration
8	1236	C–O	Stretching vibration
9	1159	C–O–C	Stretching vibration
10	1034	C–O	Stretching vibration
11	721	$(CH_2)n$	Rocking vibration

The FTIR spectrum of Glycine max seeds powder revealed the presence of hydroxyl, amine, carbonyl, amide, and aliphatic functional groups. The broad peak at 3295 cm^{-1} indicates O–H/N–H stretching, suggesting phenolic compounds and proteins. Peaks observed at 2923 cm^{-1} and 2853 cm^{-1} correspond to aliphatic C–H stretching of lipids and fatty acids. The strong absorption peak at 1740 cm^{-1} indicates carbonyl (C=O) groups of esters or fatty acids. Amide I and Amide II peaks at 1654 cm^{-1} and 1548 cm^{-1} confirm the presence of proteins. Peaks between 1236–1034 cm^{-1} correspond to C–O and C–O–C stretching vibrations associated with carbohydrates and polysaccharides. The peak at 721 cm^{-1} represents CH_2 rocking vibrations of long-chain hydrocarbons.

DISCUSSIONS

The present investigation demonstrated the significant phytochemical composition and biological activities of the n-hexane extract of Glycine max seeds through GC–MS analysis, antioxidant assay, antiurolithiatic studies, and FTIR characterization. The GC–MS chromatogram revealed the presence of 25 bioactive compounds, indicating the rich phytochemical nature of the extract. Among the identified constituents, linoelaidic acid (32.98%), cis-vaccenic acid (27.62%), n-hexadecenoic acid (17.50%), stigmast-5-en-3-ol (5.36%), octadecanoic acid (2.70%), ergost-5-en-3-ol (2.22%), and stigmasta-5,22-dien-3-ol (2.02%) were found in higher concentrations. These compounds are known to possess antioxidant, anti-inflammatory, nephroprotective, and membrane stabilizing properties. The presence of tocopherols, squalene, fatty acids, sterols, and phenolic compounds strongly supports the medicinal importance of the extract. Fatty acids such as cis-vaccenic acid and linoelaidic acid are reported to reduce oxidative stress and inflammation, which play important roles in kidney stone formation [15].The antioxidant activity evaluated using the DPPH radical scavenging assay revealed that the n-hexane extract possessed moderate free radical scavenging activity with an IC50 value of 982.6 µg/mL compared to standard ascorbic acid (21.89 µg/mL). Although the extract exhibited lower activity than the standard drug, the observed antioxidant potential may be attributed to the presence of phenolic compounds, tocopherols, and unsaturated fatty acids detected during GC–MS analysis. Oxidative stress is one of the major pathological factors involved in renal epithelial injury and calcium oxalate crystal formation. Therefore, the antioxidant potential of the extract may contribute indirectly to its antiurolithiatic effect by preventing oxidative damage in renal tissues. The antiurolithiatic activity of the n-hexane extract was confirmed through oxalate depletion and crystal aggregation inhibition assays. The extract showed remarkable inhibition of calcium oxalate crystal aggregation at all tested concentrations. Maximum oxalate depletion activity was observed at 500 µg/mL, suggesting optimal activity at this concentration. Similarly, crystal aggregation inhibition was highest at 100 µg/mL and 500 µg/mL, indicating the strong ability of the extract to prevent crystal nucleation, growth, and aggregation. These findings suggest that the phytoconstituents present in the extract may interfere with calcium oxalate crystal formation and reduce stone deposition [16]. FTIR spectral analysis further confirmed the presence of important functional groups such as hydroxyl, amine, aromatic, alkane, ester, and phenolic groups in both Glycine max seed powder and standard cystone powder. The similarity in FTIR peaks between the seed powder and cystone indicates the presence of comparable bioactive constituents responsible for antiurolithiatic activity. Overall, the study scientifically validates the traditional therapeutic potential of Glycine max seeds as a natural antioxidant and antiurolithiatic agent.

CONCLUSION

In conclusion, the present study demonstrated that the n-hexane extract of Glycine max seeds possesses significant phytochemical constituents with promising antioxidant and antiurolithiatic activities. GC–MS analysis confirmed the presence of various bioactive compounds such as fatty acids, sterols, tocopherols, and phenolic compounds that may contribute to its therapeutic effects. The extract exhibited moderate antioxidant potential and effectively inhibited calcium oxalate crystal aggregation and oxalate depletion, indicating its ability to prevent kidney stone formation. FTIR analysis further confirmed the presence of active functional groups comparable to standard cystone powder, supporting its potential as a natural antiurolithiatic agent.

REFERENCES

1. Agarwal, K. (2017). Ageratum conyzoides L. A plant with promising antilithic activity. International Journal of Applied and Universal Research, 4(1), 31-34.
2. Atmani, F., Glenton, P., & Khan, S. (1998). Identification of proteins extracted from calcium oxalate and calcium phosphate crystals induced in the urine of healthy and stone-forming subjects. Urological Research, 26, 201-207.
3. Edwin, E., Sheeja, E., Vabbav, J., & Sheweta, D. (2005). Toxicology of herbs. Pharma Times, 37(6), 27.
4. Emniyet, A. A., Avci, E., Ozcelik, B., Avci, G. A., & Kose, D. A. (2014). Antioxidant and antimicrobial activities with GC/MS analysis of the Morus alba L. Leaves. Hittite Journal of Science and Engineering, 1(1), 37-41.
5. Vandna, Trivedi, S., Dubey, A., Sahu, V. K., Dash, S. L., & Mishra, A. (2026). Valorization of Soybean (Glycine max (L.) Merr.) Processing By-Products: Pharmacological Properties and Clinical Significance: A Comprehensive Review. Journal for Research in Applied Sciences and Biotechnology, 5(2), 70–81. https://doi.org/10.55544/jrasb.5.2.9
6. Dubey, A., Ghosh, N., & Singh, R. (2023). An in-depth and in vitro evaluation of the antioxidant and neuroprotective activity of aqueous and ethanolic extract of Asparagus racemosus Linn seed. Research Journal of Chemistry and Environment, 27(10), 46–66. https://doi.org/10.25303/2710rjce046066.
7. Dubey Et Al., A. (2023). Role of aqueous and ethanolic seed extract of asparagus racemosus on acr-induced neurotoxicity in adult zebrafish: Emergence of neuroprotective results. Egyptian Journal of Aquatic Biology and Fisheries, 27(6), 285–296. https://doi.org/10.21608/ejabf.2023.329192
8. Sasikala V, Radha SR, Vijayakumari B. In vitro evaluation of Rotula aquatica Lour. for antiurolithiatic activity. J Pharm Res. 2013; 6(3); 378–382.

9. Gudulkar S, Rajbhar K, Dawda H, Mukundan U. Screening of selected plants for their effectiveness in the treatment of kidney stone. Annals of Phytomedicine: An International Journal. June,2020; 9(1); 213–217.
10. Jaiswal S, Patel M, Saxena D, Naik S. Antioxidant Properties of Piper Betel (L) Leaf Extracts from Six Different Geographical Domain of India. Journal of Bioresource Engineering and Technology. June,2014; 2(2); 12–20.
11. Das S, Parida R, Sandeep IS, Nayak S, Mohanty S. Biotechnological intervention in betelvine (Piper betle L.): A review on recent advances and future prospects. Asian Pac J Trop Med. 2016; 9(10); 938 946.
12. L M, Krishna A, Reddy J, Nirmala GS. Optimization studies on extraction of phytocomponents from betel leaves. Resource Efficient Technologies. 2017; 3(4); 385–393.
13. Saini S, Dhiman A, Nanda S. Pharmacognostical and phytochemical studies of Piper betle linn leaf. Int J Pharm Pharm Sci. January,2016; 8(5); 222 226.
14. Patel N, Jain D, Suryawanshi H, Pawar S. Phytopharmacological Study of Piper Betle Leaf. Saudi Journal of Medical and Pharmaceutical Sciences. November,2019; 5(11); 964–971.
15. Senthil Kumar R, Sabarinath.C, Kalaichelvan.VK. Phytochemical Characterization of Ethyl Acetate Extract of Bixaorellana Leaves using Gas Chromatography-Mass Spectrometry Analysis. Indian Journal of Natural Sciences. 2023; 14(80):60750 – 60753.
16. Makhawi AM, Hamadnalla H. Phytochemical screening of leaves and roots of Stylochitonborumensis: a medicinal plant. Earth Environ Sci Res Rev. 2019;2(1):1-5.