JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

QUALITATIVE, QUANTITAIVE SCREENING, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF VARIOUS PARTS OF *SOLANUM SURATTENSE*

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Abstract:

Solanum surattense which belongs to the family Solanaceae is one of the medicinal plants in India. Biological activities such as antidysentric, antiseptic, antidiuretic is reported by the whole plant. This study reveals both the qualitative and quantitative screening of phytochemicals along with antioxidant and antibacterial activity of different extracts of leaf, root and stem of *S. surattense*. Ethanolic, methanolic, petroleum ether and aqueous extracts were used for the screening purpose. Alkaloids, terpenoids, flavonoids, phenols, saponins, cardiac glycosides, resins and anthroquinones were found to be present in the extracts in reasonable qualities. In the quantitative screening aqueous leaf extract showed highest amount of total phenolics (28.10 ± 0.19 mg/gm), the same extract showed high amounts of total flavonoids (16.20 ± 0.89 mg/gm) and aqueous stem extract showed highest percentage of antioxidant activity (56.70 ± 0.10 µg/ml). Antibacterial activity was analyzed by using disc diffusion method against gram positive and negative bacteria. All the extracts revealed activity against all the strains. Aqueous leaf extract showed highest zone of inhibition against *Staphylococcus aureus*. Ethanolic extract of leaf showed minimum inhibitory concentration (MIC) values of 15.0 µg/ml against *Bacillus subtilis* and *Staphylococcus aureus*. Even ethanolic stem extract also showed lowest MIC value of 15.0 µg/ml against *Bacillus subtilis.*

Key words: *Solanum surattense,* phytochemical analysis, total phenols and flavonoids, antioxidant, antibacterial activity.

Introduction:

Plant based drugs form a common element in all traditional medicines and have substantial contribution to modern therapeutics by providing life – saving drugs such as quinine, morphine, digitoxin, reserpine and so on [1]. These herbal medicines are used to cure mental disorders, skin diseases, tuberculosis, diabetes, jaundice, hypertension, cancer etc. The emergence of new infections diseases, the resurgence of some infectious diseases that appeared to have been controlled, the increase in microbial drug resistance and the increasing failure of chemical drugs have led to the screening of several medicinal plants for their antimicrobial activity [2]. For the last 30 years Traditional, complementary and alternative medicine has been recognized by WHO as culturally acceptable, affordable and sustainable means of primary healthcare [3]. For the purpose of scientific back up of the plant-based medicines, a thorough examination of their bioactive principles for their efficacy and safety is very much essential [4].

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. Solanaceae is a huge plant family comprising of about 90 genera and 3000 species, approximately half of which belongs to a particular genus, Solanum. There are herbs, shrubs or small trees under this genus. This family includes a number of plants usually known for the occurrence of a variety of natural products with medicinal importance. Crude plant extract is beneficial in bronchial asthma and non-specific cough, influenza, difficult urination, bladder stones, rheumatism, etc [5].

Solanum surattense (*S. xanthocarpum Schr.* & *Wendl.*) of Solanaceae is known by different names in various languages in India i.e. Kantkari, Kateri, Vakudu [6]. In English it is called as Indian night shade or Yellow berried night shade. According to Indian Meteria medica leaves of *S. surattense* have anthelmintic, anti-inflammatory, digestive, carminative, appetizing, antihypertensive, antipyretic, antitussive properties and useful in curing rheumatoid arthritis, asthma, bronchitis, pharyngitis, urolithiasis, amenorrhoea, dysmenorrhea, lumbago, haemorrhoids, cardiac disorders and catarrh [7].

The detection of bioactive compounds from different parts of medicinal plants continues to be a bonus to the well-being of the human society as they do not have any contrary side effects. The literature survey discloses the significance of fact-finding study of medicinal plants for their therapeutic properties. Many exploratory reports showed that *Solanum* plants are significant source of large number of phytochemical compounds with significant curative application against human pathogens [8]. Bearing in mind, the main objective of the present study is to explore the phytochemical composition of the crude extracts (ethanol, methanol, petroleum ether and aqueous) of root, stem and leaf of *S. surattense* along with total phenol, total flavonoid content, antioxidant activity of the crude extracts (DPPH method) and also antibacterial activity against certain bacterial strains.

MATERIALS AND METHODS

Collection of plant material:

The plant material was collected from the medicinal herbal garden, Dravidian University, Chittoor dist, Andhra Pradesh. The plant parts used for the study were leaf, stem and root. Botanical credentials of plant material was done based on the data present in previous literature and properly documented.

Preparation of plant extracts:

Plant parts were collected and rinsed with double distilled water, which were then dried on a paper towel in shade for a week. The dried parts (leaf, stem and root) were powdered in a blender and then subjected for extraction. 100 gm weight of the dried plant parts were packed separately into thimble of soxhlet extractor and extracted with solvents such as ethyl alcohol, methanol, chloroform, ethyl acetate, hexane and diethyl ether separately. The extracts were concentrated to a dry mass under vacuum according to method of Harborne (1973) [9].

Qualitative phytochemical analysis of various extracts:

Phytochemical screening of the reconstructed extracts acquired after the soxhlet extraction was done by following the procedure of Indian Pharmacopoeia [10], phytochemical analysis of all the solvent extracts was conducted. By this analysis, the presence of several phytochemicals (alkaloids, terpenoids, flavonoids, saponins, phenols, cardiac glycosides, resins and anthroquinones) was tested. The concentrations of the metabolites perceived were conveyed as absent (-), less (+), moderate (++) and high (+++) depending on the color intensity.

Alkaloids were tested by using Mayer's test, presence of cream colored precipitate indicates the presence of alkaloids [11]. For terpenoids, a 0.5 ml extract + 2 ml chloroform + 3 ml conc. Sulphuric acid reddish brown coloration of the interface indicated the presence of terpenoids [12]. For flavonoids, a 2 ml filtrate + conc. HCl + magnesium ribbon pink tomato red color indicated the presence of flavonoids [13]. Formation of stable persistent froth indicated the presence of saponins [14] when mixed vigorously with distilled water. For phenols, 1 ml of each solvent extracts dissolved in alcohol was separately treated with a few ml of neutral ferric chloride solution and the color change indicated the presence of phenols [15]. For cardiac glycosides, a brown ring indicates a deoxysugar characteristic of cardenolides [16]. For resins, to one ml of extract 10 ml of distilled water was added and for 15 min ultra-sonicated at 30°C and the presence of turbidity shows the presence of resins [17]. For anthroquinones, extract is mixed with benzene and 10% ammonium solution, pink color formation in the ammonical phase indicates the presence of anthroquinones [18]. Total phenols were analyzed by Folin – Ciocalteu method in ehich the absorbance was measured against the blank at 760 nm with an UV – Visible spectrophotometer [19]. Total flavonoid content was done by adding 5% sodium nitrate and 10% aluminium chloride to the extract and the absorbance was measured at 510 nm [20].

Antioxidants were measured by DPPH (2, 2 diphenyl 1 picrylhydrazyl) free radical scavenging activity and the percentage was measured by the formula $[(A_0 – A_1)/A_0] * 100$ where A_0 is absorbance of the control and A_1 is absorbance of the extractives [21].

Antibacterial activity:

Preparation of bacterial inoculum: The gram negative bacterium *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and the gram positive bacteria *Bacillus subtilis* were cultured in nutrient broth medium and the cell density was measured spectrophotometrically.

Antibacterial assay was done by agar well diffusion method [22] with slight modifications. The zone of inhibition was measured in millimeters against test organisms with antibiotic ciprofloxacin as positive control and DMSO as negative control. Minimum inhibitory concentration was done by macro broth dilution method. 2 fold serial dilutions of ciprofloxacin antibiotic was used as the control. Least concentration (highest dilution) of the extract with no detectable growth was considered as the MIC value.

Statistical analysis:

All experimental results were carried out in six fold and were expressed as average of six analyses ± SD (Standard deviation).

RESULTS AND DISCUSSION

Phytochemical analysis of secondary metabolites in different plant parts of *S. surattense*:

The preliminary phytochemical screening of the different extracts such as aqueous, ethanol, methanol and petroleum ether of different plant parts *of S. surattense* of leaf, stem and root showed the presence of various phytoconstituents which are showed in table 1.The results showed the presence of alkaloids, terpenoids, flavonoids, saponins, phenols, cardiac glycosides, resins and anthroquinones.

Higher concentrations of alkaloids, flavonoids, saponins, phenols, cardiac glycosides was present in the aqueous and ethanol extracts of leaf. Cardiac glycosides were also seen in high quality in the stem and root ethanolic extract. Resins were seen in high quality in the ethanolic leaf extract. Petroleum ether has very less quantity of alkaloids, terpenoids, saponins, resins and anthroquinones. Resins and anthroquinones were absent in the methanolic and petroleum ether extracts of stem and root. Dar *et al.*, reported the similar results in 2017 [23].

Table 1: Phytochemical analysis of secondary metabolites in different plant parts of *S. surattense*

Phytochemicals	Different extracts	Plant parts of *S. surattense*		
		Leaf	Stem	Root
Alkaloids	Aqueous	+++	+++	++
	Ethanol	++	++	++
	Methanol	++	++	+
	Petroleum Ether	++	+	+
Terpenoids	Aqueous	++	++	++
	Ethanol	+++	++	++
	Methanol	++	++	+
	Petroleum Ether	+	+	+
Flavonoids	Aqueous	+++	+++	++
	Ethanol	+++	++	++
	Methanol	++	++	++
	Petroleum Ether	++	++	+
Saponins	Aqueous	+++	+++	++
	Ethanol	++	++	++

	Methanol	+	++	+
	Petroleum Ether	+	+	-
Phenols	Aqueous	+++	+++	+++
	Ethanol	+++	++	++
	Methanol	++	++	++
	Petroleum Ether	++	++	+
Cardiac glycosides	Aqueous	+++	+++	+++
	Ethanol	+++	+++	+++
	Methanol	++	++	+
	Petroleum Ether	+	+	+
Resins	Aqueous	++	+	++
	Ethanol	+++	++	+
	Methanol	++	++	+
	Petroleum Ether	+	+	--
Anthroquinones	Aqueous	++	+	+
	Ethanol	++	+	+
	Methanol	+	--	+
	Petroleum Ether	--	--	--
- =absent; + = less; ++ = moderate; +++ = high				

The preliminary phytochemical studies received marked importance as the crude drugs hold varied configuration of secondary metabolites [13]. The phytochemicals are produced by the leaf and stem of this plant. Though their functions are unknown they are considered to have functional and defense against plant pathogens [24].

Quantitative phytochemical analysis of different extracts:

Total phenols:

The total phenol content of different plant parts of *S. surattense* plant in different extracts were represented in table 2. The aqueous extract of leaf revealed high amount of total phenolics (28.10 ± 0.19 mg/gm) followed by the stem of same extract (24.17 ± 0.20 mg/gm). The least amount was noted in the petroleum ether extract of root (7.14 ± 0.23 mg/gm). The phenolic compounds (secondary metabolites) are present in the form of simple phenolic acids to complex polymer compounds in the plants. The phenol profile depends on the photosynthesizing mesophilic tissue, which may be the reason for the difference in the total phenolic compounds recorded [25].

Table 2: Total phenol content (mg/gm) of different plant parts of *S. surattense* plant in different extracts

Different extracts	**Plant parts of *S. surattense***		
	Leaf	**Stem**	**Root**
Aqueous	28.10 ± 0.19	24.27 ± 0.20	20.17 ± 1.80
Ethanol	24.18 ± 0.57	12.18 ± 0.71	10.20 ± 0.60
Methanol	14.05 ± 0.71	10.17 ± 0.85	11.19 ± 0.68
Petroleum ether	16.17 ± 0.80	14.18 ± 0.72	7.14 ± 0.23

Values are mean ± S.E. of six independent determinations

Total flavonoid content:

The total flavonoid content of different plant parts of *S. surattense* in different extracts was represented in table 3. The aqueous extract of leaf revealed highest amount of total flavonoids when compared to others (16.20 ± 0.89 mg/gm) which was followed by the ethanolic extract of leaf (14.17 ± 0.23 mg/gm). Low concentration was seen in the petroleum ether extract of the root (3.28 ± 0.08 mg/gm). The remaining extracts showed moderate amount of total flavonoid contents in different parts of the plant. The number of free hydroxyl groups shows the impression of flavonoid content [26]. Genetic diversity, biological, environmental and seasonal variations considerably marks the flavonoid content [27].

Table 3: Total Flavonoid content (mg/gm) of different plant parts of *S. surattense* plant in different extracts

Different extracts	Plant parts of *S. surattense*		
	Leaf	Stem	Root
Aqueous	16.20 ±0.89	13.73 ± 0.70	8.23 ± 0.53
Ethanol	14.17 ± 0.23	9.14 ± 0.30	7.19 ± 0.50
Methanol	8.30 ± 0.71	7.61 ± 0.30	8.01 ± 0.23
Petroleum ether	9.13 ± 0.90	5.14 ± 0.31	3.28 ± 0.08

Values are mean ± S.E. of six independent determinations

DPPH free radical scavenging assay:

Various parts of *S. surattense* was examined for the antioxidant activity of aqueous extract in five different concentrations (100, 200, 300, 400 and 500 µg) by considering the standard activity of antioxidant ascorbic acid which is given in table 4. The plant parts contain free radical scavengers like polyphenols, flavonoids and phenolic compounds.

Table 4: DPPH free radical scavenging assay of different plant parts of *S. surattense* plant in aqueous extract

	Concentration of the sample				
	100 µg	200 µg	300 µg	400 µg	500 µg
Ascorbic acid (Standard)	47.21 ± 0.41	56.73 ± 0.35	65.54 ± 0.97	68.43 ± 0.35	74.20 ± 0.98
Leaf	18.35 ± 0.71	28.65 ± 0.80	36.23 ± 0.31	41.18 ± 0.61	46.80 ± 0.58
Stem	18.06 ± 0.80	27.53 ± 0.71	38.13 ± 0.17	42.26 ± 0.72	48.21 ± 0.82
Root	15.84 ± 0.44	24.25 ± 0.62	31.07 ± 0.52	39.41 ± 0.91	44.30 ± 0.67

Values are mean ± S.E. of six independent determinations

The aqueous extract of stem expressed highest percentage of antioxidant activity at 500 µg concentration (48.21 ± 0.82) which is followed by aqueous extract of leaf at same concentration (46.80 ± 0.58). The DPPH activity of the aqueous extract of leaf, stem and root of *S. surattense* is less when compared to that of the standard Ascorbic acid. This may be because of the equilibrium between production and scavenging of reactive oxygen species is

perturbed under a number of stressful conditions such as salinity, drought, high light, toxicity due to metals and pathogens [28]. Natural antioxidants present in the plant parts are accountable for inhibiting the toxic consequences of oxidative stress [29].

Antibacterial Activity:

The results of preliminary antibacterial activities of various solvent extracts of leaf, stem and root of the plant against four bacterial species have been recorded in table 5.

Table 5: Susceptibility of test bacterial strains to leaf, stem and root extracts of *S. surattense* and standard ciprofloxacin

Type of extract/antibiotic	Zone of inhibition of antibacterial activity (in mm)			
	Escherichia coli	***Bacillus subtilis***	***Staphylococcus aureus***	***Klebsiella pneumoniae***
Leaf:				
Ethanol	8.34	9.43	7.73	8.52
Methanol	12.42	18.36	8.72	14.21
Petroleum ether	--	23.57	--	--
Aqueous	17.24	29.62	9.23	--
Stem:				
Ethanol	--	8.23	7.14	--
Methanol	15.73	20.23	17.46	15.76
Petroleum ether	--	--	--	--
Aqueous	16.34	16.44	16.52	11.64
Root:				
Ethanol	--	--	--	--
Methanol	15.36	13.64	15.86	14.93
Petroleum ether	--	--	--	--
Aqueous	15.78	15.86	--	11.96
Antibiotic:				
Ciprofloxacin	10.45	14.34	12.74	11.29

Values are mean ± S.E. of six independent replicates

The results showed remarkable activity against the isolated test organisms with zone of inhibition ranging from 7 to 30 mm. Maximum zone of inhibition is shown by aqueous leaf extract (29.62 mm) extract against *Bacillus subtilis* followed by petroleum ether extract of same part (23.57 mm) against same bacterial strain. Minimum zone of inhibition is shown by ethanolic extract of stem against *Staphylococcus aureus*. Ethanolic root extract and petroleum ether extract of stem and root does not show any activity. The leaf petroleum ether extract also does not show any activity aginst *E. coli, S. aureus and K. pneumoniae*. Even the aqueous extract of root also

doesnot showed activity against *S. aureus*. The results obtained from the crude extracts were compared with the standard antibiotic Ciprofloxacin. Almost all the tested organisms are highly sensitive to the aqueous and methanol extracts (29.62 – 17.46 mm) than the standard antibiotic which showed more or less activity (10.45 – 14.34 mm). Reports suggested that some bacteria like *E. coli* are known to be multi resistant to drugs, was also resistant to plant extracts [30]. The present study reveals that the active principles present in the aerial parts of the plant are active against all the bacterial strains compare to roots similar results were reported by Mazher *et al.*, in 2016 [28].

Minimum inhibitory concentrations (MIC):

The MIC were shown in the table 6. Among the various extracts tested, ethanolic extract showed lowest MIC value (15 µg/ml). Lowest MIC value was recorded against *Bacillus subtilis* and *Staphylococcus aureus* of ethanolic and methanolic stem extract (15 µg/ml) followed by ethanolic stem extract against *S. aureus* (17.5 µg/ml).

Table 6: Minimum inhibitory concentrations of the crude extracts of *S. surattense* against the test bacterial strains

Type of extract/antibiotic	MIC (µg/ml)			
	Escherichia coli	***Bacillus subtilis***	***Staphylococcus aureus***	***Klebsiella pneumoniae***
Leaf:				
Ethanol	18	15	15	25
Methanol	25	28.5	15	35
Petroleum ether	--	30	--	--
Aqueous	28	40	16	--
Stem:				
Ethanol	--	15	17.5	--
Methanol	28.5	30	23.5	22.5
Petroleum ether	--	--	--	--
Aqueous	30	25	23.5	20
Root:				
Ethanol	--	--	--	--
Methanol	30	50	25	40
Petroleum ether	--	--	--	--
Aqueous	30	60	--	35
Antibiotic:				
Ciprofloxacin	17	19	28	28

Values are mean ± S.E. of six independent determinations

From the table, it is noted that the leaf extract showed lowest MIC values followed by stem extracts. The antimicrobial showed may be because of the presence of aromatic compounds such as alkaloids, flavonoids, phenols [31].

CONCLUSION

To conclude medicinal plants contain plentiful compounds which are essential to control the growth of the microorganisms. Researchers have realized an immense prospective in natural products from medicinal plants to aid as substitute source for combating infections in humans which are low in cost and also have less toxic effect. So based on the results it can be concluded that the extracts of *S. surattense* may hold vast resource of pharmaceutical properties.

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