PHYTOCHEMICAL SCREENING AND ANTI-OXIDANT POTENTIAL OF EXTRACTS OF *SOLANUM NIGRUM*

project report submitted in partial fulfillment of the requirements for the degree of

BACHELOR OF PHARMACY

Submitted by

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GALGOTIAS UNIVERSITY

May 2023

GALGOTIAS UNIVERSITY

CERTIFICATE

This is to certify that project work entitled **"Phytochemical Screening and Anti-oxidant Potential of Extracts of *Solanum nigrum*"** done by **Mr. Ankit Sharma,** is a bonafide research work done under the supervision and guidance of **Dr. Kalpana Pravin Rahate**, Professor, School of Medical and Allied Sciences, Greater Noida. The work is completed and ready for evaluation in partial fulfillment for the award of Bachelor of Pharmacy during the academic year 2022-2023. The project report has not formed the basis for the award of any Degree/Diploma/Fellowship or other similar title to any candidate of any University.

Date:

Prof. Pramod Kumar Sharma
Dean
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BONAFIDE CERTIFICATE

This to certify that the project work entitled **"Phytochemical Screening and Anti-oxidant Potential of Extracts of *Solanum nigrum*"** is the bonafide research work done by **Mr. Ankit Sharma**, who carried out the research work under my supervision and guidance for the award of Bachelor of Pharmacy under Galgotias University, Greater Noida during the academic year 2022-2023. To the best of my knowledge the work reported herein is not submitted for award of any other degree or diploma of any other Institute or University.

Dr. Kalpana Pravin Rahate
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DECLARATION

I hereby declare that the work embodied in this project report entitled **"Phytochemical Screening and Anti-oxidant Potential of Extracts of Solanum Nigrum"** in Partial fulfillment of the requirements for the award of Bachelor of Pharmacy, is a record of original and independent research work done by me during the academic year 2022-23 under the supervision and guidance of **Dr. Kalpana Pravin Rahate,** Professor, School of Medical and Allied Sciences, Galgotias University, Greater Noida. I have not submitted this project for award of any other degree or diploma of any other Institute or University.

Date:

Place: Greater Noida **Name and Signature of Candidate**

ACKNOWLEDGEMENT

I would like to express my special thanks of gratitude to my project guide **Dr. KALPANA PRAVIN RAHATE**, Professor, SMAS, Galgotias University, who gave me the golden opportunity to do this wonderful project on the topic **"Phytochemical Screening and Anti-oxidant Potential of Extracts of Solanum Nigrum"** , which also helped me in doing a lot of research and I come to know about so many new things.

I would like to extend my gratitude to the Dean **PROF. PRAMOD KUMAR SHARMA,** Program Chair **Dr. NIRANJAN KAUSHIK,** Professor, School of Medical and Allied Sciences, Galgotias University, Greater Noida, providing me with all the facility that was required.

I would like to thanks all my teachers, laboratory technician and other staff for helping me in completion of this project.

I would also like to thank my friends and relatives, who helped me a lot in finishing this project within the limited time, it helped me increase my knowledge and skills.

At last, but not least, I would like thank GOD for giving me patience and power for the successful completion of the project.

ABSTRACT

Solanum nigrum also known as makoy plant or Black nightshade, is a commonly used herb with ethnomedicinal importance. It is a Eurasian species, widely distributed in Middle East, India, Africa. The plant is a constituent of several Indian medicines useful in relief of number of health conditions like fever, stomach issues, dysentery, ulcers, asthma, jaundice and whooping cough. It is said to have many therapeutic activities including antitumor, antioxidant, anti-inflammatory, hepatoprotective, diuretic, antipyretic activities. The present study includes evaluation of phytochemical constituents, and in vitro antioxidant activities of extracts of *Solanum nigrum* leaves with the aim to furnish more information on its therapeutic potential. Extraction of the dried leaves of *Solanum nigrum* was carried in 70% ethanol followed by partitioning in, n-haxane, Dichloromethane (DCM), ethyl acetate and methanol. Preliminary phytochemical screening of the extracts and all the fractions was carried out as per the standard methods. Antioxidant study of the fractions was carried out by DPPH scavenging assay, nitric oxide scavenging assay and by determination of reducing power. Phytochemical screening of all the extracts and fractions revealed that n-hexane and DCM fractions contained maximum secondary metabolites including alkaloids, glycosides, saponins, phenolic compounds and coumarin. The extract was found to possess potent antioxidant activity which was found to be comparable to standard antioxidant compounds. Reducing power of hexane and DCM fractions was found to increase with increase in concentration and was comparable to ascorbic acid. The fractions also scavenged the DPPH radical due to antioxidant potential. The IC_{50} value of quercetin, hexane and DCM fraction was found to be 0.477±0.002, 44.067±0.001 and 62.569±0.002 respectively. The two fractions also found to scavenge nitric oxide similar to ascorbic acid and the antioxidant capacity was observed to increase with increasing concentration. IC_{50} value of ascorbic acid, hexane and DCM fraction was found to be 116.127±0.003, 217.833±0.002 and 226.487±0.003 respectively. This study forms basis for future research activities on extracts and active compounds of *Solanum nigrum*.

Key words: *Solanum nigrum*, phytoconstituents, antioxidant, DPPH, Reducing power.

CHAPTER-1

INTRODUCTION

INTRODUCTION

1.1 Medicinal Plants

Since prehistoric times, therapeutic plants, often known as medicinal herbs, have been identified and employed in traditional medicine practices. For a variety of purposes, including defense and protection against insects, fungus, illnesses, and herbivorous mammals, plants manufacture hundreds of chemical compounds (1).

In both traditional and modern medicine, medicinal plants are utilized with the goal of promoting health, providing treatment for a specific ailment, or both. In 2002, the Food and Agricultural Organization estimated that over 50,000 different medicinal plants were utilized globally. Out of about 30,000 plants for which a usage of any type is known, 17,810 plant species are thought to have a medicinal purpose, according to a more cautious estimate from the Royal Botanic Gardens, Kew in 2016 (2).

Roughly one-fourth of the medications prescribed to patients in modern medicine are made from medicinal plants and go through extensive testing. Depending on the medical system, medicinal plants may account for the bulk of therapies that are regularly tried ad hoc and without supporting evidence (3,4).

In industrialized nations, there is an increase in the usage of plant-based materials, such as herbal or natural health products with alleged health benefits. Despite the benign reputation of herbal treatments, this entails risks of toxicity and other impacts on human health (5).

Herbal remedies have been used for a very long time, well before the advent of modern medicine; nonetheless, little, if any, is known about the pharmacological underpinnings of their activities or the safety of using them. In 1991, the World Health Organization created a policy on traditional medicine. Since then, it has released recommendations for using it as well as a number of monographs on commonly used herbal remedies (6,7).

The three primary types of benefits that medicinal plants may offer are: health benefits to those who use them as medicines; financial benefits to those who collect, process, and sell them; and societal benefits, such as job possibilities, tax revenue, and a more productive workforce.

Unfortunately, the development of plants or extracts with potential medical applications is hampered by a lack of strong scientific evidence, subpar drug development procedures, and inadequate funding (8).

All plants create chemical substances that give them an evolutionary benefit, including protecting themselves from herbivores or acting as a hormone in plant defenses, like salicylic acid does (9,10). The content and recognized pharmacological activity of these phytochemicals in medicinal plants will operate as the scientific foundation for their use in modern medicine, if confirmed by research. These phytochemicals have the potential to be used as medications. For instance, galantamine, an alkaloid approved for therapy against Alzheimer's disease, is one of nine classes of alkaloids found in daffodils (Narcissus). Alkaloids, which have a bitter taste and are toxic, are concentrated in plant portions like the stem that herbivores are most likely to consume; they may also offer parasite protection.

1.2 Family- Solanaceae

The Solanaceae, sometimes termed as the nightshades, is a family of flowering plants that includes various attractive plants, weeds, and agricultural products. They include shrubs, trees, lianas, vines, and annual and perennial herbs. The Solanaceae family, which includes over 98 genera and 2,700 species, has a diverse range of habitats, morphologies, and ecologies (11).

This family is widespread throughout the planet, with the exception of Antarctica. The continents of South America and Central America have the most species variety. There are several regularly harvested or farmed species in the Solanaceae family. The potato (S. tuberosum; the family is often known as the "potato family"), tomato (S. Lycopersicon), and aubergine or aubergine are the most economically significant members of the Solanum genus (S. melongena). Bell and chilli peppers are both produced from the Capsicum genus, another significant one.

Herbs, shrubs, trees, vines, lianas, and occasionally epiphytes are all members of the Solanaceae plant family. They can be annuals, biennials, or perennials, decumbent or erect. There are tubers underground in certain plants. They don't make colourful sap, laticifers, or latex. At the base, the end, or neither, they may bear a cluster of leaves. The leaves frequently alternate or alternate in opposition to one another (i.e., alternating near the base of the plant and opposite towards the inflorescence). The leaves may seem spine-like, leathery, or herbaceous. Usually petiolate or

subsessile, but occasionally sessile, are the leaves. Although they are often odourless, some of them can be pungent or foetid. A foliar lamina may be simple or compound, and a compound lamina may be ternate or pinnatifid. The leaves lack a basal meristem and feature reticulated venation. The laminae lack secretory cavities and are often dorsiventral. Stomata are rarely found on both sides of a leaf; they are often restricted to one of the two sides. Although certain species of the flowers are monoecious, andromonoecious, or dioecious; in general, they are hermaphrodites (12).

1.2.1 The following taxonomy overview of the Solanaceae is offered, including subfamilies, based on the most current molecular phylogenetics analyses of the family (12):

Cestroideae (Browallioideae)

Pericyclic fibres and an androecium with four or five stamens, usually didynamous, are features of this subfamily. Pericyclic fibres and an androecium with four or five stamens, usually didynamous, are features of this subfamily.

Fig 1.1: *Cestrum elegans*, (subfamily : *Cestroideae*), a shrub used as an ornamental

Goetzeoideae

Drupes, which are a kind of fruit and seed with twisted embryos and huge fleshy cotyledons, are a distinctive feature of this subfamily.

Fig 1.2*: Goetzea elegans* (subfamily *Goetzeoideae*) in bud and flower, South Miami, Florida United States.

Nicotianoideae

The Solanaceae family has a subfamily called Nicotianoideae. Although they can also be found in America and Africa, most genera are found in Australia. Eight genera and roughly 125 species make up the subfamily, 90 of which are part of the Nicotiana genus.

Fig 1.3: Tobacco inflorescence, *Nicotiana tabacum*

Petunioideae

A subfamily of the nightshade-related flowering plant family Solanaceae is the Petunioideae. There are 13 genera in it. In Chilean folk medicine, Fabiana imbricata, also known as pichi in the local dialect, is used as a diuretic and digestive. It contains sesquiterpenes with gastroprotective effects, according to studies.

Fig 1.4: *Brunfelsia pauciflora* - Brazilian species, grown as pot-plant in glasshouse, Chelsea Physic Garden

Schizanthoideae

The Schizanthoideae family includes annual and biennial plants with tropane alkaloids, no pericyclic fibres, unique hair, and pollen grains. Flowers are naturally zygomorphic. Three staminodes, two stamens, and an explosive anther make up the androecium.

Fig 1.5: Zygomorphic flowers, with bilabiate corolla of *Schizanthus pinnatus*, a schizanthoidea ornamental

Solanoideae

The Nicotianoideae subfamily of flowering plants is connected to the Solanoideae subfamily of the Solanaceae family. The Solanoideae subfamily contains some of the Solanaceae family's most economically significant genera and species, such as the tomato (Solanum lycopersicum), potato (Solanum tuberosum), eggplant (Solanum melongena), bell peppers, chilies, mandrakes (Mandragora spp.), and jimson weed (Datura stramonium).

Some of the well-known tribes that make up this subfamily are Capsiceae, Datureae, Hyoscyameae, Juanulloeae, Lycieae, Nicandreae, Nolaneae, Physaleae, Solandreae, and Solaneae.

This subfamily also includes the disputable tribes Mandragoreae and Jaboroseae.

Fig 1.6: **Solanum nelsonii**

TABLE 1.1 : Estimated number of species in family Solanaceae

Genera	**Approximate no. of species**
SOLANUM	1330
LYCIANTHES	200
CESTRUM	150
NOLANA	89
PHYSALIS	85
LYCIUM	85
NICOTIANA	76
BRUNFELSIA	45
Estimated no. of species in the family	2700

Solanum nigrum

Solanum nigrum is a Solanaceae-family medicinal plant. These are its common names: Makoi and black nightshade .There are two types of Solanum nigrum: one has fruit that is black in colour and the other is reddish brown. Fruit with a black colour is poisonous in both types. From a health standpoint, the leaves, plant as a whole, and roots are all utilized.

Before the terms "medicines" or "medical science" had ever been coined, the human race had been using many types of medicinal plants. The plant family Solanaceae, which belonged to the genus Solanum, was used by people. It belongs to a big plant family that has over 1400 different species. The tropical and tropical regions are where you can find these plants the most. Examples include Solanum aviculare, which is primarily found in Europe, Australia, and New Zealand; S. dulcamara, which is primarily found in Europe; S. incanum, which is typically found in Africa; S. Khasianum, which is primarily found in the Indian subcontinent; S. laciniatum, which is typically found in the Australian continent; and S. Nigrum, a species that is primarily found in cosmopolitan areas. Several species are also grown, some of which are well-known crops as S. pseudocapsicum, S. tuberosum (potato), and S. melongena (eggplant, aubergine). The development of the discovery and testing of various medicinal plants for their potential antibacterial action is a response to the increasing failure of synthetic medications, side effects, and development of antibiotic resistance by pathogenic microbes . Several therapeutic plants have been tested against pathogenic microbes; therefore, a thorough investigation is needed to identify the medicinal qualities of plants .

Numerous compounds in S. nigrum are in charge of its pharmacological actions. Glycoalkaloids, glycoproteins, polysaccharides, and polyphenolic substances such gallic acid, catechin, protocatechuic acid (PCA), caffeic acid, epicatechin, rutin, and naringenin are its active ingredients(13).

The shoots and berries of SN are a minor food crop in many underdeveloped nations, and they are also used for a variety of local and medicinal purposes [14]. In several regions of the world, SN is primarily used as veggies for making soup. The "vegetable black nightshade's" nutritional worth has been the subject of several studies, and it has been shown that this species is a nutrient-rich vegetable [15]. In addition to its nutritional qualities, this plant was chosen because of folklore claims about its possible medicinal use [16]. Various vegetable portions may be

healthy, according to research. SN leaves have reportedly been employed in traditional medicine to cure a range of conditions, including epilepsy, diarrhea, pain, ulcers, inflammation, and different eye infections[17,18].

In India, where an intriguing aqueous extract of SN leaves was used in a pharmaceutical investigation., the leaves are used in folk medicine to heal oral ulcers [19].

According to recent research, SN has an anti-cancer effect on human endometrial carcinoma cells [23], human colorectal carcinoma cells [22], human ovarian carcinoma cells [21], and human hepatocellular carcinoma cells [20]. In addition to methionine, an amino acid that is difficult to get in other vegetables, the leaves can provide considerable values of lipids, fibre, vitamins A and C, minerals including calcium, iron, and phosphorus. Additionally, steroid glycosides have been recognized as chemical elements in leaves [24]. Unknown steroidal alkaloids [25] and steroidal glycosides [26] have just lately been found in the unripe berries. Nitric oxide (NO) production caused by lipopolysaccharide (LPS) was significantly inhibited by those substances.

Since medicinal plants are increasingly being utilized to create reliable and secure medicines suitable for human use, several studies have examined the chemical composition of extracts from plants, their biological functions, and the optimization of methods for extraction [27,28].

The extracts from the SN are abundant in polyphenolic compounds. The leaves are rich in polyphenols, including phenolic acids and flavones [29]. They also highlighted the extract's potential for preventing or treating illnesses brought on by stress that include oxidative damage to biological components, particularly the brain [30]. According to research by Zaidi et al., administration of SN leaf extract to rats was able to reduce oxidative stress. The antioxidant properties of SN stems and leaves may be due to the presence of the polyphenolic compounds discussed above [31]. According to research by Sun et al., oxidative stress is connected to pathological conditions such diseases of the Central Nervous System (CNS) and the biological ageing of the brain [32].

According to a very intriguing study that discovered that dietary inclusions of Solanum leaves could shield against cognitive and neurochemical impairments brought on by scopolamine, this vegetable could be employed as a source of therapeutic foods and nutraceuticals that to serve the

prevention and management of illnesses like Alzheimer's disease that are linked to cognitive impairment [33].

Cell membranes change in terms of their structural and functional makeup as a result of the production and release of radical oxygen reactive species (ROS). When the mitochondrial respiratory chain is damaged, free radicals attack polyunsaturated fatty acids in bio membranes, and mitochondria become the predominant source of ROS. Under these circumstances, a substance with antioxidant characteristics may be helpful in reducing oxidative cell damage and ROS generation, which is especially intriguing if this activity is generated by a functional meal [34]. Reduced glutathione (GSH), one of the most significant scavengers of reactive species, is employed in experiments to measure ROS, and its ratio to oxidized glutathione is used as a sign of oxidative stress [35].

The central level of oxidative stress may activate several calcium-dependent enzymes, which can result in mitochondrial malfunction, a decrease in adenosine triphosphate (ATP) levels, the production of ROS, and eventually the death of neuronal cells [36]. While a prolonged incubation (excitotoxicity) leads to cell death, a brief exposure to glutamate, a major excitatory neurotransmitter in the central nervous system (CNS), may cause swelling of differentiated astrocytes as well as numerous acute and chronic brain injuries [37,38]. This phenomenon results in modifications in glutamate transport, GSH depletion, and macromolecular formation [39].

CHAPTER-2

LITERATURE REVIEW

LITERATURE REVIEW

Rani et al. (40) studies conclude, medicinal plants are frequently used to treat numerous infectious disorders in people, solanum nigrum's effectiveness against pathogens isolated from sputum samples was examined. The objective of the study was to identify and describe the bacteria isolated from patients who had respiratory tract infections. Solanum nigrum extracts in aqueous, ethanol, and diethyl ether were made, and four different strengths of each extract were tested for antibacterial efficacy against the isolated bacteria. Comparing ethanolic and aqueous and diethyl ether extracts of solanum nigrum, the ethanolic extract exhibited the strongest antibacterial activity. The plant contained alkaloids, terpenoids, flavonoids, saponins, steroids, and phenols, according to a phytochemical examination. As a result, solanum nigrum exhibits antimicrobial action and can be employed clinically to discover new antibacterial agents for diseases that affect the respiratory system.

Solanum nigrum was investigated for its antioxidant potential by campisi et al. (41).although there is little to no scientific evidence to support their use, a number of foods used in traditional medicine have antioxidant potential and may be useful in the treatment of a number of diseases. As a result, the objective of this study was to assess the antioxidant effects of two leaf extracts of solanum nigrum l. (sn), a medicinal plant that is predominantly used across the world to produce soup and is a member of the Solanaceae family. The leaves were then extracted in methanolic/water (80:20) (sn1) and water (sn2), respectively. We calculated the overall polyphenolic content as well as the concentration of phenolic acids and flavone molecules. In order to determine if sn1 and sn2 extracts could correct the oxidative state that glutamate had changed in primary cultures of astrocytes, the study measured glutathione levels, intracellular oxidative stress, and the cytotoxicity of the extracts. In an in vitro glutamate-exposed rat astroglial cell primary culture, both extracts were successful at squelching the radical and regaining the oxidative condition. These extracts prevented the increase in glutamate uptake and reduced glutamate excitotoxicity, which damages cells and has a great antioxidant potential.

Ravi et al. Studied about anti-inflammatory effect of methanolic extract of solanum nigrum linn berries(42). Study examines the anti-inflammatory properties of a methanolic extract of solanum

nigrum linn's berries. The berries of solanum nigrum, often known as "black night shades," have been used medicinally for centuries to treat inflammation-related conditions. Solanum nigrum dried and ground up berries were extracted utilizing the Soxhlet equipment with methanol. On carrageenan-induced paw edema, the effects of methanolic extracts of solanum nigrum berry were investigated. The edema caused in the hind paw was lessened by the methanolic extract. The 375 mg/kg b.w. Methanolic extract of solanum nigrum has demonstrated notable anti-inflammatory effects. It has been determined that the solanum nigrum linn berry methanolic extract (375 mg/kg b.w.) Has good anti-inflammatory efficacy against carrageenan-induced paw edema.

Hadi et al. Reviewed the solanum nigrum l. Antimicrobial, antioxidant properties and hepatoprotective effects and analysis of bioactive natural compounds (43). Together with the other components of the entire plant, the berries and leaves are the principal sections utilized for medicinal purposes. The leaves are claimed to cause diaphoresis and are used as a poultice for skin conditions, antituberculosis, and rheumatoid and gouty joint pain. Furthermore, leaves are utilized for neurological problems, nausea, and dropsy. The berry and flower decoction can help with coughing. These diuretics are a treatment for bronchitis and pulmonary tb. The berry juice is utilized for hydrophobia, ophthalmopathy, and antidiarrhea. Heart disease is treated with it as well. Berries are thought to have cathartic, diuretic, and tonic qualities. Inflammations and skin conditions can benefit from them as well. The roots are helpful for hepatitis, osteopathy, ophthalmopathy, and rhinopathy. The entire plant is used to treat edema, cough, asthma, inflammation, expectoration, cardiotonicity, digestion, diuretics, laxatives, and diaphoresis. The herb is also beneficial in treating general debility, dropsy, nephropathy, ophthalmopathy, hemorrhoids, leprosy, and cardiopathy. The CNS and spinal cord reflexes are lowered by the plant's decoction. The methanol extracts of all the plant samples were found to have high antibacterial activity against the two investigated bacteria, Xanthomonas campestris (a plant disease) and aeromonashydrophila (an animal pathogen), in antibacterial screening using the disc diffusion method. The studied bacteria were clearly inhibited by the s. Nigrum methanol extracts. The potential antibacterial activity of the dried fruit of solanum nigrum l.'s ethanolic extract was evaluated. Both gram-positive and gram-negative bacteria were somewhat resistant to the ethanolic extract's antibacterial action. Solanum nigrum was found to have antibacterial action against pseudomonas aeruginosa, Escherichia coli, klebsiella pneumonia, and bacillus subtilis.

Prakash et al. Studied about antifungal activity and preliminary phytochemical studies of leaf extract of solanum nigrum linn (44). Solanum nigrum linn. Is extensively used in Indian traditional and folk medicines to cure various skin ailments. The present study aims to evaluate the possibility for the presence of novel bio-active compounds against fungal pathogens. To determine antifungal activity, aqueous and crude extracts from leaves of s. Nigrum linn. Was used against a. Niger, a. Flavus, c. Albicans by dry weight method. Extracts prepared using crude solvents exhibited higher antifungal activity as compared to their corresponding aqueous extracts. No good activity was observed in the aqueous extract. The pathogen inhibiting activity was found to be dose dependent. The preliminary phytochemical screening of the leaves revealed the presence of alkaloids, flavanols, flavones, flavanols, saponins and steroids.

CHAPTER-3

AIM &

OBJECTIVES

AIM AND OBJECTIVES

Solanum nigrum is an annual or short-lived perennial herb. The leaves and immature green berries of this plant contain alkaloid known as solanine. Once the berries mature and turn black, the fruits are edible, in limited quantities. In Indian traditional medicine, this plant is a key ingredient. Its infusion is useful in dysentery, stomach complaints, and fever. In Tamil Nadu, leaves of this plant are used to treat mouth ulcers that happen during winter periods, where as in Northern parts of India, extracts of leaves and berries are useful to treat lever-related ailments, including jaundice and the juice of the roots in asthma and whooping cough (45). The plant contains high amount of polyphenols, which indicate that it shows antioxidative and antitumor properties. Hence in this study an attempt has been taken to investigate in vitro antioxidant potential of various extracts of leaves of *Solanum nigrum.*

Aim of the study

Aim of the present research work was to investigate preliminary phytochemical and *in vitro* antioxidant potential of the extracts of leaves of *Solanum nigrum.*

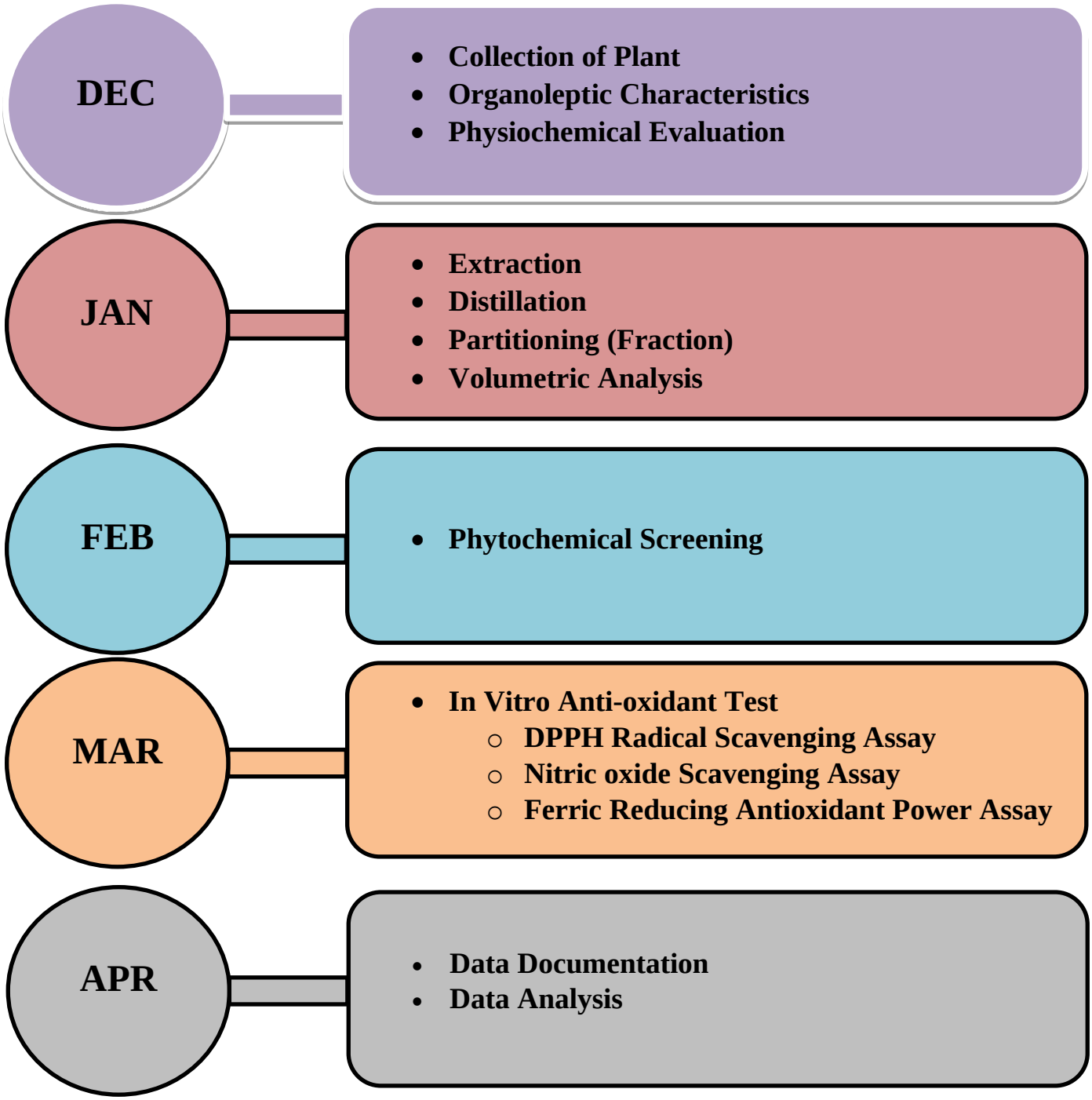
Objectives

1. To prepare the hydroalcoholic extract followed by its fractionation
2. To perform preliminary phytochemical screening of the extracts and fractions
3. To examine in vitro antioxidant potential of the extracts.

CHAPTER-4

PLAN OF WORK

PLAN OF WORK


DEC
Collection of Plant
Organoleptic Characteristics
Physiochemical Evaluation
JAN
Extraction
Distillation
Partitioning (Fraction)
Volumetric Analysis
FEB
Phytochemical Screening
MAR
In Vitro Anti-oxidant Test
DPPH Radical Scavenging Assay
Nitric oxide Scavenging Assay
Ferric Reducing Antioxidant Power Assay
APR
Data Documentation
Data Analysis

CHAPTER 5

PLANT PROFILE

PLANT PROFILE

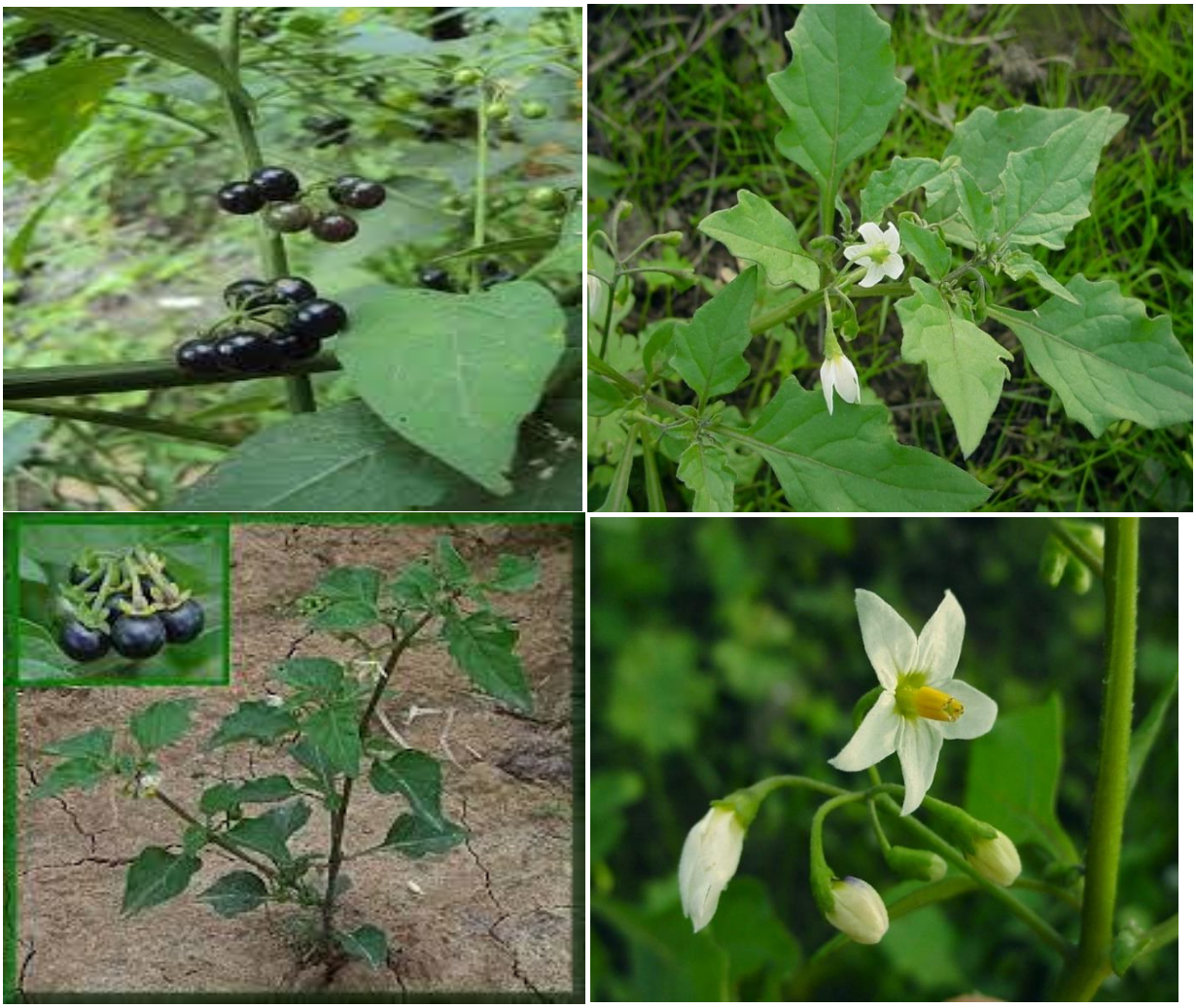


Fig 5.1: Solanum nigrum plant, fruits, leaves, flowers

Biological source: It contains full grown berries of Solanum Nigrum in dried form.

Geographical Source: It is a part of Medical Botany, National Institute of Siddha, located in Chennai, Tami Nadu, and India.

Family: It belongs to the family Solanaceae

Common name: Black nightshade, Makoi

Synonyms: It is known in different names in different places:

In Australia it is famous as Blackberry nightshade and Black nightshade.
In Europe it is known as Annual nightshade, Black nightshade, Common nightshade, and Garden nightshade.
In New Zealand it is known as Black nightshade
In South Africa it is known as Nightshade
In different language and different locations in India it is called in different names:
Sanskrit: Dhvansamaci
Urdu: Mako
Bengali: Gudakamai
Hindi: Makoya, Kakamachi, Kali makoy
Kannada: Ganikesopu
Malayalam: Manatakkali
Marathi: Kamoni
Punjabi: Mako, Peelak, Mamoli

TAXONOMY

Following is the complete taxonomy of the plant Solanum Nigrum:

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta - Vascular plants

Superdivision: Spermatophyta - Seed plants

Division: Magnoliophyta - Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Asteridae Order: Solanales

Genus: Solanum – nightshade

Species: SolanumNigrum L. - black nightshade

Authority - Linn.

Macroscopic characteristics

The plant's bark is delicate and flexible. The root's outermost covering is peeled off, revealing the wood's pale-yellow inside. The emerging flowers have five petals. The shape of the petals is often regular. Although the flowers are typically bell-shaped, they can also be star-, tubular-, or flat-shaped. This plant family typically consists of climbers, while some members may also be scrambling plants. The steams are covered in leaves and have hair. Stipules are not present, yet the leaves are whole or divided. They are alternately oriented in the stems. The morphological investigation clearly demonstrates that the roots have numerous little branches and have a small, brown appearance on the outside. The diameter of mature fruits is roughly 6 mm, and they are often obtuse. Fruits are short, papery, pulpy, and their seeds are free to float around in the pulp.

Distribution

According to Edmonds (1979a), the Solanum nigrum plant is primarily a Eurasian species that does not normally grow in South America. It is remarkably adapted to the Mediterranean environment and may have its roots in the Middle East or possibly India. It may also be native to Africa, where it is believed to be extensively spread, however there hasn't been much accurate taxonomic research done on individuals from this enormous continent. However, the species has not yet been discovered in South or Central America, in the Pacific Islands, or in places like North America, New Zealand, or Australia. (1984, Edmonds).

Biology and Ecology

Black nightshade (*Solanum nigrum*) is a common weed of vegetable and spring legume crops. Flowers are pollinated by insects and are self-fertile. It germinates in spring and summer, fruiting in the same year. The seeds are distributed by birds.

Black nightshade-related species are typically unable to endure drought conditions and instead thrive in regions that receive irrigation (Burgert et al. 1973). In the tropics, they do best during the rainy seasons, yet they can flourish with an annual rainfall of 500–1200 mm. In temperate regions, frequent watering is necessary during dry spells, although glasshouse-grown plants often need twice-daily irrigation. These plants grow best at temperatures between 20 and 30 °C, however the majority of species can tolerate temperatures between 15 and 35 °C. High fertility soils, especially those rich in nitrogen and phosphorus, are optimal for the plants' growth. These plants do best in soils with an ampH range of 6.0 to 6.5, ranging from sandy loam to friable clay (Edmonds,1997).

USES

- S. nigrum has been consumed widely throughout history, and in 15th-century China, the fruit was mentioned as a famine food. Eatable strains' ripe berries and boiling leaves are consumed despite some forms' toxicity problems. The totally cooked leaves are utilized in fataya pies and quiches as well as horta, despite having strong, slightly bitter flavors similar to spinach. The flavor of the mature blackberries is characterized as sweet and salty with traces of melon and licorice.
- The berries are farmed and occasionally eaten in India, but not for business. In South India, the berries and leaves are frequently used as food after being cooked with tamarind, onion, and cumin seeds.
- Traditional Indian medicines frequently contain S. nigrum as a key component. Fever, stomach issues, and dysentery are treated with infusions. The plant's juice is applied to ulcers and other skin conditions. The fruits are used to cure asthma and "excessive thirst," as well as being tonics, laxatives, hunger stimulants, and more. The plant has historically been used to cure tuberculosis. In Tamil Nadu, India, mouth ulcers that develop during the winter months are treated with the leaves of this plant. Boiling extracts of leaves and

berries are also employed in North India to treat liver-related conditions, such as jaundice. Its roots' juice is used to treat whooping cough and asthma.

- In eastern medicine, S. nigrum, a commonly used herb, is said to have antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic properties.
- Moreover, it is used to treat stomach ulcers.
- Solanum nigrum water extracts have demonstrated a cytotoxic action in decreasing ROS production in the human MM cell line A-375.
- Solanine, the plant's active component, prevents the growth of many cancer cells in vitro, including pancreatic and breast cancer. Its anti-tumor action primarily involves the activation of various cell and molecular pathways, which impede tumour spread by causing cell and molecular death and autophagy.

Phytoconstituents

Table 5.1: Phytoconstituents reported in Solanum nigrum

S.No.	Class of compound	Phytoconstituents	Molecular formula	References
1.	Steroidal saponins	Diosgenin	C27H42O3	(46)
2.	Steroidal saponins	Degalactotigonin	C50H82O22	(47)
3.	Steroidal saponins	Stigmasterol	C29H48O	(48)
4.	Steroidal saponins	Pterosterone	C27H44O7	(48)
5.	Steroidal saponins	Solanigroside I	C62H104O31	(49), (50)
6.	Steroidal saponins	Hypoglaucin H	C39H60O15	(50)
7.	Steroidal saponins	Nigrumnin I	C55H90O25	(49), (50)
8.	Steroidal saponins	Tigogenin	C27H44O3	(49), (50)
9.	Steroidal saponins	Dumoside	C40H62O16	(49), (50)

10.	Steroidal saponins	Cholesterol	C27H46O	(51)
11.	Alkaloids	Solamargine	C45H73NO15	(49), (50)
12.	Alkaloids	Solanigroside P	C39H63NO12	(49), (50)
13.	Alkaloids	Solanigroside Q	C45H69NO15	(49), (50)
14.	Alkaloids	Solasodine	C27H43NO2	(49), (50)
15.	Alkaloids	N-methyl solasodine	C28H45NO2	(49), (50)
16.	Alkaloids	Tomatidenol	C27H43NO2	(49), (50)
17.	Alkaloids	Solasonine	C45H73NO16	(49), (50)
18.	Alkaloids	Adenine	C5H5N5	(46)
19.	Alkaloids	Nicotinic acid	C6H5NO2	(46)
20.	Alkaloids	Glutarylcarnitine	C12H21NO6	(46)
21.	Phenylpropanoids	Trans-4-Hydroxycinnamic acid	C9H8O3	(52)
22.	Phenylpropanoids	Cis-4-Hydroxycinnamic acid	C9H8O3	(53)
23.	Phenylpropanoids	Caffeic acid	C9H804	(46)
24.	Phenylpropanoids	Chlorogenic acid	C16H18O9	(46)
25.	Phenylpropanoids	Scopoletin	C10H8O4	(48), (47)
26.	Phenylpropanoids	Pinoresinol	C20H22O6	(48)
27.	Phenylpropanoids	Medioresinol	C21H24O7	(48)
28.	Flavonoids	Quercetin	C15H10O7	(54)

29.	Flavonoids	Quercitrin	C21H20O11	(54)
30.	Flavonoids	Isoquercitrin	C21H20O12	(54)
31.	Flavonoids	Kaempferol	C15H10O6	(53)
32.	Benzoic acids	Gallic acid	C7H6O5	(46)
33.	Benzoic acids	2,4-Dihydroxybenzoic acid	C7H6O4	(46)
34.	Benzoic acids	2,5-Dihydroxybenzoic acid	C7H6O4	(46)
35.	Other compounds	Galacturonic acid	C6H10O7	(46)
36.	Other compounds	Pyruvic acid	C3H4O3	(46)
37.	Other compounds	Formic acid	CH2O2	(46)
38.	Other compounds	Succinic acid	C4H6O4	(46)
39.	Other compounds	Oleic acid	C18H34O2	(47)
40.	Other compounds	Palmitic acid	C16H32O2	(47)

CHAPTER 6

MATERIALS AND METHODS

MATERIALS AND METHODS

6.1 Materials:

Table 6.1:Apparatus, equipment and instruments

Name of instrument	Specifications, Model, Make & country
Analytical electronic balance	High precision Axis LCGC AGN 204, LCGC, Shimadzu ATX224R, India
Centrifuge	Remi CPR-30 Plus, India
Desiccator	Borosil, Fisher Scientific Pvt Ltd. India
Heating mantle	KHM-5, Kemi Scientific, India
Hot air oven	HICON Grover Enterprises, India
Hot Plate	Kemi Scientific, India
Melting point apparatus	Roy Instruments Pvt Ltd, India
Micropipette (High precision)	VVCS-1000 (100-100l), Beacon Diagnostics Pvt Ltd. Gujarat, India
Muffle furnace	KMF-1, Kemi Scientific, India
Rotary vacuum evaporator	Trident Labortex
Soxhlet Apparatus	Borosil, Magnum, India
Ultra Sonicator	CD-4800, Shenzhen Codyson Electrical Co Ltd, China
UV Cabinet for TLC	KUVC-2, Kemi Scientific, India
UV-Visible Double Beam Spectrophotometer	UV 1800-240v Shimadzu, USA
Water bath	KSWB-1, Kemi Scientific, India

6.2 Collection Of Plant

Solanum nigrum plants were collected from north Delhi area in December 2022. The plant specimen was authenticated by former Chief Scientist, Dr. Sunita Garg and Chief Scientist, Mr. R.S. Jayasomu, CSIR-NIScPR, Pusa, New Delhi (Ref. NIScPR/RHMD/Consult/2023/4314-15). The Fresh leaves were shade dried at room temperature (24 ± 2^0C) and powdered.

6.3 Organoleptic Characters

Colour : Buff green

Taste : Indistinct

Odour : Indistinct

Fineness of powder: The coarseness or fineness of leaves powder of *Solanum nigrum* was classified according to the nominal aperture size expressed in µm of the mesh of sieve. Sieve no. 250 and 500 were used for the study.

Foreign matter: Macroscopic examination was done for determining the presence of foreign matter. 100 g of air-dried leaves powder of the plant *Solanum nigrum* was weighed and spread into a thin layer. The foreign matter was sorted into groups by visual inspection using a magnifying lens. The remaining sample was sifted through sieve no. 250. Dust was considered as mineral admixture. Weight of the portion of sorted matter was found to be within 0.05 g.

6.4 Physicochemical Evaluation

6.4.1. Moisture Content

About 1.5 g of the leaves powder was transferred into a weighed flat and thin porcelain dish. It was kept in oven at 100- 105^{o}C for one hour. Then cooled in a desiccator and weighed again. The loss of weight was recorded as moisture content.

6.4.2. Foaming Index

One g of the coarse leaves powder weighed accurately and transferred to 500 ml conical flask containing 100 ml boiling water. Moderate boiling was maintained for 30 min. The mixture was cooled and filtered into 100 ml volumetric flask. The volume was made up with sufficient water. The decoction was poured into 10 ml stoppered test tubes in successive portion of 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml and 10 ml. The volume of each tube was adjusted to 10 ml with water. The tubes were stoppered and shaken well for 15 sec in length wise direction and then allowed to stand for 15 min. Height of foam generated on the was measured

Result assessed as follows:

- If the height of foam in each tube is < 1 cm, foaming index is less than 100.
- If the height of foam of 1cm is measured in any tube, the volume of the decoction is used to determine the index.
- If the height of foam is >1cm in each tube, foaming index is more than 1000

Foaming Index = 1000/a

Where a = volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm was observed.

6.4.3 Ash Value

6.4.3.1. Total ash

The total ash is produced by incinerating the root powder at the temperature possible to remove all of the carbon. A higher temperature may result in the conversion of carbonates to oxides. The total ash usually consists of carbonates, phosphates, silicates and silica which include both physiological ash, which is derived from plant tissue itself and non-physiological ash, which is the residue of the adhering material to the plant, e.g., sand and soil.

About 2-4 g of accurately weighed root powder was placed in a previously ignited and tare crucible. The material was spread in a layer and ignited it by gradually increasing heat to 500-600^{o}C until it was white indicating absence of carbon. The crucible was cooled in a desiccator and weighed. The content in mg/g of the air-dried material was calculated.

6.4.3.2 Acid insoluble ash

Acid-insoluble ash is determined by treating the total ash with dilute hydrochloric acid and weighing the residue. This limit particularly indicated contamination with silicious materials such as earth and sand by comparison with the total ash value for the same sample. Differentiation can be made between contaminating material and in the natural ash of the drug.

The crucible containing total ash, 25 ml of hydrochloric acid was added. The crucible was covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml hot water and added the liquid in the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until filtrate was neutral. The filter paper containing the insoluble matter was transferred to original crucible, dried on a hot plate and ignited to constant

weight. The desiccator was cooled for 30 min. Acid insoluble ash in mg/g of air-dried material was calculated.

6.4.3.3. Water soluble ash

Water soluble ash is that part of the total ash content which is soluble in water.

To a crucible containing total ash 25 ml water was added and boiled for 5 min. The insoluble matter was collected in an ash less filter paper. It was washed with hot water and ignited for 15 min at temperature not exceeding 450^0C. The content of water-soluble ash in mg/g of air-dried material was calculated.

6.5 Extraction And Fractionation

Maceration extract: 20 g leaves powder of Solanum nigrum was kept for maceration in 100 ml of 70% ethanol for 48 hours. After filtration the filtrate was evaporated in Rotary vacuum evaporator under reduced pressure. The concentrated residue was transferred into a flat-bottomed dish and evaporated to dryness on a water bath, cooled in desiccators for 30 min and weighed immediately. Content of the extractable matter was calculated.

$$\text{Percent 70\% ethanol soluble extractive} = \left[\frac{\text{weight of extract x volume of ethanol}}{\text{weight of drug powder x volume of extract}} \right] X\ 100$$

6.5.1 Hydroalcoholic extract and its fractionation:

150 g of leaves powder was mixed in 2Lof 70% ethanol and extracted in Soxhlet extractor for 12 hours on water bath. The solvent was evaporated in Rotary vacuum evaporator under reduced pressure. The concentrated residue was transferred into a flat-bottomed dish and evaporated to dryness on a water bath, cooled in desiccators for 30 min and weighed immediately. Content of the extractable matter was calculated. The hydroalcoholic (mother) extract obtained was suspended in distilled water (1g/10ml) and sonicated for 30 min at 45^0C. The resulting suspension in water was partitioned with equal volume of hexane in separating funnel for three

times. The whole hexane suspension was transferred into a flat-bottomed dish and evaporated to dryness on a water bath, cooled in desiccators for 30 min and weighed immediately. Content of the extractable matter was calculated. Further partitioning of the extract was done in dichloromethane, followed by evaporation in Rotary vacuum evaporator under reduced pressure and content of the extractable matters was calculated. The aqueous suspension left after partitioning was evaporated to dryness and the residue was further sonicated with ethyl acetate and methanol separately for 30 min (thrice each). The solvent fractions then evaporated in Rotary vacuum evaporator under reduced pressure. Content of the extractable matter in all the partitioned solvents was calculated. All the extracts were stored at 4^0C till further use

6.6 Volumetric Analysis

6.6.1 Acid Value

2-4 g of substance was added to 50 ml mixture of equal volume of 95% ethanol and ether, previously neutralized with 0.1M potassium hydroxide to phenolphthalein solution. Warm the solution gently with shaking. The solution was cooled to room temperature, filtered and titrated with 0.1M KOH using phenolphthalein as indicator until solution remains faintly pink after shaking for 30 sec.

$$\text{Acid value} = \frac{\text{volume of KOH x molarity of KOH x 5.61}}{\text{Weight taken}}$$

6.6.2 Saponification Value

1g of substance was added to a 200 ml flask. 25 ml of 0.5 M ethanolic potassium hydroxide and little pumice powder was added to it. The mixture was boiled under reflux on a water bath for 30 min, cooled to room temperature and titrated with 0.5M HCl using phenolphthalein as indicator. Blank determination was also performed.

$$\text{Saponification value} = \frac{\text{(B-A) x molarity of HCl x 28.05}}{\text{Weight taken}}$$

where B is the titre value for blank and A is the titre value for sample solution.

6.6.3 Iodine Value

About 2 g of sample was taken in a 500 ml iodine flask. 10 ml of CCl_4 and 20 ml iodine monochloride solution was added and the flask was stoppered. The flask was kept in the dark at 15-25^{0}C for 30 min. 15 ml of potassium iodide solution was added on cup top. The stopper was removed and 100 ml water was added. The resultant solution was titrated with 0.1M sodium thiosulphate using starch solution as indicator. Blank determination was also performed.

$$\text{Iodine value} = \frac{(B-A) \times 1.269}{\text{Weight taken}}$$

Where B is the titre value for blank. A is the titre value for sample solution.

6.7 Preliminary Phytochemical Screening

6.7.1 Chemical Tests

Preliminary phytochemical studies (55) were performed on the extract and hexane, dichloromethane, ethyl acetate and methanol fractions of the leaves of *Solanum nigrum*.

Reagents and chemicals

Dragendroff's reagent (Merck Millipore), Hydrochloric acid, ethanol, Hager's reagent, Wagner's reagent (Alpha Labs Chemicals, India), Mayer's reagent (Sigma-Aldrich), Anthrone reagent (Fine Chemicals), Benedict's solution (Merck), Fehling's solution A and B (Merck), Molisch's reagent, sulphuric acid, sodium hydroxide, Millon's reagent (LobaChemie), magnesium, sodium bicarbonate, chloroform, $FeCl_3$ solution, Iodine.

1. Alkaloids

(a) Dragendroff's test

To 2 mg of the extract in 5 ml distilled water, 2 M Hydrochloric acid was added till the solution becomes acidic. To this 1 ml of Dragendroff's reagent was added. Formation of orange or orange red precipitate indicates the presence of alkaloids.

(b) Hager's test

To 2 mg of the extract, few drops of Hager's reagent was added. Formation of yellow precipitate confirms the presence of alkaloids.

(c) Wagner's test

Two mg of extract was acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner's reagent was added. A yellow or brown ppt. indicates the presence of alkaloids.

(d) Mayer's test

Ten mg of extract was dissolved in conc hydrochloric acid and filtered. A few drops of this solution were poured into the center of a watch glass. Mayer's reagent was added in drops to the sides of the watch glass with the help of a glass rod. Formation of gelatinous white precipitate at the junction of two liquids shows the presence of alkaloids.

2. Carbohydrates

(a) Anthrone test

The filtrate was concentrated after two mg of extract and 10 ml of water were shaken together. Anthrone reagent in the amount of 2 ml was added. The presence of carbohydrates is indicated by the development of green or blue hue.

(b) Benedict's test

The filtrate was concentrated after two mg of extract and 10 ml of water were shaken together. Benedict's solution (5 ml) was added to this and heated for 5 minutes. Carbohydrates are present when a precipitate with a brick red hue form.

(c) Fehling's test

The filtrate was concentrated after two mg of extract and 10 ml of water were shaken together. Equal portions of Fehling's solutions A and B were added to this 1 ml mixture and heated for a short period of time. Brick red precipitate's formation denotes the presence of reducing sugar.

(d) Molisch's test

The filtrate was concentrated after two mg of extract and 10 ml of water were shaken together. This was then mixed with 2 drops of newly made Molisch's reagent (20% alcoholic solution of -naphthol). Conc sulphuric acid in a volume of 2 ml was added to the test tube's sidewalls. When two liquids converge, a reddish-violet ring forms, indicating the existence of carbohydrates that vanish with the addition of too much alkali.

3. Proteins

(a) Biuret's test

A drop or two of 3% w/v copper sulphate solution was added to one ml of hot extract after adding 5-8 drops of 10% w/v sodium hydroxide solution beforehand. Proteins were present, as evidenced by the formation of violet red hue.

(b) Millon's test

4. Flavonoids

(a) Shinoda's test

Five milliliters of ethanol were mixed with two milligrams of ethanolic extract, and then ten drops of diluted hydrochloric acid and a trace amount of magnesium were added. Flavonoids are present when pink, reddish, or brown hue forms.

5. Glycosides

Molisch's test

10 ml of water and 2 mg of ethanolic extract were shaken together, then the filtrate was filtered and concentrated. 2 ml of concentrated sulfuric acid was then gently introduced through the test tube's side after 2-3 drops of Molisch's reagent had been added and stirred. Glycosides are present when reddish violet rings develop.

6. Triterpenoids

Liebermann - Burchard's

Test one ml of concentrated sulfuric acid was applied along the test tube's sides after two mg of dry extract had been mixed with acetic anhydride and heated to boiling. Triterpenoids can be detected by the emergence of a pink tint.

7. Resins

One milliliter of ethanolic extract was mixed in acetone before being added to distilled water as a solution. Turbidity is a sign of resins in the environment.

8. Saponins

Approximately 5 ml of an ethanolic extract were placed in a test tube, and a drop of sodium bicarbonate solution was added. After three minutes of vigorous shaking, the test tube was removed. Saponins are present when froth begins to form in the shape of a honeycomb.

9. Steroids

(a) Liebermann-Burchard's test

One ml of concentrated sulfuric acid was applied along the test tube's sides after two mg of dry extract had been dissolved in acetic anhydride and heated to boiling. Steroids are present when green coloration develops.

(b) Salkowski reaction

Sulphuric acid was progressively introduced via the test tube's sides to the chloroform layer after two mg of dry extract had been mixed with the solvent. Steroids were present because of the formation of the hue red.

10. Tannins

1-2 ml of the ethanolic extract were mixed with a few drops of a 5% w/v FeCl3 solution. Gallotannins were represented by a green tint, whereas pseudotannins were shown by a brown colour.

11. Starch

In 5 ml of distilled water, 0.01 g of iodine and 0.075 g of potassium iodide were dissolved, and 2-3 ml of ethanolic extract were added. Starch was present, as shown by the formation of the blue hue.

6.8 *In Vitro* Antioxidant Activity

Reagents and chemicals

6.8.1. Determination Of Reducing Power

The presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The colour of the test solution changes from yellow to various shades of green and blue, depending on the reducing power of each compound. The amount of Fe^{2+} complex can then be monitored by measuring the formation of Prussian blue colour at 700 nm. Increasing absorbance indicates an increase in reductive ability. The reducing power of the extract was evaluated according to the method of Bhalodia *et al*. (56).

Preparation of standard ascorbic acid solution

In a 50 ml volumetric flask, 50 mg L-ascorbic acid was dissolved in methanol and the volume was made up with methanol. Various concentrations of ascorbic acid solution (50-300 µg/ml) were prepared by diluting 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ml to 10 ml using methanol.

Preparation of sample solution

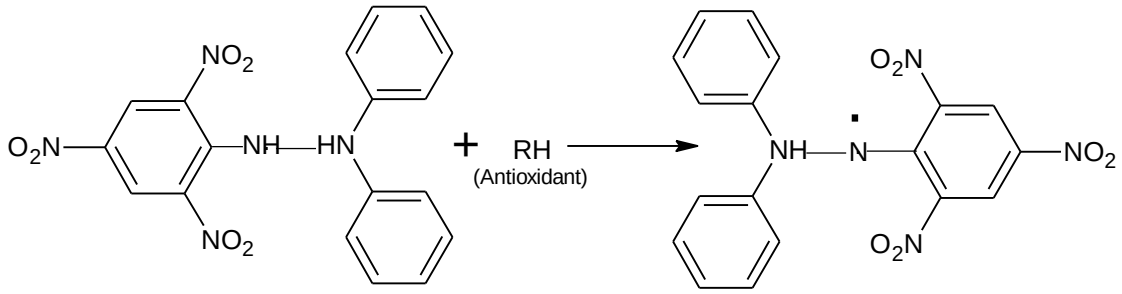
In a 50 ml volumetric flask, 50 mg of extract was dissolved in methanol and the volume was made up with methanol. Various concentrations of extract solutions (50-300 μg/ml) were prepared by diluting 0.5, 1, 2, 3, 4 and 5 ml to 10 ml using methanol.

Method

The mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml $K_3Fe(CN)_6$ (1 % w/v) was added to 1.0 ml of the extract and standard solution of different concentrations. The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 2.5 ml of trichloroacetic acid (10% w/v). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 ml), mixed with distilled water (2.5 ml) and 0.5 ml of $FeCl_3$ (0.1 %, w/v). The absorbance was measured at 700 nm against blank sample. The higher the absorbance value the stronger is the reducing power. All measurements were made in triplicate. Ascorbic acid was used as standard.

6.8.2. DPPH Radical Scavenging Assay

Radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The reduction capacity of DPPH radical was determined by decrease in absorbance. The changes in colour from deep-violet to light-yellow were measured at 517 nm on a UV-visible light spectrophotometer (57).



DPPH **DPPH$_2$**

Figure 6.1: DPPH radical scavenging assay principle

Preparation of standard Quercetin solution

In a 50 ml volumetric flask, 50mg of quercetin was dissolved in ethanol and the volume was made up with ethanol. Various concentrations of quercetin solutions (10-50 μg/ml) were prepared by diluting 0.1, 0.2, 0.3, 0.4 and 0.5 ml to 10 ml using ethanol.

Preparation of sample solution

In a 50 ml volumetric flask, 50 mg of extract was dissolved in ethanol and the volume was made up with ethanol. Various concentrations of extract solutions (50-250 μg/ml) were prepared by diluting 0.5, 1.0, 1.5, 2.0 and 2.5 ml to 10 ml using ethanol.

Preparation of 0.3mM DPPH solution

Accurately weighed 5.91 mg DPPH was transferred to 50 ml volumetric flask. It was dissolved in ethanol and the volume was made up with ethanol.

Method

One ml of a 0.3 mM DPPH ethanolic solution was added to 2.0 ml of sample solutions of different concentrations, and allowed to react at room temperature for 30 minutes. The absorbance was measured at 517 nm. All determinations were performed in triplicate. The difference in absorbance between test and control was calculated and expressed as % of radical scavenging activity. The results are expressed as mean ± SD. % DPPH radical scavenging activity was calculated using the following formula

Percent DPPH radical scavenging activity

$$= \left[\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100 \right]$$

6.8.3. Nitric Oxide Scavenging Assay

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss

reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. (57)

Preparation of standard ascorbic acid solution

In a 50 ml volumetric flask, 50 mg of L-ascorbic acid was dissolved in methanol and the volume was made up with methanol. Various concentrations of ascorbic acid solution (50-250 µg/ml) were prepared by diluting 0.5, 1.0, 1.5, 2.0 and 2.5 ml to 10 ml using methanol.

Preparation of sample solution

In a 50 ml volumetric flask, 50 mg of extract was dissolved in methanol and the volume was made up with methanol. Various concentrations of extract solutions (50-300 µg/ml) were prepared by diluting 0.5, 1.0, 1.5, 2.0 and 2.5 ml to 10 ml using methanol.

Method

Two ml of (10 mM) sodium nitroprusside and 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of methanolic extract at various concentrations (50 – 250 µg) and the mixture incubated at 25^0C for 150 min. 0.5 ml of this solution was added into 1.0 ml sulfanilic acid reagent and incubated at room temperature for 5 min. Finally, 1.0 ml (1 % w/v) naphthyl ethylenediamine dihydrochloride was mixed and incubated at room temperature for 30 min. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthyl ethylenediamine dihydrochloride was measured spectrophotometrically at 546 nm. Each determination was made three times. Mean SD was used to express the results. Percent nitric oxide radical scavenging was calculated using the following formula.

Percent Nitric oxide radical scavenging activity

$$= \frac{[\text{Absorbance of control} - \text{Absorbance of test}]}{\text{Absorbance of control}} \times 100$$

CHAPTER 7

RESULT & CONCLUSION

RESULTS AND CONCLUSIONS

7.1 Organoleptic Characters

Colour : Buff green

Taste : Indistinct

Odour : Indistinct

Foreign matter

No foreign matter in the powder of *Solanum nigrum* leaves was observed upon visual inspection and after passing through sieve no. 250.

7.2 Physicochemical Evaluation

The quality and purity of the powder of *Solanum nigrum*leaves was determined on various parameters. Results of physicochemical evaluation is mentioned in table 7.1.

Table 7.1: Physicochemical evaluation of powder of *Solanum nigrum*leaves

	PHYSICOCHEMICAL PARAMETERS	**VALUES (as per Ayur. Pharmacopoeia of India)**
7.2.1	MOISTURE CONTENT*	2.7 %w/w.
7.2.2	FOAMING INDEX*	<100
7.2.3	ASH VALUE*	
	i. Total ash	4.36 %w/w (≤ 9 %)
	ii. Acid insoluble ash	1.81 %w/w (≤ 7 %)
	iii. Water soluble ash	2.55 %w/w
7.2.4	EXTRACTIVE VALUE*	
	Maceration extract	22.95 % w/w
	Hydroalcoholic extract	27.06 % w/w
	Hexane fraction	41.2 % w/w
	Dichloromethane fraction	44.7 % w/w
	Ethyl acetate fraction	8.9 % w/w
	Methanol fraction	1.2 % w/w

* values are expressed as mean (n=3)

7.3 Volumetric Analysis

Volumetric analysis of the powder of *Solanum nigrum*leaves was carried out to know the extent of contamination of the powder in terms of fatty acid generated due to hot moist atmosphere and by attack of microorganisms. The number of carboxylic groups (of fatty acids), average mass of fatty acids and amount of unsaturation in fatty acids was assessed by determining the acid value, saponification value and iodine value respectively. The values obtained are shown in table 7.2.

Table 7.2: Volumetric analysis of powder of *Solanum nigrum*leaves

	PARAMETERS	VALUES
7.3.1	ACID VALUE*	0.3018 mg KOH/g
7.3.2	SAPONIFICATION VALUE *	132.26 mg KOH/g
7.3.3	IODINE VALUE*	3.94 mg/100g

* values are expressed as mean (n=3)

7.4 Preliminary Phytochemical Screening

7.4.1 Screening By Chemical Tests

The preliminary phytochemical screening of the extract and the fractions of *Solanum nigrum* revealed the presence of various phytoconstituents. Ethanolic maceration extract and the n-hexane, Dichloromethane, ethyl acetate, methanolic fractions of *Solanum nigrum* extracts showed the presence of flavonoids, tannins and proteins. Alkaloids were absent all the extracts. n-hexane and chloroform extracts contained less amount of phytoconstituents. The results are depicted in table 7.3.

Table 7.3: Phytochemical screening of extracts and fractions of *Solanum nigrum*

Chemical tests	Maceration extract	n-hexane	Dichloro methane	Ethyl acetate	Methanol
Alkaloids					
Dragendroff's test	-	++	+	++	+
Hager's test	-	-	-	-	-
Carbohydrates					
Selwinoff's test	+	-	-	-	-
Reducing sugar					
Fehling's test	-	-	-	-	-
Benedict's test	++	+++	+	++	-
Monosaccharide					
Barfoed's test	-	-	-	-	-
Proteins					
Biuret's test	-	-	-	-	-
Millon's test	+	-	-	-	-
Flavonoids					
Alkaline reagent test	-	+	-	+++	-
Lead acetate test	++	+	-	-	-
Ferric chloride test	-	-	-	-	-
With Conc.H_2SO_4	-	-	-	-	-
Glycosides					
Aqueous NaOH	++	+++	++	+	-
Conc. H_2SO_4	++	+++	++	+	+
Triterpenoids/ Steroids					
Salkowski reaction	-	-	-	-	-
Resins					
Turbidity test	-	-	-	-	-
Saponins					
Foam formation	+++	+++	+++	-	-
Phenolic compounds					
Iodine test	++	+++	++	+	+
Potassium dichromate test	+	++	-	-	-
Tannins					
Gelatin test	-	-	-	-	-
Coumarin					
NaOH	+++	++	+++	+	-

- Phytochemical screening of extracts and fractions of *Solanum nigrum* revealed that maceration extract contains reducing sugar, proteins, flavonoids, glycosides, saponins, phenolic compounds, and coumarin.
- n-hexane fraction contains alkaloids, reducing sugar, flavonoids, glycosides, saponins, phenolic compounds and coumarin.
- DCM fraction contains alkaloids, reducing sugar, glycosides, saponin, phenolic compound and coumarin.
- Ethyl acetate fraction contains alkaloids, reducing sugar, flavonoids, glycosides, phenolic compounds and coumarin.
- Methanol fraction contains alkaloids, glycosides and phenolic compounds.
- Phytochemical screening of all the extracts and fractions revealed that n-hexane and DCM fractions contained maximum secondary metabolites including alkaloids, glycosides, saponins, phenolic compounds and coumarin.

7.5 *In Vitro* Antioxidant Activity

7.5.1 Determination of Reducing Power

Reducing power of hexane and DCM fraction of *Solanum nigrum* was determined by its capacity to reduce Fe^{3+} to Fe^{2+}. Results showed that with increase in concentration the reducing capacity of the fractions also increased (table 7.4, figure 7.1, figure 7.2). The activity of the fractions was comparable to the reducing capacity of ascorbic acid.

Table 7.4: Reducing power of fractions of *Solanum nigrum*

Concentration (µg/ml)	ABSORBANCE (700 nm)		
	Hexane fraction*	DCM fraction*	Ascorbic acid*
1	0.211 ± 0.002	0.191 ± 0.002	0.314 ± 0.001
2	0.428 ± 0.001	0.381 ± 0.001	0.489 ± 0.000
3	0.731 ± 0.001	0.684 ± 0.001	0.806 ± 0.003
4	1.049 ± 0.000	0.982 ± 0.001	1.142 ± 0.001
5	1.374 ± 0.000	1.229 ± 0.000	1.392 ± 0.000

*values are expressed as mean±SD of three replicates.

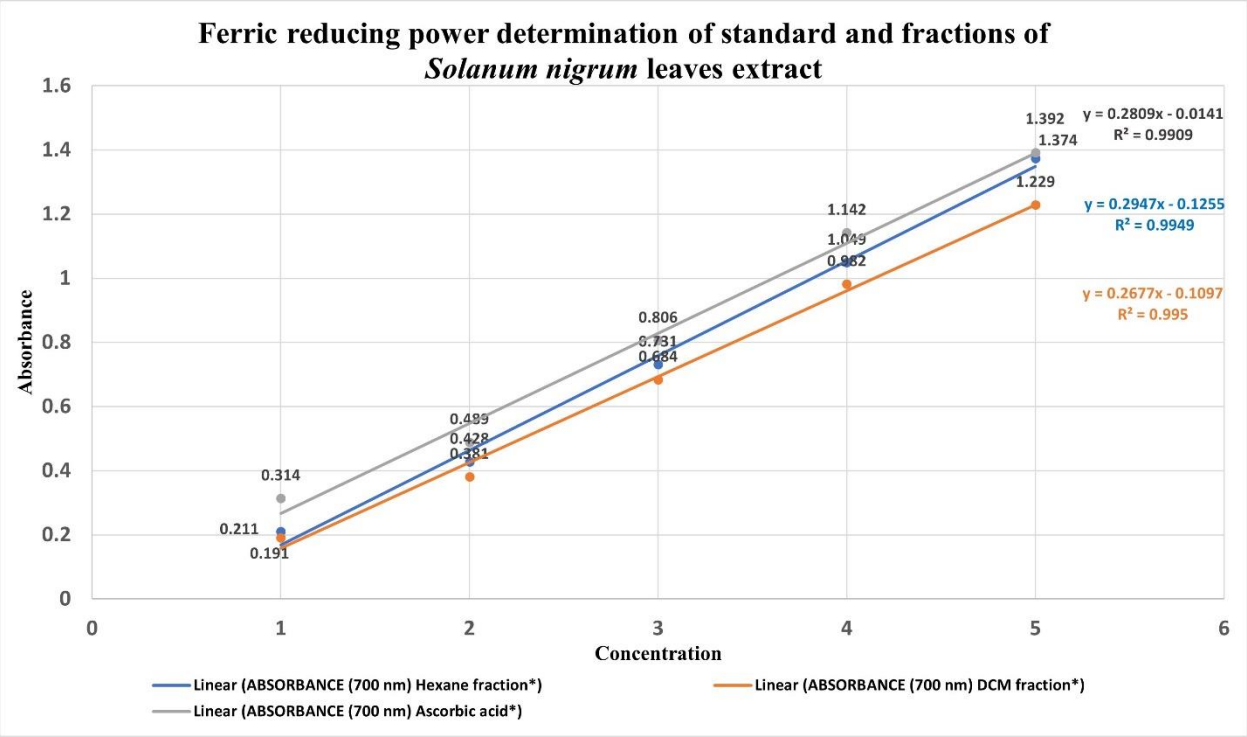


Figure 7.1
Reducing power of fractions of *Solanum nigrum*

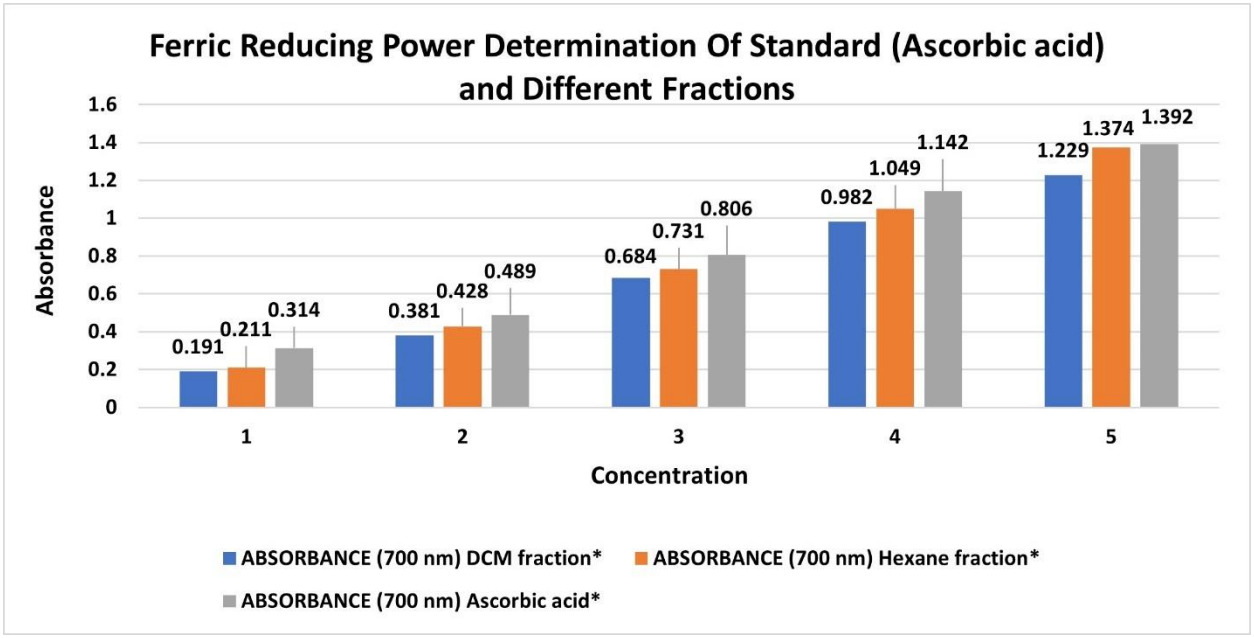


Figure 7.2
Reducing power of fractions of Solanum nigrum (Bar Graph)

7.5.2 DPPH Radical Scavenging Assay

The model of scavenging stable DPPH radical is a widely used method to evaluate free radical scavenging ability of various extracts. Figure 7.2 shows that the hexane and DCM fractions of *Solanum nigrum* leaves have antioxidant activity with the IC_{50} value 44.067±0.001µg/ml and 62.569±0.002µg/ml respectively, which was comparable to quercetin (0.477±0.002 µg/ml). DPPH radical scavenging assay revealed that n hexane fraction have more radical scavenging power than DCM fraction. Results are shown in table 7.5, figure 7.3 & figure 7.4.

Table 7.5: DPPH radical scavenging by fractions of *Solanum nigrum*

CONCN (µg/ml)	ABSORBANCE			PERCENT INHIBITION		
	Quercetin	Hexane fraction	DCM fraction	Quercetin *	Hexane fraction*	DCM fraction*
20	0.467	0.611	0.736	53.065±0.002	38.592±0.002	26.030±0.003
40	0.309	0.511	0.621	68.944±0.002	48.643±0.000	37.587±0.000
60	0.241	0.454	0.506	75.778±0.003	54.371±0.002	49.145±0.002
80	0.198	0.302	0.412	80.100±0.001	69.648±0.001	58.592±0.003
100	0.143	0.194	0.284	85.628±0.000	80.502±0.000	71.457±0.001
IC_{50} (µg/ml)				0.477±0.002	44.067±0.001	62.569±0.002

*values are mean±SD of three replicates.

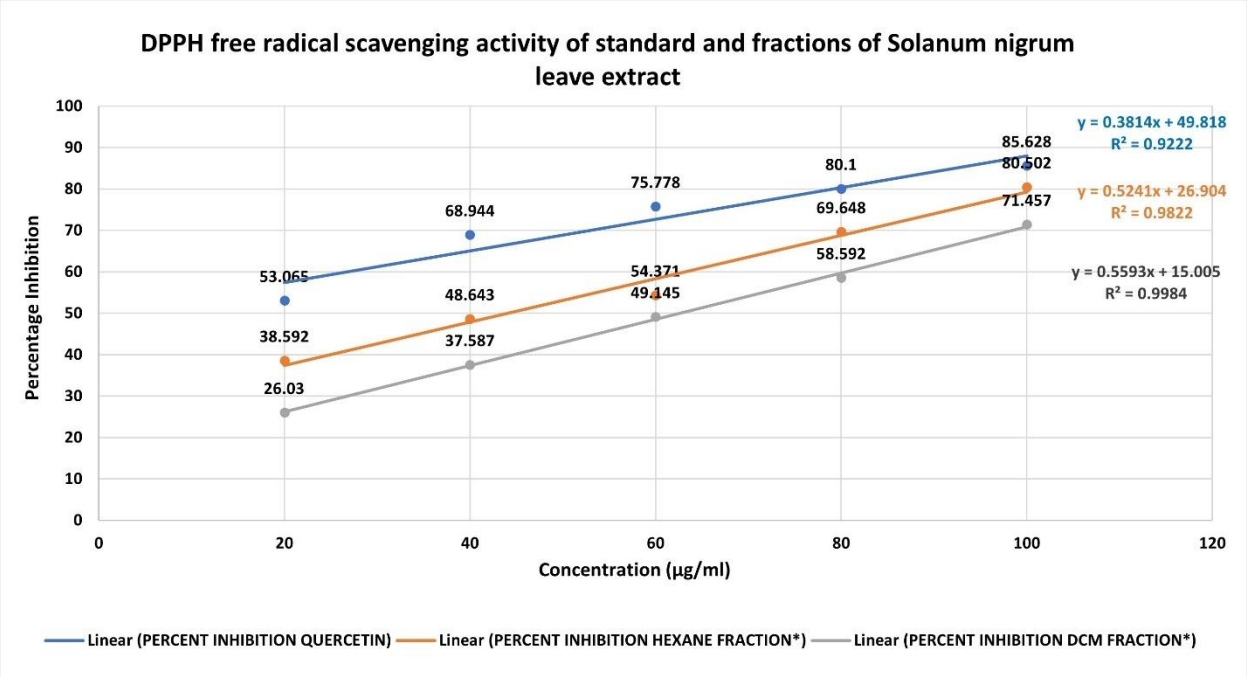


Figure 7.3
DPPH scavenging activity of fractions of *Solanum nigrum*

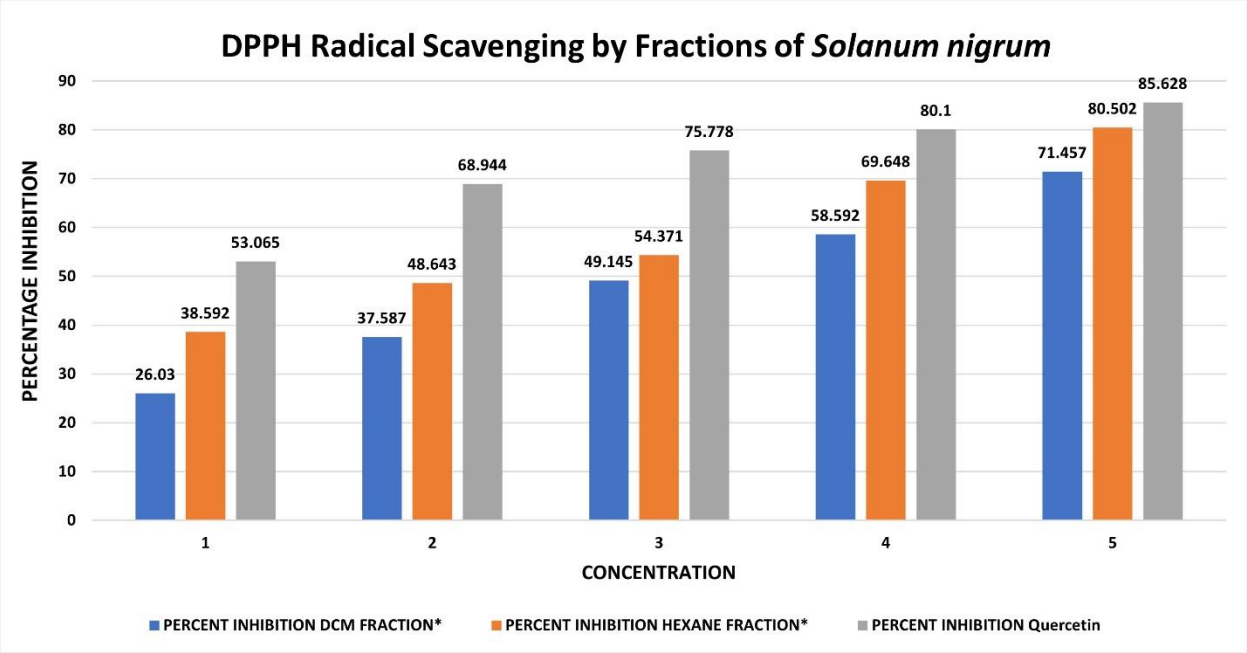


Figure 7.4
DPPH scavenging activity of fractions of *Solanum nigrum* (Bar Graph)

7.5.3 Nitric Oxide Scavenging Assay

The extract of *Solanum nigrum* leaves exhibited strong capacity to scavenge nitric oxide (IC_{50} 217.833±0.002µg/ml(Hexane) and IC_{50}226.487±0.003 µg/ml (DCM)). The activity of the extract increased with increasing concentration. However the activity of standard ascorbic acid was more pronounced than the extract (IC_{50} 116.127±0.003 µg/ml). Nitric oxide scavenging assay revealed that n hexane fraction has more nitric oxide scavenging power than DCM fraction. Results are shown in table 7.6 and figure 7.5 and table 7.6.

Table 7.6: Nitric oxide scavenging by fractions of *Solanum nigrum*

CONCN (µg/ml)	ABSORBANCE			PERCENT INHIBITION		
	Ascorbic acid	Hexane fraction	DCM fraction	Ascorbic acid *	Hexane fraction*	DCM fraction*
50	0.376	0.532	0.576	35.060±0.002	8.117±0.003	0.518±0.001
100	0.311	0.485	0.477	46.286±0.001	16.234±0.001	17.616±0.002
150	0.252	0.402	0.399	56.476±0.003	30.569±0.000	31.088±0.000
200	0.167	0.344	0.305	71.157±0.000	40.587±0.002	47.322±0.003
250	0.118	0.213	0.278	79.620±0.001	63.212±0.003	51.986±0.002
IC_{50} (µg/ml)				116.127±0.003	217.833±0.002	226.487±0.003

*values are mean±SD of three replicates.

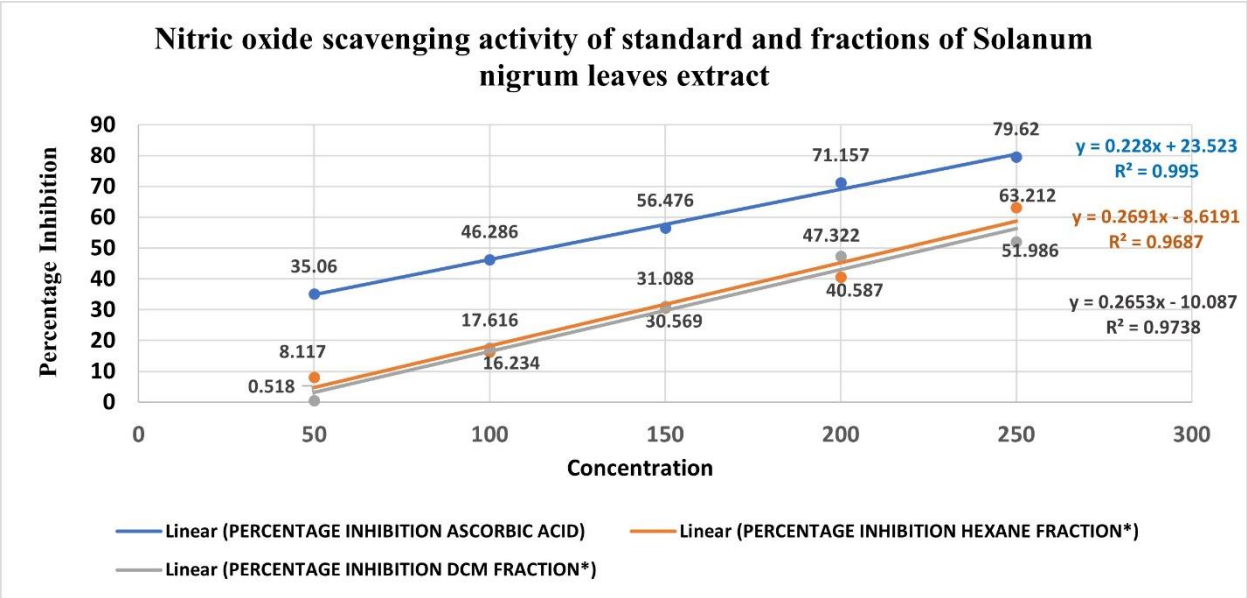


Figure 7.5
Nitric oxide scavenging by fractions of *Solanum nigrum*

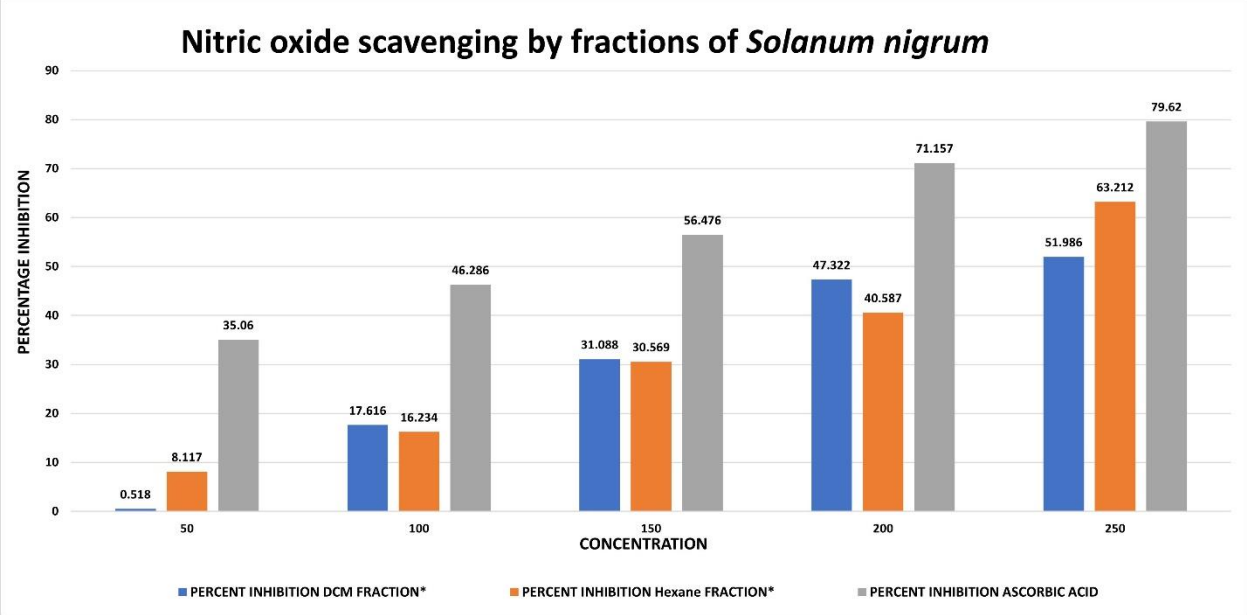


Figure 7.6
Nitric oxide scavenging by fractions of *Solanum nigrum (Bar Graph)*

CHAPTER 8

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CHAPTER 9

ANNEXURE

Azadi Ka Amrit Mahotsav

सीएसआईआर - राष्ट्रीय विज्ञान संचार एवं नीति अनुसंधान संस्थान
CSIR - National Institute of Science Communication and Policy Research
(Erstwhile NISCAIR & NISTADS)
वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद् Council of Scientific & Industrial Research
(विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार Ministry of Science & Technology, Govt. of India)

NISCPR सीएसआईआर-निस्पर

RAW MATERIALS HERBARIUM AND MUSEUM, DELHI (RHMD)

Authentication No.- NIScPR/RHMD/Consult/2023/4314-15 13/1/2023

CERTIFICATE FOR CRUDE DRUG SAMPLE AUTHENTICATION

This is to certify that whole plant ssample of *Solanum nigrum*, Black Nightshade, Makoy, received from Dr. Kalpana P. Rahate vide letter No. Nil, Dated 12th January 2023 has been **found correct as fresh whole plant of *Solanum nigrum* L. which are commonly known as Black Nightshade, Makoy.** The identification has been done on the basis of macroscopic studies of the sample followed by detailed scrutiny of literature and matching the sample with authentic samples deposited in the Raw Material Herbarium and Museum, Delhi (RHMD).

Identification pertains to the quantity/quality of specimen/sample(s) received in RHMD. CSIR-NIScPR. This certificate is not issued for any judicial purpose.

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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF EXTRACT OF SOLANUM NIGRUM

CHAPTER 1: Introduction

1.1 MEDICINAL PLANTS

Therapeutic plants, sometimes referred to as medicinal herbs, have been identified and utilised in traditional healing practises since prehistoric eras Plants produce hundreds of chemical compounds for a range of uses, such as defence and protection against insects, fungi, diseases, and herbivorous mammals(1).

Plants that are medicinal are used in modern as well as conventional medicine with the aim of boosting health, treating a particular illness, or both. According to estimates from the Food and Agricultural Organisation, approximately fifty thousand, distinct plants for medicine were used worldwide in 2002. A more conservative estimate from the Royal Botanic Gardens, Kew in 2016 (2) found that 17,810 plant species are believed to have therapeutic properties out of the approximately 30,000 plants for which a usage of any form is documented.

In contemporary medicine, around one-fourth of the drugs that are given to patients are created from plants for medicinal purposes and put to rigorous testing. The majority of therapies that are routinely tried ad hoc and without supporting evidence may be accounted for by medicinal plants, relying on the medical system (3,4).

Plant-based materials, such as herbal or natural health products with claimed health advantages, are increasingly used in industrialised countries. Although herbal treatments have a good reputation for being benign, there are toxicity risks and other effects on human health(5).

Well before the development of modern medicine, herbal remedies have been used for a very long time; however, very little, if any, is known about the pharmacological basis of their actions or the safety of using them. The World Health Organisation developed a conventional medicine strategy in 1991. It has now published advice on how to use it as well as other monographs on frequently used herbal remedies(6)(7).

The three main categories of advantages that medical plants can provide are: health advantages for individuals who use them as medications; financial advantages for those who gather, process, and sell them; and social advantages, such as employment opportunities, tax income, and workforce productivity improvements. Unfortunately, a lack of robust scientific evidence, poor drug development practises, and insufficient funding hinder the development of plants or extracts with potential medical applications(8).

All plants produce chemical compounds that provide them with an evolutionary advantage, such as salicylic acid's ability to act as a hormone in plant defences or as a means of protecting themselves from herbivores(9)(10). If research supports these claims, the content and known pharmacological activity of these phytochemicals in medicinal plants will serve as the scientific basis for their use in contemporary medicine. These phytochemicals may one day be utilised in medicine. One of the nine types of alkaloids found in daffodils (Narcissus) is galantamine, an alkaloid authorised for treatment of Alzheimer's disease. Alkaloids are concentrated in plant parts like the stem that herbivores are most likely to ingest; they have a bitter taste and are poisonous; they may also provide parasite protection.

1.2 FAMILY- SOLANACEAE

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