

CODEN [USA]: IAJPBB

ISSN: 2349-7750

INDO AMERICAN JOURNAL OF PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187 https://doi.org/10.5281/zenodo.7026040

Available online at: <u>http://www.iajps.com</u>

Research Article

EVALUATION OF INVITRO ANTI-OXIDANT, TOTAL FLAVONOID CONTENT AND ANTI-MICROBIAL ACTIVITY OF SOLANUM SURATTENSE (WILD EGG PLANT) BURN LEAVES

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Article Received: July 2022	Accepted: July 2022	Published: August 2022

Abstract:

The present research work relates to the preparation of ethanoic extraction of solanum surattense leaves and evaluation of extract for the anti-oxidant and anti-microbial activity The phytochemical analysis of ethanolic extract solanum surattense has revealed the presence of various phytochemical constituents such as Alkaloids, Flavonoids, Saponins, sterols, carbohydrates, glycosides. The Antioxidant activity was analysed using hydrogen peroxide assay method for the ethanolic extracts of solanum in concentrations of 10 - 50µg/Ml. The results revelaed that the ethanolic extract of solanum had showed the 60% activity The prepared extract was evaluated for Antimicrobial activity by using Agar well diffusion by Zone Inhibition method The antimicrobial of ethanolic extract of Solanum surattense leaves against two bacterial species at various concentrations were (20, 40,60,80,and 100µg/mL concentrations were evaluated. The results revealed that the Solanum surattense leaves showed good and better results for Antioxidant and Anti microbial activity. Thus it serves as an encouragement towards development of new drugs for the benefit of mankind.

Key words: solanum surattense, Leaves, Ethanolic extract, Anti-oxidant, Anti- microbial activity.

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Please cite this article in press Podili Niranjan Rao et al **Evaluation Of Invitro Anti-Oxidant, Total Flavonoid Content** And Anti -Microbial Activity Of Solanum Surattense (Wild Egg Plant) Burn Leaves., Indo Am. J. P. Sci, 2022; 09(8).

www.iajps.com

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INTRODUCTION:

Medicinal plants, also known as medicinal herbs, have been used in traditional medicine since prehistoric times, hundreds of compounds for a variety of functions can be synthesised from plants, including protection against insects, fungi, diseases and herbivorous mammals. The plant kingdom is a treasure house of potential drugs and in the recent years, there has been an increasing awareness about the importance of medicinal plants. Numerous phytochemicals with potential or established biological activity have been identified. However, a single plant contains wide diverse phytochemicals. The effects of using a whole plant as medicine are uncertain.

Herbal medicine is the foundation for about 75–80% of the World population, mainly targeting primary health care for in the developing countries because of better cultural acceptability, compatibility with human body and lesser side effects. However, there is a drastic increase in the usage of herbal medicine was found in last few years from the developed countries . The World Health Organization (WHO) has also recommended the evaluation of plants for effectiveness against human diseases and for the development of safe modern drugs.

Solanum surattense belongs to the family of Solanaceae. It is a commonly growing perennial herbaceous weed. In Hindi it is known as Katai, Katali, Ringani, Bhatakataiya, Chhotikateri and in English as Febrifuge plant, Yellow berried nightshade. Solanum surattense is distributed throughout India, Sri Lanka, South East Asia, Malaysia and tropical Australia. It has been used traditionally for curing various ailments such as fever, cough, asthma and diabetes in south Indian traditional medicines. The antidiabetic potential of the fruit was studied in diabetic rats The ethanol and methanol extracts of S. surattense showed strong antibacterial activity against Pseudomonas aeruginosa. Wound healing activity, physicochemical activity and antioxidant potential of the plant is also evaluated.¹⁻⁴

MATERIAL AND METHODOLOGY:

1.1 Collection & identification of Plant materials:

The plant samples such as leaves of Solanum surattense were collected from SV University, Tirupathi, Andhra Pradesh,I ndia during ethnobotanical surveys in 2020 to 2021. Specimen was labeled, numbered, annotated with the date of collection, the locality and their medicinal uses. The voucher specimens were then identified, and authenticated by K.Madhava chetty, Plant taxonomist, Division of Botany, Sri Venkateswara University, Andhra Pradesh. After authentification leaves and fruits were collected in bulk, washed, shade dried and extracted with different solvents such as ethanol for 48 hrs in a Soxhlet assembly⁵⁻⁷.

Drying and Grinding:

The fresh leaves of these species were collected and separated from undesirable material and then washed with water to remove dust and foreign matter and allowed to shade dry at room temperature for 10-15 days for long term storage purpose. The shade dried leaves were ground into a coarse powder with a suitable grinder. The powder was stored in air tight container until was commenced.

Preparation of ethanolic leaf extract of solanum surattense

50gms coarsely powdered plant material was weighed accurately and extracted with 1000ml of 95% of ethanol by using soxhlet Apparatus. The solvent was completely evaporated under reduced pressure at 50° C and dried in vacuum. The material thus obtained was filtered and dried. These was used as an extract to carry out preliminary phytochemical screening⁸.

1.2 Preliminary screening of photochemicals ⁹⁻¹²:

The preliminary phytochemical studies were performed for testing the different chemical groups present the drug, 10% (w/v) solution of extract was taken unless otherwise mentioned in the respective individual test. General screening of various extract of the plant material was carried out for the qualitative determination of the groups of organic compound.

Alkaloids

Preparation of test solution: The test solution was prepared by dissolving extracts in the dilute hydrochloric acid solution.

1. Mayer's test: The acidic test solution with Mayer's reagent (Pot. Mercuric iodide) gave cream coloured precipitate.

2. Dragendorff's test: The acidic solution with Dragendorff's reagent (Potassium bismuth iodide) showed reddish brown precipitate.

3. Wagner's test: The acidic test solution treated with Wagner's reagent (Iodine in Potassium iodide) gave brown precipitate.

Carbohydrates

Preparation of test solution: The test solution was prepared by dissolving the test extract with water. Then it was hydrolyzed with 1 volume of 2N HCl and subjected to following chemical tests.

1. Fehling's test: The test solution when heated with equal volume of Fehling's A and B solutions, gave orange red precipitate, indicating the presence of reducing sugars.

2. Tests for pentoses: The test solution with equal volume of HCl containing little phloroglucinol was heated. Formation of red colour indicated presence of pentose.

Flavonoid

1. Preparation of test solution:

a. To a small amount of extract, equal volume of 2M HCl was added and heated in a test tube for 30 to 40 min, at 100 $^{\circ}$ C.

b. The cooled extract was filtered, and extracted with ethyl acetate.

c. The ethyl acetate extract was concentrated to dryness, and used to test for flavonoids.

Shinoda test: Test solution with few fragments of magnesium ribbon and conc. HCl showed pink to magenta red colour.

Test for Proteins:Biuret test:

Test solutions were treated with 40% sodium hydroxide and dilute copper sulphate solution which gave blue colour.

Test for Saponin: Foam test:

Test solution when shaken with water showed formation of foam, which was stable for at least 15 min confirms saponin.

Test for Steroids: Preparation of test extract solution:

The extracts were refluxed separately with alcoholic solution of potassium hydroxide till complete saponification. The saponified extract was diluted with water and unsaponifiable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (unsaponifiable matter) was subjected to the following test by dissolving the residue in the Chloroform.

Test for Triterpenoids: Salkowski test:

To the test extract solution add few drops of conc. H2SO4, shaken and allowed to stand, lower layer turned red indicating the sterols.

Tannins and phenol compounds:

To 2-3 ml of alcoholic or aqueous extract, added few drops of following reagents: 5% FeCl3 solution: Deep blue-black colour.

Lead acetate solution: White precipitate.

Tests for Glycosides: Legal's test:

Extract were treated with sodium Nitroprusside in pyridine and sodium hydroxide.

1.3 *Invitro* Evaluation Studies

Preparation of standard solution:

10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with Methanol to get 100mcg/ml Quercetin standard solution (stock solution). From the stock solution, concentration of 100, 200, 300, 400, 500, mcg/ml were prepared. To each of these 4ml water was added followed by 0.3ml of 5% sodium nitrite. After 5min, 0.3ml of 10% Aluminium chloride solution and at the 6th minute 2ml of 1M Sodium hydroxide was added. The total volume was made up to 10ml with distilled water, a blank was prepared without addition of aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 510nm using UV-Visible spectrophotometer, a standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

Estimation of Total Flavonoids Content:

The concentrations of total flavonoids in crude extracts were determined according to the Aluminium Chloride method.1mg/ml plant extract was dissolved in distilled water for the estimation of flavonoid content. The plant extract solution was mixed with 5% NaNO₂ after 5 minutes, of which 10% AlCl₂.6H₂O

was added. To the mixture solution, 1 Molar NaOH was added, and the volume was adjusted to 1 ml. The same procedure was prepared for the standard solution of Rutin, and the calibration line was constructed. The reaction mixtures were then incubated for 30 min at room temperature. The absorbance was determined using a UV-VIS spectrophotometer at 510 nm. Flavonoid content was calculated from the graph made with standard Rutin and finally expressed in terms of quercetin equivalent (mg/g of extract).¹³⁻²⁰

1.4. In-Vitro Antioxidant Activity of Ethanolic Extract Of Plant:

Quantitative measurement of antioxidant potential of a drug using specific free radicals like DPPH freeradical, H2O2 free radical.

Hydrogen Peroxide Assay

Aliquot of 0.1 ml of extract (25-400µg/ml) was transferred into the Eppendroff tubes. The volume was made up to 0.4 ml with 50 mm phosphate buffer (PH 7.4).To the above solution add 0.6 of Hydrogen peroxide solution (2mm).The sample was placed in 10 mints. After 10 mints the absorbance was measured at 230 nm. Ascorbic acid was used as the positive control, Hydrogen peroxide scavenging activity in percentage was measured by using this following equation

 $= [(A0 - A1)/A0] \times 100$ A0 = Absorbance of control A1= Absorbance of sample

1.5 Screening Anti-Bacterial Activity of Ethanolic Extract Of Solanum Surattense ²¹⁻²⁵

Procurement of Bacteria

Bacterial strains used for determining antimicrobial activity of leaf extracts of S. surattense procured from Department of Biotechnology, S.V University, Tirupati, Andhra Pradesh, India. Pathogens used for the study were Escherichia coli, Staphylococcus aureus.

Revival of Pathogen

The collected pathogens were revived in nutrient broth and stored in nutrient agar slants at 4°C.Screening of leaf extract (ethanol) of Solanum surattense was done using agar-well diffusion method. Nutrient agar medium ingredients

- Beef extract -1g,
- Yeast extract -2g,
- Sodium Chloride -1g,
- Peptones 5g, Agar -20g,
- Distilled Water -1000 ml

The medium was autoclaved at 121.6°C for 30 minutes and poured into petriplates. Bacteria were grown in nutrient broth for 24 hours.

A 100µl of bacterial suspension was spread on each nutrient agar plate. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each petriplate. The wells in each plate were loaded with 20%, 40%, 60%,80% and 100% concentration of prepared extracts of Solanum

surattense and delivered into them. The plates were incubated at 37°C for 24h. Clear inhibition zones around the wells indicated the presence of antibacterial activity. After incubation time, the zone of inhibition was measured precisely in millimeters (mm). The same procedure was followed for standard antibiotics ampicillin (50 μ l) to compare the efficacy of extracts against test organisms. Each experiment was repeated three times, and the average values were calculated. The amoxicillin stock solution was prepared at the concentration of 1mg/mL. The controls were prepared using the same solvents employed to dissolve the extracts. The petriplate kept as control contained pure solvent in the well. The plates were incubated at 37±20C for 24 hours in the incubation chamber. The readings were taken in perpendicular direction for all the three replicates and the average values were tabulated. Percentage inhibition of growth of bacterial microorganisms was calculated after subtracting control from the values of inhibition diameter using control as standard.

Percentage of growth inhibition= (Control Test/Control) x100

Control=average diameter of bacterial colony in control.

Test=average diameter of bacterial colony in treatment sets.

RESULTS & DISCUSSION:

2.2 Preliminary Phytochemical Analysis:

The phytochemical analysis of ethanolic extract solanum surattense has revealed the presence of various phytochemical constituents such as Alkaloids, Flavonoids,

Saponins, sterols, carbohydrates, glycosides and other which are tabulated below in Table 1.

S/NO	Qualitative Phytochemical Test	Tests	Results[Ethanolic extract]
1	Test for Alkaloids	Mayer test	Present
		Dragendroff test	present
2	Test for tannins	Gelatin test	Absent
3	Test for sterols	Salkowski test	present
4	Test for saponins	Forth test	Present
		Foam test	present
5	Test for Glycosides	Legal test	present
6	Test for Flavonoids	Alkaline reagent test	present
7	Test for Phenols	Ferric chloride test	Absent
8	Test for Carbohydrates	Benedicts test	Absent
9	Test for Proteins	Biuret's test	Present

	Xanthoproteic test	Absent
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2.3 INVITRO ANTI-OXIDANT ACTIVITY

Antioxidant activity was performed by two different methods such as DPPH, Hydrogen peroxide scavenging assays. In this we discuss hydrogen peroxide scavenging assay ,Ascorbic acid was used as standard for the determination of antioxidant potential of S. surattense. Results obtained with the hydrogen peroxide assay given in Table 2 and Figure 1 from the hydrogen peroxide assay, The IC50 value of ethanolic extract of the plant was 57.5% . Thus results obtained from this techniques show strong evidence of the antioxidant potential of the plant Solanum surattense. Wherein the Table 3 figure 2 and explains the Percentage inhibition of Solanum surattense by using Hydrogen peroxide scavenging assays

Table 2: Hydrogen peroxide free radical scavenging assay			
Con	Absorbance (EE)	Absorbance (standard)	Absprbance(aq)
20	1.08	1.2	1
40	0.85	1.1	0.8
60	0.69	0.95	0.6
80	0.6	0.88	0.5
100	0.35	0.73	0.2

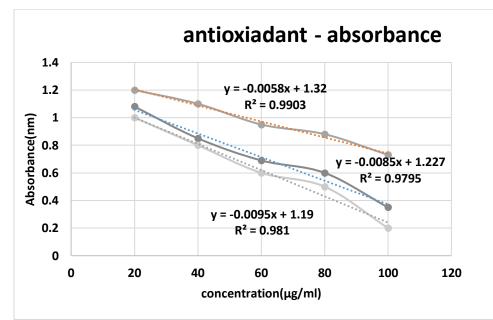


Fig 1: Graph showing the hydrogen peroxide assay.

Table 3: Percentage inhibition of Solanum surattense by using	Hydrogen peroxide scavenging assays

Concentration	% of Ethanolic Extract	% of Aqueous Extract
20	10	16.6
40	22.7	27.2
60	27.3	36.8
80	31.81	43.1
100	52.05	72.06

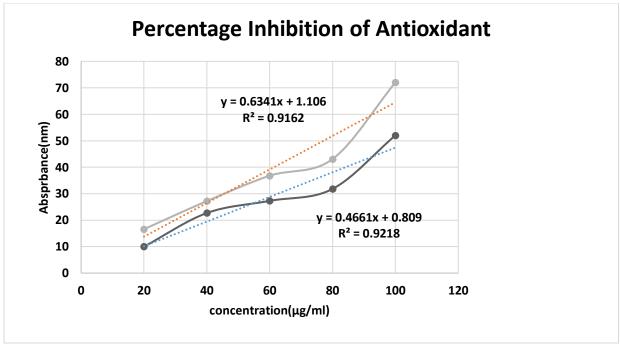


Fig 2: Percentage Inhibition of anti-oxidant activity of prepared Extract

2.4. Estimation of Total Flavonoids Content:

The concentrations of total flavonoids in crude extracts were determined according to the Aluminium Chloride method. In this Rutin used as standard for the estimation of flavanoid content in solanum surattense leaves. Results obtained with these are given in table 4. From Alcl3 method. Thus results obtained from this techniques show strong evidence of the Flavanoid content of the plant Solanum surattense.

Concentration	Absorbance (EE)	Absorbance(standard)	Absorbance (aq)	
10	0.11	0.15	0.1	
20	0.24	0.35	0.21	
30	0.347	0.56	0.3	
40	0.432	1.1	0.4	
50	0.54	1.5	0.5	

Table 4: Results of total flavonoid content of the prepared extracts

*EE-Ethanolic extract; AE-Aqueous extract; Standard -Rutin



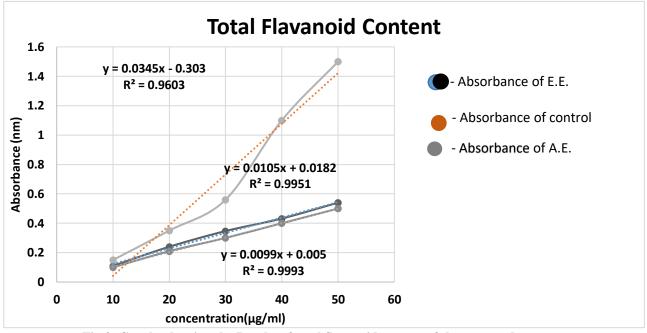


Fig 3: Graphs showing the Results of total flavonoid content of the prepared extracts

The results of Percentage Inhibition of Flavonoid Content In Solanum Surattense Leaves were shown in table 5 and figure 4.

Concentration	% of Flavanoid Content In EE	% of Flavanoid Content In AE
10	26.6	33.3
20	31.42	40
30	38.03	46.42
40	60.72	63.63
50	64	66.6

Table 5: Percentage Inhibition of Flavonoid Content In Solanum Surattense Leaves

EE-Ethanolic extract; AE-Aqueous extract

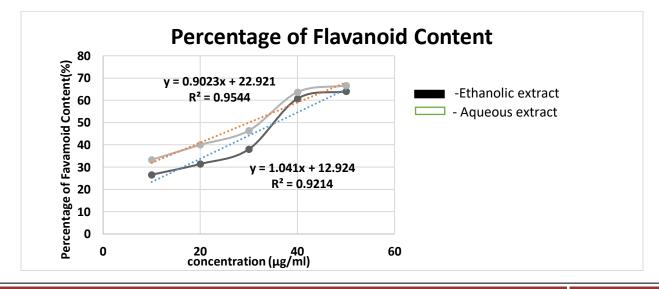


Fig 4: Graph showing the Percentage Inhibition of Flavonoid Content In Solanum Surat tense Leaves 2.5 Anti-Bacterial Activity

Minimum Inhibitory Concentration (MIC)

This test was carried out using serial broth dilution technique. It indicates the lowest concentration of an antimicrobial agent that inhibits growth of a certain microorganism after 18to24hours of incubation. Anti-microbial agents with low activity against an organism had a high MIC while highly active anti-microbial agent gave a low MIC. The MIC tests were carried out for crude extracts that showed antibacterial activities based on the agar well-diffusion method. Table 6: Antibacterial activity by zone of inhibition method at different concentrations of ethanolic extract of Solanum surattrnse leaves

Con In(µg/Ml)	Zone of Inh	ibition	Amoxicillin (Standard)Mm	
	E.coli	S.aureus		
control	Nill	Nill		
20	10	12	22	
40	11	13	34	
60	14	16	29	
80	15	17	25	
100	17	19	28	

E.COLI –Escherichia coli; S.Aureas-Staphylococcus aureas; Standard-Amoxicilin

The present study brings out that ethanolic leaf extract of Solanum surattense proved itself as good antibacterial agent. The ethanolic extracts of S. surattense showed considerable growth inhibition of test bacteria at different concentrations (20, 40, 60,80 100μ g/ml) leaf extract of the plant. The ethanolic extract of Solanum Surattense was found to be most effective against S. aureus at (19mm at 100µg/ml) followed by (17mm at 80µg/ml), (16mm at 60µg/ml), (13mm at 40µg/ml), and (12mm at 20µg/ml)it offered minimum inhibition compared to the ethanolic extract of Solanum surattense was found to be against E.coli at (17mm at 100µg/ml) followed by (15mm at 80µg/ml), (14mm at 60µg/ml), (11mm at 40µg/ml), (10mm at 20µg/ml)and it showed minimum inhibition towards E.coli as shown in table.6 and fig 5.

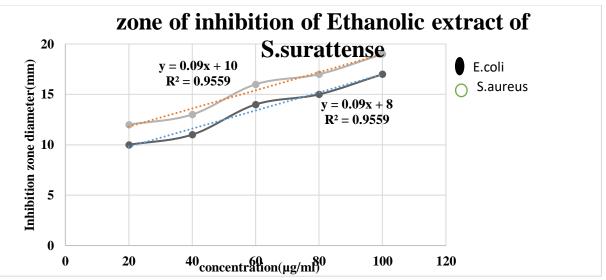


Fig 5: Anti bacterial activity of ethanolic leaf extract of Solanum surattense against various Bacterial strains

It was concluded from the results that ethanolice leaf extract of Solnum surattense leaves were quite effective in inhibiting the growth of Staphylococcus aureus which is considered as a serious human pathogen causing infections in wounds. Possible reason for this antibacterial activity of Solanum surattense are presence of alkaloids, saponins and flavanoids in its leaves. Majority of phytochemical components are known to produce the therapeutic activity like antibacterial, antifungal and antioxidant etc. These finding are in accordance with the work carried out by Salie etal. Our study was also found to correlate with the results of on phytochemicals extracted from the leaves of solanum surattense. Thus it serves as an encouragement towards development of new drugs for the benefit of mankind.

CONCLUSION:

The present research work relates to the preparation of ethanoic extraction of solanum Surat tense leaves and evaluation of extract for the anti-oxidant and antimicrobial activity The study was completely worked on a comparative basis to find out which extract(in particularly ethanoic and aqueous extract) is more efficient. The phyto chemical screening of the prepared extract were done for presence of alkaloids, flavonoids, phenolic, proteins, amino acid, sterols, carbohydrate, terpenoids, tannin, cardiac glycosides, and saponins The Antioxidant activity was analyzed using H2O2 method for species of Solanum using ethanolic extracts in concentrations of 10 - 50µg/mL which showed 60% activity in Solanum surattense. The prepared extract was evaluated for Antimicrobial activity by using Agar well diffusion by Zone Inhibition Method From the results it was concluded that the Solanum surattense leaves showed good and better results for Antioxidant and Anti-microbial activity. The antimicrobial activity showed very good results in and Solanum surattense leaves against two bacterial species when the ethanolic extracts (20, 40,60,80, and 100µg/Ml) concentrations. Thus it serves as an encouragement towards development of new drugs for the benefit of mankind.

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