



Evaluation of phytochemical screening, total phenols, flavonoids, antioxidant and antibacterial activity of various plant parts of extracts of *Solanum nigrum* L.

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ABSTRACT

Solanum nigrum is one of the Indian medicinal plants which belong to the family Solanaceae. The whole plant is reported to have many biological activities such as antiseptic, antidysentric, antidiuretic and also has gastric ulcerogenic activity. The present study was carried out to investigate possible qualitative and quantitative phytochemical screening, antioxidant and antibacterial activity of various extracts of *S. nigrum* leaf, stem and root. Qualitative phytochemical screening of the extracts (ethanol, methanol, petroleum ether and aqueous) was performed to detect the presence of different kinds of phytoconstituents. Phytochemical screening exposed the presence of alkaloids, terpenoids, flavonoids, phenols, saponins, cardiac glycosides, resins and anthroquinones amid all the tested samples. Quantitative screening showed that the aqueous extract of leaf has high amount of total phenolics (28.03 ± 1.20 mg/gm), ethanolic extract of leaf showed highest amount of total flavonoids (48.19 ± 0.07 mg/gm) and aqueous extract of stem expressed highest percentage of antioxidant activity (56.70 ± 0.10 μ g/gm). The antibacterial activity was scrutinized against gram positive and gram negative bacteria by using disc diffusion method. All the extracts showed high activity against all the strains. Maximum zone of inhibition is shown by ethanolic stem extract against *Klebsiella pneumonia*. Among various types of extracts tested petroleum ether and aqueous extract showed lowest minimum inhibitory concentration values (4.25 μ g/ml) against *E. coli*. A lowest MIC value was recorded against *Bacillus subtilis*.

Key words: *Solanum nigrum*, solvent extracts, phytochemical analysis, total phenols, total flavonoids, antioxidant, antibacterial activity.

INTRODUCTION

Plants have been used in various sophisticated traditional medical systems that were predominant in the world for thousands of years. Since ancient times, India has been renowned for medicinal and aromatic plants [1]. The various indigenous therapeutic systems in India such as siddha, ayurveda, unani and naturopathy use several plant species in the treatment of different complaints [2]. In India, out of 20,000 medicinal plants that have been recorded [3] only 800 species are used by more than 500 traditional communities for their health care needs [4]. People throughout the globe have been using hundreds to thousands of indigenous plant species for treating various ailments since antiquity [5]. Renewed interest on phytomedicines emerged in early 1980's as the Council of Scientific and Industrial Research (CSIR) has published the information on the screening of biochemicals of many medicinal plants [6].

S. nigrum (black nightshade), a medicinal plant of Solanaceae family is well known for its therapeutic properties. It usually grows as moist weed in various kinds of soils which is cultivated in tropical and subtropical agro climatic regions. Different parts of this plant is used to treat numerous diseases such as inflammation, fever, pain etc. [7]. As seen in *S. surattense*, Solanine is the major constituent in almost all parts of this plant [8]. The identified compounds possess many medicinal activities including antioxidant, anti-inflammatory, diuretic, hepatoprotective and anti-pyretic agent [9]. The main active components includes glycoalkaloids, glycoproteins, and polysaccharides and also comprises polyphenolic compounds such as gallic acid, catechin, protocatechuic acid, caffeic acid, epicatechin, rutin, and naringenin [10].



Solanum nigrum

The literature survey reveals the relevance of investigative study of medicinal plants for their therapeutic properties. The discovery of bioactive compounds from different parts of medicinal plants continues to be a boon to the welfare of the human society at large as they do not have any adverse side effects. Hence it is a clarion call to carry research on herbal medicines. Many investigative reports exposed that *Solanum* plants are important source of large number of phytochemical compounds with substantial remedial application against human pathogens [11]. In this connection the main objective of the present study is to investigate the phytochemical composition of the crude extracts (ethanol, methanol, petroleum ether and aqueous) of root, stem and leaf of *S. nigrum* along with total phenol, flavonoid content, anti-oxidant activity of the crude extracts using DPPH and also antibacterial activity against selected bacterial strains.

MATERIALS AND METHODS

Plant sample and extraction of crude extracts

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

Preparation of plant extracts

The plants were collected from the field and initially rinsed with distilled water to free from soil and dried on paper towel under shade for one week and stored in airtight containers at room temperature. The dried leaves were coarsely powdered in a blender before subjecting for extraction.

A 50 gm weight of the dried leaf, stem and root powders were packed separately into thimble of soxhlet extractor and extracted with different polar and non-polar solvents such as ethyl alcohol, methanol, chloroform, ethyl acetate, hexane and diethyl ether separately, Hexane and chloroform extracts were made at the temperatures less than 60°C means 30 - 40°C and methanol and ethyl acetate extracts were made at temperatures 60 - 80°C for 12-16 hours. The extracts were concentrated to a dry mass under vacuum according to method of Harborne (1973).

Qualitative phytochemical analysis of different extracts

Phytochemical screening of the reconstituted extracts thus obtained was done qualitatively for the presence of various phytochemical constituents like alkaloids, flavonoids, tannins, steroids and phenols by using standard phytochemical methods [12, 13]. The concentrations of the metabolites detected were expressed as absent (-), less (+), moderate (++) , high (+++) and very high (+++++) depending on the intensity of the color reaction.

Detection of alkaloids (Mayer's test): The presence of cream colored precipitate indicates the presence of Alkaloids when the extract was treated with Mayer's reagent (1.36 g of Mercuric Chloride was dissolved in 20 ml of distilled water. Five grams of potassium iodide was dissolved in 20 ml of distilled water. The first solution was added to second solution and volume was made up to 100 ml with distilled water) [15]

Detection of terpenoids: 2 ml of chloroform was added to 0.5 ml of each extract which was then treated with 3 ml of concentrated Sulphuric acid. Reddish brown coloration of the interface indicates the presence of Terpenoids [16].

Detection of flavonoids: The immediate formation of red color of the extract indicated the presence of Flavonoids when treated with a few drops of concentrated hydrochloric acid [17].

Detection of saponins: Formation of stable persistent froth indicates the presence of saponins when mixed vigorously with distilled water [18].

Detection of phenols: Change in color of the extract indicates the presence of Phenols when treated with a few drops of neutral ferric chloride solution after dissolving the extract in alcohol or water [19].

Detection of cardiac glycosides: About 5 ml of aqueous extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This mixture was underlaid with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides. A violet

ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer [20, 16].

Detection of resins: To 10 ml of distilled water 1 ml of extract was added and was ultra-sonicated for 15 min at 30°C. The filtered mixture shows turbidity which shows the presence of resins [21].

Detection of anthroquinones: To the extract (3 ml), benzene (3 ml) and 5 ml of 10% Ammonium solution was added. Presence of Anthroquinones was indicated by the formation of pink or violet color in the ammonical phase [22].

Quantitative phytochemical analysis of different extracts

Detection of total phenols (Folin – Ciocalteu method): An aliquot (least amount) (1 ml) of extracts or standard solution of gallic acid (100, 200, 300, 400, and 500 µg /ml) was added to 25 ml of volumetric flask, containing 10 ml of decontaminated water. A blank reagent using distilled water was prepared. One mL of Folin - Ciocalteu phenol reagent was added to the mixture and shaken. After 5 mins 10 ml of 7.5% Na₂CO₃ solution was added to the mixture. The volume was then made up to the mark. Upon completion of incubation for 30-45 min at room temperature, the absorbance against the reagent blank was determined at 760 nm with an UV-Visible (Shimadzu Japan). TPC was expressed as mg gallic acid equivalents (GAE) [23].

Detection of total flavonoid content: To 1 ml of the extract 4 ml of water was added, to which 0.3 ml of 5% Sodium nitrite and 0.3 ml of 10% Aluminium chloride was added. After incubation of 5 min at room temperature 1 ml of 1M NaOH was added and the final volume was made up to 10 ml with distilled water. Absorbance was measured against the blank at 510 nm using spectrophotometer [24].

DPPH free radical scavenging assay: The donating ability of hydrogen atom by the plant extracts was determined by the decolorisation of methanol solution of 2, 2 diphenyl 1 picrylhydrazyl (DPPH). DPPH produces purple color in methanol solution and in the presence of antioxidants the color fades to yellow. A solution of 0.1 mM DPPH was prepared in methanol and 2.4 ml of this is mixed with 1.6 ml of aqueous extract at different concentrations was added. The mixture was kept in dark at room temperature for 30 min. Absorbance was measured at 517 nm spectrophotometrically. Ascorbic acid (1mM) was used as reference compound [25].

Percentage DPPH free radical scavenging activity = $[(A_0 - A_1)/A_0] * 100$

A₀ = Absorbance of the control

A₁ = Absorbance of the extractives

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: The antibacterial activity was tested by agar well diffusion method [26] as adopted earlier [27] with little modifications. Nutrient agar medium was prepared which was sterilized and allowed to cool so that the medium gets solidified. Just before solidification 0.1 ml of diluted inoculums (10⁵ cfu/ml) of test organism was added to the medium and then it was poured into sterilized petri dishes under aseptic conditions.

These plates were then allowed to cool and 6 mm filter paper disk impregnated with known concentration (50 µl of plant extract of 500 µg/ml) of antibacterial compound is on the agar plate [28]. The plates were incubated at 37°C for 18 hours. The antibacterial activity was evaluated by measuring the zone of inhibition (in millimeters) against test organism. The antibiotic Ciprofloxacin at 500 µg/ml concentration each were used in the test system as positive controls and DMSO is used as negative control.

Determination of Minimum Inhibitory Concentration (MIC): By using macro broth dilution method, minimum inhibitory concentration was determined. After serially diluting two fold in nutrient medium, inoculation with 5×10^6 cells (colony forming units) of the test bacterial strain was done and incubated at 37°C for 18 hrs. Two fold serial dilutions of antibiotic Ciprofloxacin was incorporated in each experiment as control. MIC was taken as the least concentration (highest dilution) of extract showing no detectable growth in the assay (macro broth).

Statistical analysis

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

RESULTS AND DISCUSSION

Qualitative phytochemical analysis of different extracts

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

Higher concentrations of alkaloids, flavonoids, cardiac glycosides was present in the ethanol extract of leaf. Flavonoids were also seen in very high quality in the ethanolic extract of root. Cardiac glycosides were observed very high in the aqueous extract of leaf and also in the stem ethanolic extract.

Alkaloids were recorded in high quality in the aqueous extract of leaf, stem, root and the leaf methanolic and petroleum ether extracts. Similarly the leaf aqueous, ethanol and methanol extracts recorded high quality of terpenoids. Cardiac glycosides were seen high in the ethanolic extract of stem. Saponins were absent in the petroleum ether extract of leaf. Resins and anthroquinones were recorded in less quality in almost all the extracts. Lower concentration of saponins were present in methanolic and petroleum ether extracts of stem and root. Similar results were reported by Dar *et al.*, 2017 [29].

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

Phytochemicals	Different extracts	Plant parts of <i>S. nigrum</i>		
		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; ++++ = very high				

Cardiac glycosides were seen in moderate amount and also very less amount of terpenoids were recorded in the methanolic and petroleum ether extract of root. The preliminary phytochemical studies received pronounced importance, because the crude drugs possess varied composition of secondary metabolites [13]. The secondary metabolites which are popularly known as phytochemicals are produced mainly by the leaf part of this plant. Though their functions are unknown they are considered to have functional and defense against plant pathogens [30].

Quantitative phytochemical analysis of different extracts

Total phenols:

The total phenol content of different plant parts of *S. nigrum* plant in different extracts were represented in table 2. The aqueous extract of leaf revealed high amount of total phenolics (28.03 ± 1.20 mg/gm) followed by the petroleum ether extract of leaf (22.15 ± 0.06 mg/gm). The least amount was noted in the methanolic extract of root (2.18 ± 0.05 mg/gm). Phenolic compounds are a class of secondary metabolites that are widely distributed in plant organisms which includes several thousand compounds with different structures ranging from simple phenolic acids to complex polymer compounds. The difference in the total phenolic compounds

recorded in different parts of the plant may be due to the contribution in the formation of phenol profile depends on the photosynthesizing mesophilic tissue or the metabolism of a typical phloem exudate [31].

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

Different extracts	Plant parts of <i>S. nigrum</i>		
	Leaf	Stem	Root
Aqueous	28.03 ± 1.20	22.01 ± 1.10	9.04 ± 0.04
Ethanol	18.34 ± 0.53	10.14 ± 0.03	7.16 ± 0.07
Methanol	16.10 ± 0.08	12.14 ± 0.06	2.18 ± 0.05
Petroleum ether	22.15 ± 0.06	4.17 ± 0.09	8.14 ± 0.06

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

Total flavonoid content:

The total flavonoid content of different plant parts of *S. nigrum* in different extracts was represented in table 3. The ethanolic extract of leaf revealed highest amount of total flavonoids when compared to others (48.19 ± 0.07 mg/gm) which was followed by the root of same extract (40.91 ± 0.07 mg/gm). Low concentration was seen in the petroleum ether extract of the root (5.14 ± 0.18 mg/gm). The remaining extracts showed moderate amount of total flavonoid contents in different parts of the plant. Flavonoids are secondary metabolites with antioxidant activity, the impact of which depends on the number of free hydroxyl groups [32]. As reported by Kumar and Roy, 2018[33], genetic diversity and biological, environmental, seasonal and year-to-year variations significantly affects the flavonoid content.

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

Different extracts	Plant parts of <i>Solanum nigrum</i>		
	Leaf	Stem	Root
Aqueous	21.17 ± 0.70	23.24 ± 0.62	20.15 ± 0.14
Ethanol	48.19 ± 0.07	25.91 ± 0.64	40.91 ± 0.70
Methanol	23.14 ± 0.29	14.16 ± 0.31	10.18 ± 0.40
Petroleum ether	20.17 ± 0.14	16.19 ± 0.12	5.14 ± 0.18

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

DPPH free radical scavenging assay:

The plant parts contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. The antioxidant activity of aqueous extract in 5 concentrations (100µg, 200µg, 300µg, 400µg and 500µg) of

various parts of *S. nigrum* was examined by comparing it to the activity of standard antioxidant Ascorbic acid (Table -4).

Table 4: DPPH free radical scavenging assay of different plant parts of *S. nigrum* 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	28.15 ± 0.74	36.54 ± 0.57	42.32 ± 0.54	47.33 ± 0.90	53.31 ± 0.13
Stem	26.04 ± 0.65	34.15 ± 0.40	41.26 ± 0.60	49.09 ± 0.87	56.70 ± 0.10
Root	26.10 ± 0.32	32.34 ± 0.32	39.61 ± 0.38	46.18 ± 0.94	52.01 ± 0.92

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

The aqueous extract of stem expressed highest percentage of antioxidant activity at 500 µg concentration which is followed by aqueous extract of leaf and root. The DPPH activity of the aqueous extract of leaf, stem and root of *S. nigrum* 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 [34]. Natural antioxidants present in the plant parts are accountable for inhibiting the toxic consequences of oxidative stress [35].

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. nigrum* and standard ciprofloxacin

Type of extract/antibiotic	Zone of inhibition of antibacterial activity (in mm)			
	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>
Leaf:				
Ethanol	12.21	17.43	16.24	20.46
Methanol	12.30	13.54	13.36	14.55
Petroleum ether	–	–	–	–
Aqueous	–	–	–	–
Stem:				
Ethanol	15.72	19.66	17.27	17.33
Methanol	12.84	11.33	15.43	18.27
Petroleum ether	–	–	–	–
Aqueous	–	–	–	–
Root:				
Ethanol	18.26	15.76	17.52	18.38
Methanol	12.31	12.67	15.43	6.27
Petroleum ether	7.42	–	–	–
Aqueous	7.26	–	–	–
Antibiotic:				
Ciprofloxacin	10.45	14.34	12.74	11.29

Values are mean \pm 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 ethanolic leaf (20.64 mm) extract against *Klebsiella pneumoniae* followed by ethanolic stem extract (19.66 mm) against *Bacillus subtilis*. Minimum zone of inhibition is shown in petroleum ether and aqueous extract of root against *Escherichia coli*. Among the leaf and stem solvent extracts, petroleum ether and aqueous extract does not show any activity against the tested organisms. The root solvent extract showed very less activity (7.42 and 7.26 mm) against *E. coli* and it does not have any effect on other organisms. From the table it is evident that petroleum ether and aqueous extract of leaf, stem and root does not exhibited any zone of inhibition. The results obtained from the crude extracts were compared with the standard antibiotic Ciprofloxacin. Almost all the tested organisms are highly sensitive to the ethanol and methanol extracts (11.33 – 20.64 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 [36]. 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 [37].

Minimum inhibitory concentrations (MIC)

The minimum inhibitory concentrations were shown in the table 6. Among the various extracts tested, petroleum ether and aqueous extract showed lowest MIC value (4.25 µg/ml). Lowest MIC value was recorded against *Bacillus subtilis* methanolic stem extract (6.25 µg/ml) followed by methanolic root and stem extract against *E. coli* (7.0 µg/ml).

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

Type of extract/antibiotic	MIC (µg/ml)			
	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>
Leaf:				
Ethanol	27.50	14.25	9.0	30.0
Methanol	27.50	9.0	7.5	9.25
Petroleum ether	–	–	–	–
Aqueous	–	–	–	–
Stem:				
Ethanol	9.0	15.0	12.5	8.0
Methanol	7.0	6.25	10.0	9.5
Petroleum ether	–	–	–	–
Aqueous	–	–	–	–
Root:				
Ethanol	10.0	11.25	10.0	18.5
Methanol	7.0	7.5	9.50	14.25
Petroleum ether	4.25	–	–	–
Aqueous	4.25	–	–	–
Antibiotic:				
Ciprofloxacin	17	19	28	28

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

From the table, it is obvious that lowest MIC values were recorded in the root extracts, followed by stem and leaf (30.0 µg/ml). The antimicrobial showed may be because of the presence of aromatic compounds such as alkaloids, flavonoids, phenols [38].

CONCLUSION

Habitually medicinal plants contain abundant 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. nigrum* may hold vast resource of pharmaceutical properties.

REFERENCES

1. Chopra RN, Nayar SL, Chopra IC., 1956, In Glossary of Indian medicinal plants. Council of Scientific and Industrial Research; 1: 197.
2. Rabe T, Staden JV., 1997, Antibacterial activity of South African plants used for medicinal purposes. *J Ethnopharmacol*; 56: 81-87.
3. Dev S, 1997, Ethnotherapeutic and modern drug development: The potential of Ayurveda. *Curr Sci*; 73: 909-928.
4. Kamboj VP, 2000, Herbal medicine. *Curr Sci*; 78: 35-39.
5. Levetin E, McMahan K, 2000, Plants and Society. McGraw Hill Higher Edu., New York: P. 508.
6. Somchic M R, Sulaiman M R, Zurain A.D., 2004, Antinociceptive and anti-inflammatory effect of *Centella asiatica*. *India J Pharmacol*; 36(6): 377-80.
7. Rajani P, Sridevi V and Lakshmi MVVC, 2012, A review on biological control of aflatoxin crop contamination. *Int J Chem Environ Pharm Res*; 3: 83-86.
8. Sikdar Mithun and Dutta Uzzal, 2008, Traditional Phytotherapy among the Nath People of Assam. *Studies on Ethno-Medicine*; 2: 39-45. 10.1080/09735070.2008.11886313.
9. Acharya and Pokhrel B, 2007, Ethno-Medicinal Plants Used by Bantar of Bhaudaha, Morang, Nepal. *Our Nature*; 4(1): 96–103. <https://doi.org/10.3126/on.v4i1.508>
10. Zakaria Zainul Amiruddin, Somchit MN, Zaiton Hala, Jais AM, Sulaiman Mohd Roslan *et al.*, 2006, The in vitro Antibacterial Activity of *Corchorus olitorius* Extracts. *Int J Pharmacol*; 2: 10.3923/ijp.2006.213.215.
11. R Udayakumar, K Velmurugan, D Srinivasan, Raghu Ram Krishna, 2003, Phytochemical and antimicrobial studies of extracts of *Solanum xanthocarpum*. *Anc Sci Life*; 23: 90.
12. Kaufman PB, Cseke LJ, Warber S, Duke JA, Brielmann HL, 1999, Natural Products from Plants. *Phytomedicines: Back to the future*. 2nd ed CRC Press; P. 1589-1592.
13. Wink M, 1999, Function of plant secondary metabolites and their exploitation in biotechnology. Sheffield Academic Press and CRC Press, *Annual Plant Reviews*; Vol. 3, p. 362.

14. Harborne JB, 1984, Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall Ltd, London.
15. Humayun S, Ibrar M, Barkatullah, Ahmad I, 2012, Comparison of three extracts of *Fumeria indica* for the evaluation of cytotoxic and phytotoxic activities. *Int J Biosci*; 2(12):112-119.
16. Ajiboye BO, Ibukun EO, Edobor G, Ojo AO, Onikanni SA, 2013, Qualitative and quantitative analysis of phytochemicals in *Senecio biafrae* leaf. *Int J Invent Pharm Sci*;1(5):428-432.
17. Sheel R, Nisha K, Kumar J, 2014, Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. *IOSR J Appl Chem*; 7(1): 10-13.
18. Djaafar Z, Ridha OM, 2014, Phytochemical study of selected medicinal plant, *Solanum nigrum*, the Algerian Dessert. *Int Let Chem Phys Astron*; 1: 25-30.
19. Raaman N, 2006, Phytochemical techniques. New India Publishing, New Delhi.
20. Jaradat N, Hussen F, Ali AA, 2015, Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata Decne*. *J Mater Environ Sci*; 6(6): 1771-1778.
21. Mir SA, Mishra AK, Reshi ZA, Sharma MP, 2013, Preliminary phytochemical screening of some Pteridophytes from District Shopian (J & K). *Int J Pharm Sci*; 5(4): 632-637.
22. Rajesh H, Rao SN, Rani MN, Shetty PK, Rejeesh EP, Chandrashekar R, 2013, Phytochemical analysis of methanolic extract of *Curcuma longa Linn rhizome*. *Int J Uni Pharm Bio Sci*; 2(2): 39-45.
23. Singleton VL and Rossi Jr JA, 1965, Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am J Enol Vitic*; 16: 144 – 158.
24. Olajire A and Azeez L, 2011, Total antioxidant activity, phenolic, flavonoid and ascorbic acid contents of Nigerian vegetables. *Afr. J. Food Sci Technol*; 2(2): 22-29.
25. Shimada K., Fujikawa K., Yahara K., Nakamura T, 1992, Antioxidative properties of xanthone on the auto oxidation of soybean in cyclodextrin emulsion. *J. Agr. Food Chem*; 40: 945–948.
26. Perez C, Pauli M and Bazerque P, 1990, An Antibiotic Assay by Agar Well Diffusion Method. *Acta Biologicae et Medicinae Experimentalis*; 15: 113-115.
27. Ahmad I and Beg AZ, 2001, Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J Ethnopharmacol.*; 74(2):113-23. doi: 10.1016/s0378-8741(00)00335-4. PMID: 11167029.
28. Kirby WMM, Yoshihara GM, Sundsted KS and Warren JH, 1957, Clinical usefulness of a single disc method for antibiotic sensitivity testing. *Antibiot Annu*; 892.

29. Dar KB, Bhat AH, Amin S, Zargar MA, Masood A, *et al.*, 2017, Evaluation of Antibacterial, Antifungal and Phytochemical Screening of *Solanum nigrum*. *Biochem Anal Biochem* ; 6: 309.
30. Misawa M, 1994, Plant tissue culture. An alternative for production of useful metabolites. FAO. Agriculture Services Bull, Rome; 87 PP.
31. Feduraev P, Chupakhina G, Maslennikov P, Tacenko N, Skrypnik L, 2019, Variation in phenolic compounds content and antioxidant activity of different plant organs from *Rumex crispus* L. and *Rumex obtusifolius* L. at different growth stages. *Antioxidants (Basel)*; 8(7): 237. doi:10.3390/antiox8070237
32. Manishaben Jaiswal, "ANDROID THE MOBILE OPERATING SYSTEM AND ARCHITECTURE", International Journal of Creative Research Thoughts (IJCRT), ISSN:2320-2882, Volume.6, Issue 1, pp.514-525, January 2018, Available at: <http://www.ijcrt.org/papers/IJCRT1134228.pdf>
33. Panche AN, Diwan AD, Chandra SR, 2016, Flavonoids: An overview. *J Nutr Sci*; 5: e47. doi: 10.1017/jns.2016.
34. Kumar V, Roy BK, 2018, Population authentication of the traditional medicinal plant *Cassia tora* L. based on ISSR markers and FTIR analysis. *Sci Rep*; 8: 10714. doi: 10.1038/s41598-018-29114-1.
35. Pallavi Sharma, Ambuj Bhushan Jha, Rama Shanker Dubey, Mohammad Pessaraki, 2012, Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *J Bot*; 12: 26 pages <https://doi.org/10.1155/2012/217037>.
36. Nooman A. Khalaf, Ashok K. Shakya, Atif Al-Othman, Zaha El-Agbar, Husni Farah, 2008, Antioxidant Activity of Some Common Plants. *Turk J Biol*; 32: 51-55.
37. Gislene G. F. Nascimento, Juliana Locatelli, Paulo C. Freitas, Giuliana L. Silva, 2000, Antibacterial Activity of Plant Extracts and phytochemicals on Antibiotic resistant Bacteria. *Braz J Microbiol*; 31: 247-256.
38. Manishaben Jaiswal, "CYBERCRIME CATEGORIES AND PREVENTION", International Journal of Creative Research Thoughts (IJCRT), ISSN:2320-2882, Volume.7, Issue 1, pp.526-536, February 2019, Available at: <http://www.ijcrt.org/papers/IJCRT1134229.pdf>
39. Mazher, Mubsher & Zahid, Nafeesa & Malik, Muhammad & Hussain, Adil & Ali, Yasir & Noshad Qum, 2016, Phytochemistry and Antibacterial Assay of Fruit, Leaf and Stem Extracts of *Solanum nigrum* L. in Different Solvent. *Int J Biosci*; 9: 129-136. 10.12692/ijb/9.6.129-136.
40. Arunachalam, Pradeep, Dinesh, M, Govindaraj, A & Ng, Ramesh, 2014, Phytochemical analysis of some important medicinal plants. *Int J Biol Pharm Res*; 5.