CHAPTER 1

CHAPTER 1

Screening of Selected Medicinal Plants; Qualitative and Quantitative Phytochemical Analysis

1.1 Introduction

The meticulous selection of plants for pharmacological and *in vitro* anticancer studies represents a pivotal phase in developing novel drugs. Numerous considerations underpin this selection process (Souza,1996). Firstly, the plant should boast a rich history of traditional medicinal use, supported by ethnomedical data, suggesting its potential therapeutic efficacy. Secondly, accessibility and abundance are paramount, ensuring a sustainable supply for research endeavours and subsequent drug development. Moreover, priority should be accorded to plants harbouring known bioactive compounds or phytochemicals, as these may have anticancer properties. Finally, a thorough evaluation of the plant's safety profile is indispensable to mitigate any potential risks to human health associated with its use in drug development (Ibrahim et al., 2017; Fabricant & Farnsworth, 2001; Simmonds, 2014).

Following the selection process based on these criteria, the subsequent step entails comprehensive phytochemical and pharmacological screening (Barbosa et al., 2012). Phytochemical screening identifies the plant's bioactive compounds that could possess anticancer properties. This is achieved through many extraction and isolation techniques, followed by meticulous chemical analysis.

Concurrently, pharmacological screening is indispensable, involving an evaluation of the biological activity of these compounds through *in vitro* anticancer studies. These studies ascertain the efficacy of plant extracts or isolated compounds in impeding the growth of cancer cells or inducing apoptosis. The execution of these screenings demands meticulous attention to detail and adherence to established protocols to uphold the validity and reliability of the results

obtained. Subsequently, the most promising plant extracts or compounds identified can be evaluated through *in vivo* studies to delineate their efficacy and safety profiles in living organisms (Patil et al.,2019).

Plants are rich sources of bioactive compounds crucial for healing various ailments. These compounds, categorized into primary (like chlorophyll and proteins) and secondary (such as terpenoids and alkaloids), possess diverse medicinal properties, including anti-fungal, anti-bacterial, and anti-inflammatory effects. Exploring phytochemicals holds immense economic importance for pharmaceutical companies in their quest for novel medications (Thomford et al.,2018). Since ancient times, plant-derived substances have been pivotal in traditional medicine (Fridlender et al.,2015). They are sourced from different parts of plants like bark, leaves, flowers, roots, fruits, and seeds. The prevalence of free radical reactions poses significant health risks, including cancer (Harborne,1998, Martemucci et al.,2022). Synthetic and natural antioxidants are extensively utilized to counteract oxidative stress (Zhishen et al.,1999, Atta et al.,2017). Traditional medicinal plants, known for their antioxidant and anti-inflammatory properties, offer promising avenues for discovering potent antioxidants to combat cancer effectively (Dar et al.,2023).

1.1.1 *Butea monosperma* (Lam.)Taub.

Figure 1.1: *Butea monosperma* leaf and bark

Taxonomical classification

Kingdom	Plantae
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	*Butea*
Species	*monosperma*

Habitat and Botanical Description

Butea monosperma, commonly known as the flame of the forest, thrives primarily in dry and deciduous forests. It is characterized by its robust stature, capable of reaching heights up to 15 meters. The tree possesses a sturdy trunk enveloped in greyish-brown bark, imparting its structure's resilient and enduring quality. The foliage of *Butea monosperma* is distinguished by its compound and trifoliate leaves, a hallmark feature wherein each leaf comprises three leaflets. These leaflets are oval with pointed tips, boasting a smooth texture and a deep, lustrous green hue (Kumari et al,2022). Furthermore, the fruit of *Butea monosperma* manifests in flat pods, each encapsulating a singular seed within. This distinctive trait lends the species its botanical epithet, "monosperma," denoting its characteristic of bearing a solitary seed within each pod. Beyond its aesthetic allure, *Butea monosperma* plays a pivotal role within its ecosystem, serving as a vital sustenance and refuge for diverse wildlife. Its presence contributes significantly to the overall biodiversity of its habitat, underscoring its value as an integral component of the natural landscape.

1.1.2 .*Melia azedarach* L.

Figure 1.2: *Melia azedarach*

Taxonomical classification

Kingdom	Plantae
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	*Melia*
Species	*azedarach*

Habitat and Botanical Description

Melia azedarach, commonly known as the chinaberry tree or Persian lilac, thrives in subtropical and tropical regions, flourishing across diverse soil types and altitudes. Typically found in open woodlands, along riverbanks, and in disturbed habitats, this deciduous tree can soar to heights of up to 15 meters (Sultana et al., 2014). Distinguished by its pinnately compound leaves and clusters of fragrant lilac-coloured flowers, *Melia azedarach* bears round, yellow fruits known as chinaberries, prized for their medicinal and pesticidal properties. Beyond its practical uses, the tree boasts ornamental appeal thanks to its captivating foliage and blooms. Moreover, *Melia azedarach* has entrenched itself in traditional medicine and is revered for its anti-inflammatory properties and efficacy as an insect repellent. *Melia azedarach* is a compelling and invaluable species in botanical realms with its remarkable adaptability to diverse environments and multifaceted utility.

Melia azedarach is characterized by its deciduous nature, featuring pinnately compound leaves and clusters of petite, aromatic lilac-coloured flowers. The tree bears round,

yellow-green fruits housing sturdy, brown seeds within. Its grey and rugged bark envelops a medium-sized stature with a wide-spreading canopy.

1.1.3 *Saraca asoca* (Roxb.) Wild.

Figure 1.3: *Saraca asoca* (Roxb.) Wild.

Taxonomical classification

Kingdom	Plantae
Class	Equisetopsida
Order	Fabales
Family	Fabaceae
Genus	*Saraca*
Species	*asoca*

Habitat and Botanical description

Saraca asoca, commonly known as the Ashoka tree, thrives in the central and eastern regions of the Indian subcontinent, particularly in deciduous forests. It is well adapted to the region's tropical climate. It can be found in parts of India, Nepal, and Sri Lanka, where it is valued for both its ornamental and medicinal properties (Saklani & Jain, 1989). The tree, known for its striking bright orange to red-flowers, holds significant importance in the traditional Ayurvedic system of medicine.

Saraca asoca prefers well-drained soil and is often sighted near riverbanks and in foothills. Its botanical distribution extends to the Western Ghats in India and the lowland rainforests of Sri Lanka. Revered for its historical and cultural significance, the Ashoka tree is celebrated during religious and festive occasions throughout the Indian subcontinent.

Besides its ornamental value, *Saraca asoca* is highly prized for its medicinal uses, particularly in treating gynaecological disorders and promoting overall uterine health. This has established it as a sought-after plant in traditional herbal medicine practices (Debnath et al., 2010).

1.1.4 *Solanum virginianum* L.

Figure 1.4: *Solanum virginianum* L.

Taxonomical classification

Kingdom	Plantae
Class	Asterids
Order	Solanales
Family	Solanaceae
Genus	*Solanum*
Species	*virginianum*

Habitat and Botanical Description

Solanum virginianum, commonly referred to as Virginia nightshade, an herbaceous plant belongs to the Solanaceae family. It is versatile in habitat, being found in fields, meadows, and disturbed areas. Thriving in well-drained soils, this species exhibits adaptability to a diverse range of environmental conditions. *Solanum virginianum* is recognized by its slender, wiry stems, reaching heights up to 3 feet. Its ovate-shaped leaves feature varying sizes and distinctively toothed margins. This plant's small, star-shaped flowers span from white to pale lavender. Additionally, it yields small spherical fruits that initially appear green, maturing to a dark purple or black hue. *Solanum virginianum* inhabits diverse regions of India, spanning from North India to Central India and the Eastern Ghats. Its presence is notable in the country's open grasslands, roadside areas, and agricultural lands. This plant's ability to adapt to various soil types and climates enables it to flourish across various Indian ecosystems. In traditional

medicine, Virginia nightshade is esteemed for its anti-inflammatory and analgesic attributes, often utilized in treating ailments like arthritis, rheumatism, and skin disorders. Moreover, it holds promise as an alternative reservoir for insecticidal compounds.

In this chapter the detailing of selected plants (*Butea monosperma , Melia azedarach, Saraca asoca* and *Solanum virginianum*) for the study were explained. Additionally, the preliminary phytochemical analysis of *Bm, Ma,Sa* bark and leaf & *Sv* unripe fruit,ripe fruit and leaf were explained.

1.2 Materials and method

1.2.1 Collection and Authentication of plant material

Butea monosperma (*Bm*) (22°32'33" N 73°46'22") and *Melia azedarach* (*Ma*) (22°30'65" N 73°15'71") were collected from the Vadodara district in February-March. In contrast, *Saraca asoca* (*Sa*) was collected from the Kerala district (9°61'11" N 76°52'32") in January. Fresh plant parts of *Solanum virginianum* were collected during February-March from the Vadodara district (22°17'30" N 73°07'54"). The plant samples were identified and authenticated by Dr. Karan Rana, Division of Biomedical and Life Sciences, School of Science, Navrachana University, Vadodara. The voucher specimen (KU1,KU2,KU3,KU4, and KU5) was deposited at Navrachana University,Vadodara.

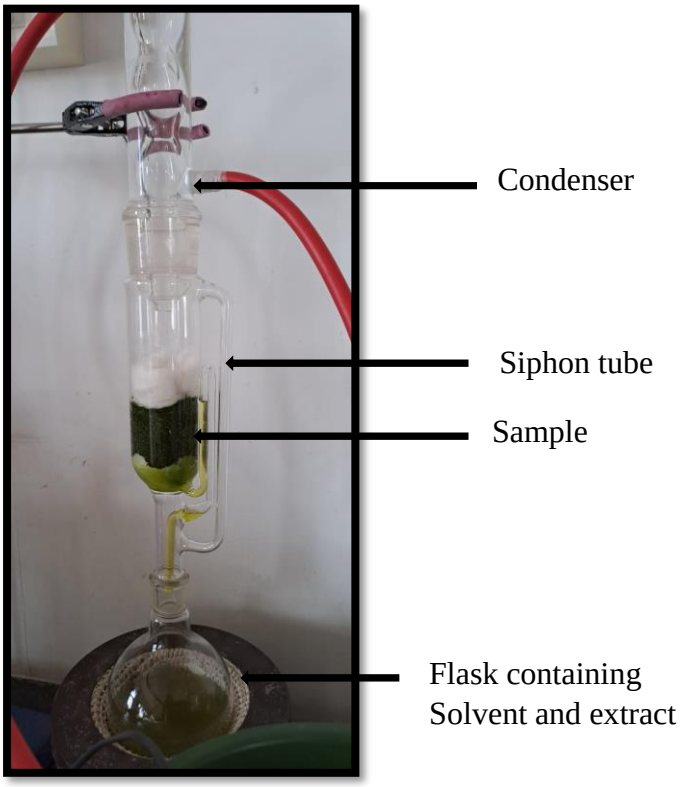
1.2.2 Preparation of Plant Powder: Drying and Maceration

The leaves (L) and bark (B) of *Bm*, *Ma*, and *Sa*, along with the leaves (L), ripe fruit (RF), and unripe fruit (UNRF) of *Sv*, were meticulously separated from the whole plant. Following this, they underwent a thorough wash with distilled water and were subjected to shade drying at room temperature, maintaining a relative humidity of approximately 25-30%. Subsequently, the separated plant parts were finely powdered using a clean mixture grinder. The resultant powder was safely stored in an airtight container in a moisture free place at room temperature,

ensuring its preservation for future processing. Notably, all plant powders' moisture content ranged from 4.90 to 5.20%.

1.2.2.1 Extraction

The selection of an appropriate extraction solvent and method plays a crucial role in determining bioactive compounds' quality, quantity, and biological activity. Key considerations include solvent toxicity, inertness, and ease of removal post-extraction. In the current study, both hot extraction methods (solid-liquid extraction) and aqueous extraction were employed (Danel and Mammen, 2016). Water served as the polar solvent, while methanol was the semi-polar solvent. Using a Soxhlet apparatus, 70% methanol in deionized water was employed for methanolic extraction, while aqueous extraction was conducted through the reflux method using deionized water as the solvent (Salleh et al.,2014). Extraction duration ranged from 40 to 48 hr until the plant material became colourless. Following extraction, crude extracts were filtered and evaporated. Post-extraction, the weight of the extracts was determined, and yield percentages were calculated relative to the initial weight of plant materials. The dried extracts were then securely stored in sterile, airtight containers for further analysis.



Soxhlet Assembly for extraction

1.2.3 Solubility of extracts

All methanolic extracts exhibited solubility in a 0.2% DMSO solution (dH_2O v/v), while aqueous solutions were prepared using dH_2O. Stock solutions of all extracts were maintained at a concentration of 10 mg/mL in their respective solvents and stored at -20°C for subsequent analysis. Working solutions were prepared by diluting stock solutions in their respective solvents according to predetermined concentration series for all assays.

1.2.4 Preliminary phytochemical analysis

The preliminary phytochemical evaluation aimed to assess the presence or absence of secondary metabolites in the plant extracts. It's worth mentioning that the extraction of secondary metabolites can vary based on the polarity of the solvents thus influencing the analysis outcomes (Harborne, 1984 & Lefebvre et al., 2021). Preliminary studies provide

crucial insights into the content of secondary metabolites, aiding in the potential applications of the extracts in further studies.

1.2.4.1 Preliminary qualitative and quantitative analysis of phytochemicals

The qualitative phytochemical analysis detected various primary and secondary metabolites in different plant parts. A range of chemical constituents was evaluated, including carbohydrates, reducing sugars, amino acids, alkaloids, tannins, phenolic compounds, steroids, glycosides, flavonoids, saponins, quinones, terpenoids, cardiac glycosides, anthocyanins, coumarins, and anthraquinones . This method is a cost-effective and efficient means of rapidly assessing the presence of various phytochemicals in a combination, providing valuable insights into bioactive compounds.

Experimental Methodologies for Phytochemical Screening: (Singleton et al., 1999 ;Yadav and Agrawal,2011;Kavita et al.,2013)

Carbohydrates

a) Molisch Test

- 1 ml plant extract + 1 ml concentrated H_2SO_4 and 1 ml Molisch Reagent

  Observation: Red or dull violet colour at the interface of the 2 layers will be present.

Reducing sugar

a) Fehling Test

- Take 1 ml extract + 1 ml Fehling reagent. Warm at 40℃ in water bath.

  Observation: Brick red ppt will be formed at bottom.

Proteins and Amino Acids

a) Ninhydrin Test

- Take 1 ml of plant extract + 1 ml Ninhydrin reagent. Keep in boiling water bath .

  Observation: Purple colour will be developed .

b) Biuret Test

- Take 1 ml plant extract + 1 ml Biuret reagent.

  Observation: Violet or pink colour will be observed.

Terpenoids

- Take 1 ml plant extract + 1 ml of chloroform + add 2 ml concentrated sulfuric acid to form layer.

  Observation: Reddish brown colour of interface indicates presence.

Saponins

- Take 1 ml plant extract + 5 ml Distilled water.

  Observation: Foam formation occurs if shaking.

Cardiac Glycosides

- Take 1 ml plant extract + 2 ml of Glacial Acetic acid + few drops of $FeCl_3$ + 1 ml concentrated H_2SO_4.

  Observation: brown ring formation at interface.

Glycoside

a) Take 1 ml plant extract + 1 ml of chloroform + 1 ml of acetic acid. Cool in refrigerator for 60 sec. Add concentrated H_2SO_4.

   Observation: violet to blue to green colour change.

b) Take 1 ml plant extract + 1 ml of Glacial Acetic acid dissolve + 1 ml $FeCl_3$ + 1 ml concentrated H_2SO_4.

Observation: Brown ring at junction forms.

Alkaloids

a) Dragendroff's

- Take 1 ml of plant extract + 1 ml Dragendroff's reagent.

  Observation: Reddish brown precipitates will be formed.

b) Hager's

- Take 1 ml of plant extract + 1ml saturated picric acid.

  Observation: Yellow precipitates will be observed.

Phenolic Compound

- Take 1 ml plant extract + 2 ml 10% lead acetate.

  Observation: Bulky white colour precipitates will be seen.

Tannins

- Take 1 ml plant extract + 1 ml 5% Ferric chloride solution .

  Observation: Violet colour will be observed.

Flavonoids

- Take 1 ml plant extract + 1ml of 2% NaOH. Formation of yellow colour turns colourless on addition of few drops of 1 % H2SO4.

  Observation: Formation of yellow colour turns colourless.

- Take 1 ml plant extract + few drops of 10% lead acetate.

Observation: Yellow colour formation.

Anthocyanine

- Take 1 ml plant extract + 1 ml 2N NaOH + Heat for 5 min at 100℃ .

  Observation: Bluish green colour formation.

Quinones

- Take 1 ml plant extract + 1 ml concentrated H_2SO_4.

  Observation: Red precipitates forms.

Anthraquinones

- Take 1 ml plant extract + 1 ml 10% HCL + Few min water bath + Filter out + Filtrate allow to cool + Add equal volume of $CHCl_3$ + Few drops of NH_3 solution + Heat

  Observation: Pink colour formation

Coumarins

- Take 1 ml plant extract + 1 ml 10% NaOH.

  Observation: Formation of yellow colour.

Steroids

- Take 1 ml plant extract + 1 ml chloroform + 1ml concentrated H_2SO_4 sidewise.

  Observation: Red colour form in the lower layer of chloroform.
- Take 1 ml plant extract + 1 ml chloroform + 1 ml concentrated H_2SO_4 + 1 ml of acetic acid.

  Observation: Greenish colouration will observe.

The purpose of these analysis ascertains the presence or absence of different phytochemicals in the plant extracts, providing valuable insights into their potential bioactivity and therapeutic applications.

1.2.4.2 Determination of Total phenolic content (TPC) (Singleton et al.,1999)

Phenolic compounds, essential primary metabolites derived from the metabolism of phloxpropanoids in pentose phosphate and the plant's shikimic acid, exhibit notable hydrogen peroxide scavenging activity, thus possessing high antioxidant potency. Gallic acid is a standard for estimating overall phenolic contents due to its stability and cost-effectiveness compared to other phenolic compounds. The total phenolic content of the plant parts was assessed using the Foline-Ciocalteu method (FCR) (Singleton et al.,1999). This method relies on the reduction of Folin–Ciocalteu reagent (FCR) in the presence of phenolics, resulting in molybdenum–tungsten blue, quantified spectrophotometrically at 760 nm. The intensity of the dye correlates linearly with the concentration of phenolics. Given the association between high phenolic content and potent antioxidant capacity, this assay holds significance in determining the overall antioxidant potential (Singleton et al., 1999).

Materials

10% Na_2CO_3 Solution: Prepared by dissolving 10 gm of Na_2CO_3 in 100 mL of dH_2O.

FCR Reagent: Foline-Ciocalteu Reagent.

Gallic Acid Standard: 1 mg/mL stock solution prepared in autoclaved dH_2O. Working concentration range: 20, 40, 60, 80, 100 µg/mL.

Procedure

For the determination of the calibration curve, 0.1 mL of each gallic acid standard solution (20, 40, 60, 80, 100 µg/mL) was taken in separate clean volumetric flask. Each flask was treated with 1 mL of FCR reagent (diluted 1:10 v/v) was added to each flask and incubated in the dark

for a duration of 30 min. 10% Na_2CO_3 solution was added to each flask to bring it up to the mark of a volumetric flask. incubated the sample system at room temperature in the dark for 60 min. Absorbance was recorded at 765 nm using a visible spectrophotometer against a blank (only dH_2O in place of standard or extract solution). 10 mg of plant extract was taken directly into a volumetric flask. The same method were performed as described above for the Gallic acid standards. The phenolic content of the extract is quantified in terms of gallic acid equivalents (mg/g extract). The standard curve equation of gallic acid is y = 0.0111x - 0.0115, where R^2 = 0.9978. Here, y indicates absorbance, while x indicates the standard concentration of gallic acid.

1.2.4.3 Determination of Total flavonoid content (TFC) (Zhishen J et al., 1999)

Flavonoids are important bioactive chemicals that occur naturally. The colorimetric test using aluminium chloride is used to quantitatively measure the total flavonoid content (TFC) by assuming that all flavonoids have the same reaction. The flavonoids possess keto and hydroxyl groups in their structures. When we mix aluminium chloride with flavonoids, it reacts hydroxyl groups of flavones and flavonols, which has absorption maxima at 415 nm. The absorbance of sample extracts was taken at his wavelength and compared with the standard graph. After solvent extraction, the total flavonoid content in extracts is determined by performing the colourimetric method. The total flavonoid content of the plant parts was determined by aluminium chloride colourimetric analysis using a spectrophotometer (Zhishen J et al., 1999). Quercetin is considered a suitable standard for TFC determination.

Materials

5% $NaNO_2$ Solution: Prepared by dissolving 5 gm of $NaNO_2$ in 100 mL of distilled water (D/W).

10% $AlCl_3$ Solution: Prepared by dissolving 10 gm of $AlCl_3$ in 100 mL of dH_2O.

1mM NaOH Solution: Prepared by dissolving 4 mg of NaOH in 100 mL of dH_2O.

Quercetin standard: 1 mg/mL stock solution prepared in 80% ethanol. Working solution range: 20, 40, 60, 80, 100 µg/mL.

Procedure

For calibration curve determination, 1 mL of each quercetin standard solutions (20,40,60,80,100 µg/mL) was taken in a volumetric flask. All standard solutions were diluted by adding 4 mL dH_2O. Then 300µl of 5% $NaNO_2$ and 300µl 10% $AlCl_3$ was added to all tubes. The tubes were kept in dark for 5 min at room temperature. Then 2 ml of 1M NaOH was added to the reaction mixture. Immediately by adding dH_2O, final volume was made up. The flavonoid content of all extracts was determined in terms of quercetin equivalents (mg QE/g extract). The absorbance of the reaction mixture was measured at 510 nm against a reagent blank using spectrophotometer .The standard curve equation is $y = 0.0105x + 0.0119$, where $R^2 = 0.996$.

1.2.5 Determination of antioxidant activity: Free radical scavenging assay (DPPH assay)

Antioxidants counteract the DPPH radical by donating a proton, leading to a reduction in DPPH. This reduction causes a colour change from purple to yellow, which can be quantified by measuring the decrease in absorbance at a wavelength of 517 nm. As the percentage of free radical inhibition increases, the scavenging activity also increases (Sakat et al.,2010). Discolouration measures the sample's or antioxidant's ability to donate hydrogen and scavenge free radicals. The antioxidant activity of plant parts was assessed by determining free radical scavenging activity using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay. This assay is based on a reduction in absorption value at 517 nm upon adding reagents, with methanol as a control blank solution (Gulcin ,2020).

Materials

DPPH (1mM): Prepared by dissolving 20 mg of DPPH in 50 mL of methanol. (This stock solution was stored at -20°C)

Solution preparation for ascorbic acid (standard) and extracts (samples)

Stock Solution: Prepared at 1 mg/mL concentration in dH_2O.

Working Solution range: 150, 250, 500, 750, 1000 µg/mL.

Procedure

In various concentration (150,250,500,750,1000 µg/mL) of sample solutions as well as standard solution of ascorbic acid taken in test tubes, freshly prepared 2 mL of 1mM DPPH solution was added in each test tube and the reagent mixture was incubated in dark at room temperature for 30 min. After incubation, absorbance was determined at 517 nm spectrophotometrically against blank solution (only methanol). The percentage of free radical scavenging activity was calculated using the following formula:

% Inhibition = {(Absorbance of control – Absorbance of sample)/Absorbance of Control} x 100

1.2.6 Inhibition of Protein denaturation activity determination (Sakat et al.,2010)

Inflammation is a vital defence mechanism in the body, protecting against infections, burns, allergens, and other potentially harmful stimuli. It manifests as heat, redness, pain, swelling, and disruptions in normal physiological functions. Protein denaturation, a process triggered by external forces like heat, strong acids or bases, organic solvents, or concentrated inorganic salts, leads to structural alterations in proteins, affecting their tertiary and secondary structures (Mizushima Y and Kobayashi M. ,1968).

Materials

Egg albumin Solution: 1%

Aspirin (1mg/mL): 5 mg of aspirin in 5 mL alcohol.

Procedure

The anti-inflammatory activity of plant extracts was evaluated using the albumin denaturation assay, following the method described by Mizushima et al. and Sakat et al. with some

modifications. The reaction mixture comprised 1 mL of plant extract (1 mg/mL) and a 1% solution of egg albumin. The extracts were incubated at 37°C for 20 minutes, and then heated at 51°C for an additional 20 min. After cooling,the intensity of turbidity was determined at 660 nm. The percentage inhibition of protein denaturation was calculated using the formula:

Percentage Inhibition (%) =(Abs Control−Abs Sample) ×100 /Abs Control

Aspirin served as the standard.

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Date: 01/06/2022

CERTIFICATE OF PLANT AUTHENTICATION

This is to certify that the plant herbarium (KU3, KU4 & KU5) provided by **Ms. Khushali Upadhyay** from Division of Biomedical and Lifesciences, School of Science, Navrachana University, Vadodara are of the following species:

1. *Butea monosperma* (Lam.) Kuntze
2. *Saraca asoca* (Roxb.) W.J.de Wilde
3. *Melia azedarach* L.

The voucher specimens have been deposited at the Herbarium of Navrachana University.

Specimens identified and verified by

Dr. Karan Rana
Assistant Professor
Division of Biomedical and Life Sciences
School of Science
Navrachana University

NAVRACHANA UNIVERSITY VADODARA

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Certificate of Plant authentication

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a UGC recognized University

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NUV/SOS/HERBARIUM/2019/01

21st May 2019

CERTIFICATE OF PLANT AUTHENTICATION

This is to certify that the plant herbarium (KU1 & KU2) provided by Ms. Khushali Upadhyay from School of Science, Navrachana University Vadodara is that of

Solanum virginianum L. (Specimen examined: K001153104!)

The voucher specimen KU1 has been deposited at the Herbarium of School of Science, Navrachana University Vadodara.

Specimen identified and verified by

Dr. Karan Rana
Assistant Professor
Division of Biomedical and Life Sciences
School of Science
Navrachana University Vadodara

Vasna-Bhayli Road, Vadodara - 391410, Gujarat, India
Telephone: +91 265 2617000 / 100 e-mail: nuv@nuv.ac.in

Certificate of Plant authentication

1.3 Results

The yield of all plant extracts were calculated after completion of methanolic and aqueous extraction which has been shown in Table 1.3.1 and 1.3.2, *Sv* depicts highest yield in both the extracts with compared to other plants.

Table 1.3.1: Percentage yield of plant extracts (Aqueous)

Sr. No.	Plant extract	Percentage Yields of the plant extracts (%)
1	*Bm* L	4.5 %
2	*Bm* B	5.0 %
3	*Ma* L	2.5%
4	*Ma* B	5.0%
5	*Sa* L	8.0%
6	*Sa* B	5.0%
7	*Sv* L	11.0%
8	*Sv* RF	12.2%
9	*Sv* URF	16.4%

Table 1.3.2: Percentage yield of plant extracts (Methanolic,70% , v/v)		
Sr. No.	**Plant extract**	**Percentage Yields of the plant extracts (%)**
1	*Bm* L	6.6 %
2	*Bm* B	4.0 %
3	*Ma* L	5.0%
4	*Ma* B	5.0%
5	*Sa* L	10.8%
6	*Sa* B	5.0%
7	*Sv* L	12.0%
8	*Sv* RF	13.5%
9	*Sv* URF	15.4%

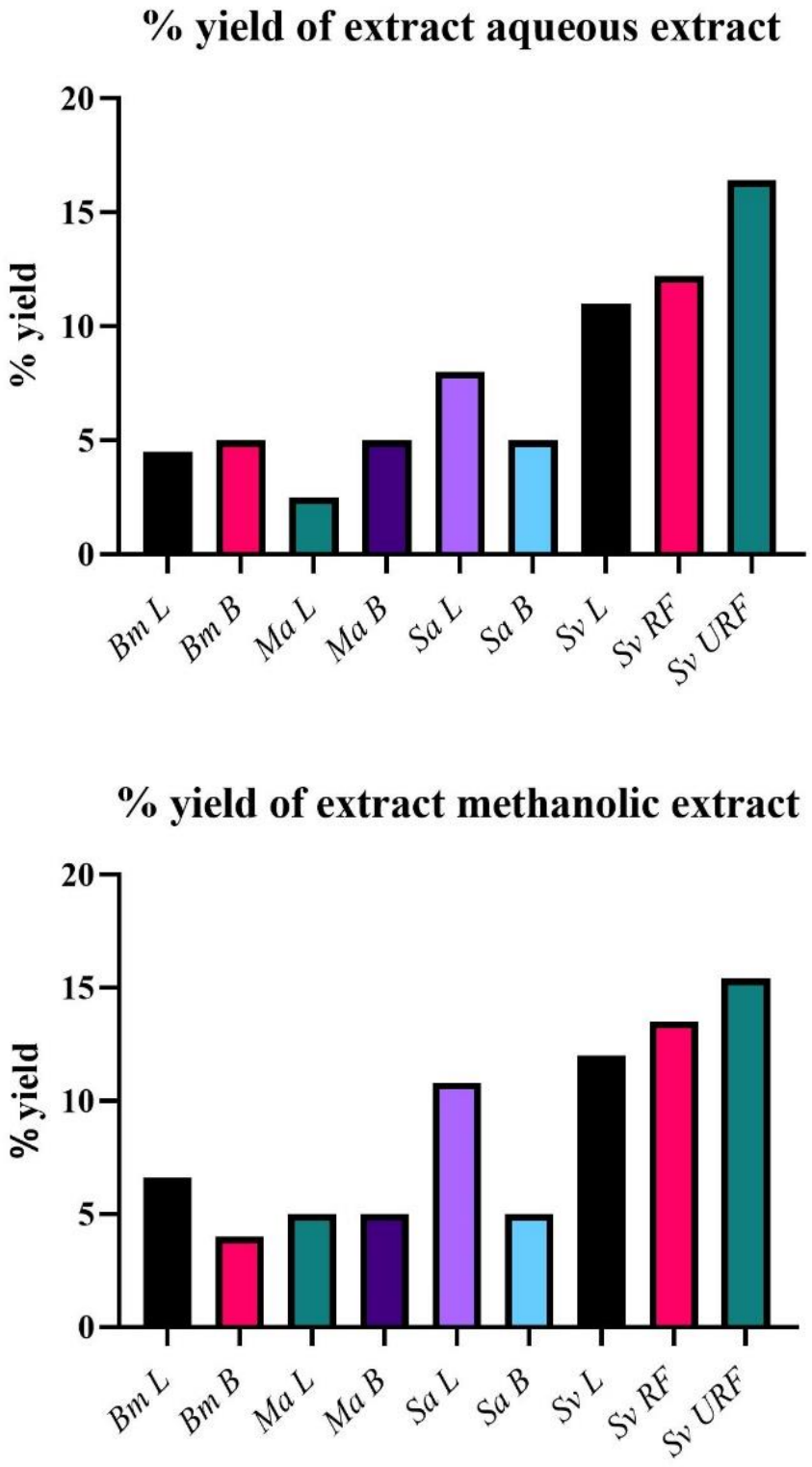

Figure 1.3.1: % Yield of extract: After completion of extraction process, the % yield of aqueous and methanolic extracts were calculated and presented in graph.

Table 1.3.3 (A): Qualitative analysis of selected plant parts : Aqueous extract; *+ : present, - : absent*										
Sr. No.	**Test**	***Bm* L**	***Bm* B**	***Sa* L**	***Sa* B**	***Ma* L**	***Ma* B**	***Sv* leaf**	***Sv* RF**	***Sv* URF**
1	Carbohydrate	+	-	+	+	+	+	+	-	-
2	Reducing sugar	-	-	+	+	+	+	-	-	-
3	Amino acids	-	-	-	-	-	-	-	+	+
4	Alkaloids	-	+	-	-	+	+	-	-	-
5	Tannins	+	+	+	+	+	+	-	-	-
6	Phenolics compounds	+	+	+	+	+	+	+	+	+
7	Steroids	-	-	+	+	+	+	+	+	+
8	glycosides	-	+	-	-	+	-	-	+	+
9	Flavonoids	+	+	+	+	+	+	+	+	+
10	Saponin	+	+	+	+	+	+	+	+	+
11	Quinones	-	-	+	+	-	-	+	+	+
12	Terpenoids	-	+	-	-	+	+	+	+	+
13	Cardiac glycoside	+	-	+	+	+	+	-	+	+
14	Anthocyanine	-	+	+	+	-	-	+	+	+
15	Coumarins	-	-	+	+	-	-	+	+	+
16	Anthraquinones	-	-	+	+	+	+	-	-	-

Table 1.3.3 (B): Qualitative analysis of selected plant parts : Methanolic extract; *+ : present ,- : absent*										
Sr. No.	**Test**	***Bm* L**	***Bm* B**	***Sa* L**	***Sa* B**	***Ma* L**	***Ma* B**	***Sv* leaf**	***Sv* RF**	***Sv* URF**
1	Carbohydrate	+	+	+	+	+	+	-	-	-
2	Reducing sugar	-	-	-	-	-	-	-	-	-
3	Amino acids	-	-	-	-	-	-	-	-	-
4	Alkaloids	+	+	-	-	+	+	+	+	+
5	Tannins	+	+	+	+	+	+	+	+	+
6	Phenolics compounds	+	+	+	+	+	+	+	+	+
7	Steroids	-	-	-	-	+	+	+	+	+
8	glycosides	+	+	-	-	+	+	-	-	+
9	Flavonoids	+	+	+	+	+	+	+	+	+
10	Saponin	+	+	+	+	+	+	+	+	+
11	Quinones	-	-	-	-	-	-	-	-	-
12	Terpenoids	+	+	+	-	+	+	-	-	-
13	Cardiac glycoside	+	-	+	+	+	+	+	+	-
14	Anthocyanine	-	+	+	+	-	-	-	+	+
15	Coumarins	-	-	+	+	-	-	+	+	-
16	Anthraquinones	-	-	+	+	+	+	-	-	-

Table 1.3.4: Total phenol estimation in plant extract through FCR Method, n=3, mean ± SEM				
	Plant	**Plant parts**	**Methanolic extract**	**Aqueous extract**
1.	***Butea monosperma (Bm)***	Leaf	27.61±0.011	21.75±0.013
		Bark	38.87±0.017	97.43±0.015
2.	***Saraca asoca (Sa)***	Leaf	93.82±0.025	127.16±0.002
		Bark	87.52±0.040	107.34±0.023
3.	***Melia azedarach (Ma)***	Leaf	34.09±0.000	14.45±0.007
		Bark	25.90±0.016	26.35±0.005
4.	***Solanum virginianum (Sv)***	Unripe fruit	73.44±0.000	38.0±0.000
		Ripe fruit	92.54±0.020	60.3±0.002
		Leaf	234±0.003	222.9±0.020

Table 1.3.5: Total flavonoid estimation in plant extract ,n=3, mean ± SEM				
	Plant	**Plant parts**	**Methanolic extract**	**Aqueous extract**
1.	***Butea monosperma (Bm)***	Leaf	10.11±0.013	09.54±0.032
		Bark	16.98±0.073	37.33±0.054
2.	***Saraca asoca (Sa)***	Leaf	12.34±0.044	37.64±0.022
		Bark	09.24±0.098	48.41±0.049
3.	***Melia azedarach (Ma)***	Leaf	6.03±0.10	2.56±0.078
		Bark	7.89±0.078	6.45±0.012
4.	***Solanum virginianum (Sv)***	Unripe fruit	13.6±1.09	12.12±0.30
		Ripe fruit	16.36±1.05	9.09±0
		Leaf	80±1.17	66.6±1.09

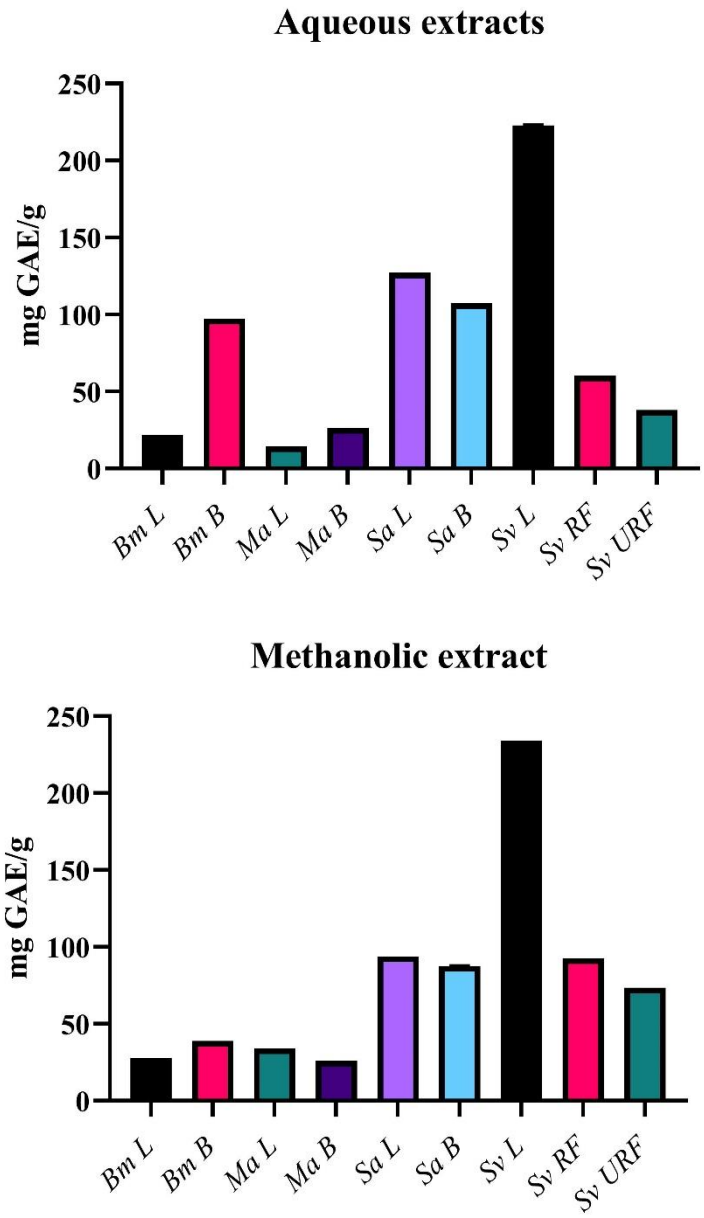

Figure 1.3.2: Total phenol estimation: Each bar represents the total phenol estimation, performed using FCR method. Gallic acid was used as a standard and calculation were done for all aqueous and methanolic extracts.

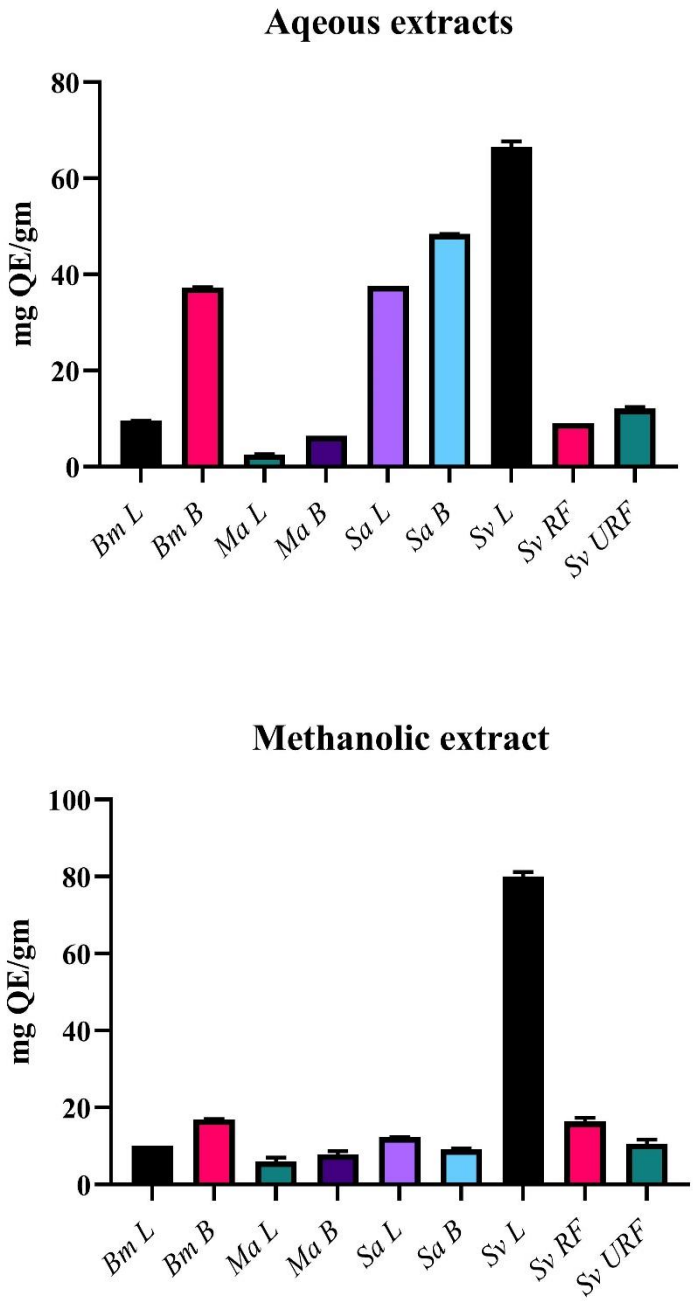

Figure 1.3.3: Total flavonoid estimation : Each bar represent total flavonoid for plant extracts, performed using Aluminum chloride method. For aqueous and methanolic extracts, the total flavonoid was calculated using quercetin as a standard.

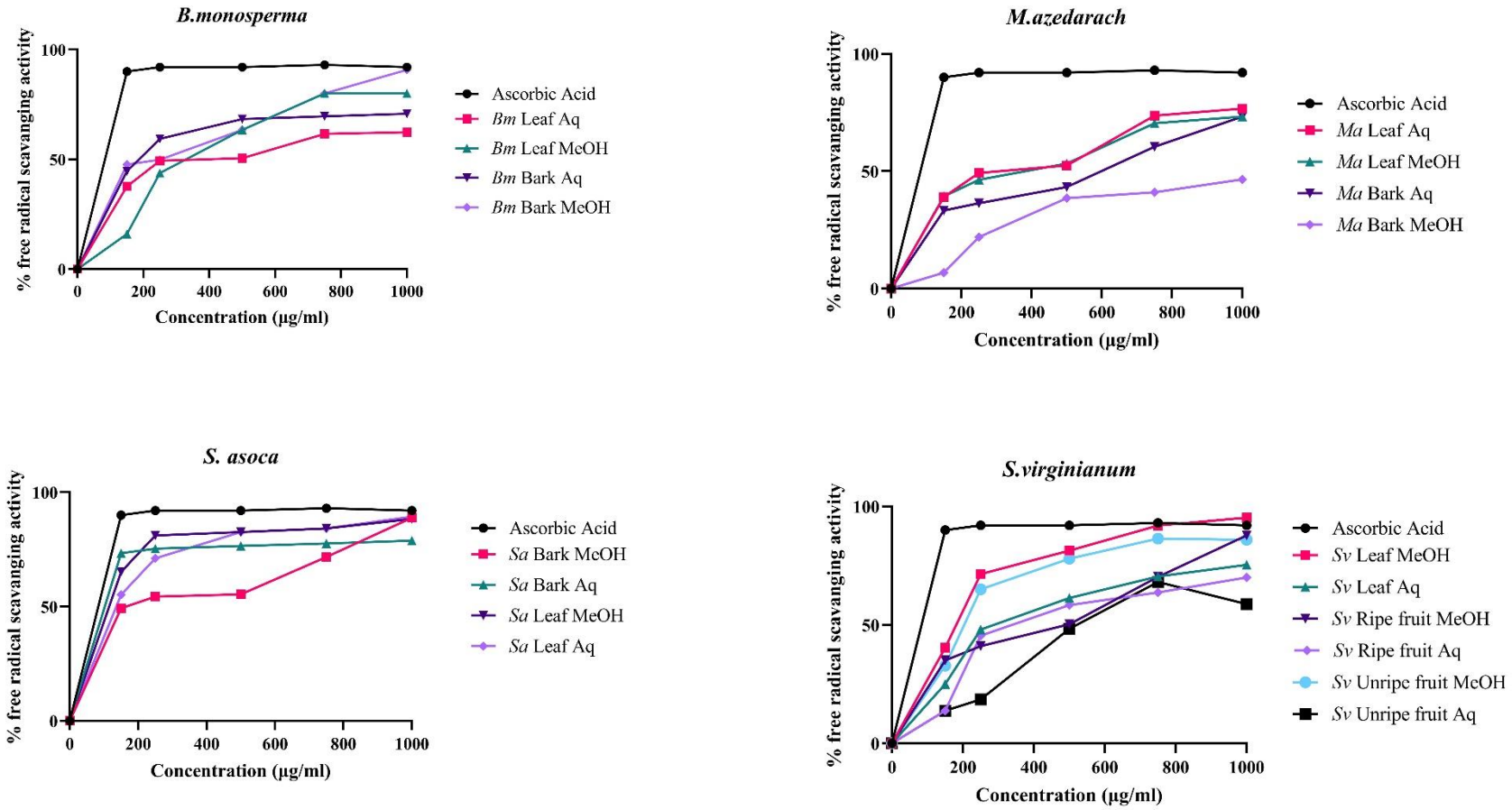

Figure 1.3.4: Anti-oxidant assay: The anti-oxidant properties of plant extracts were evaluated by performing DPPH assay. This assay determines the free radicle scavenging ability of plant extracts. Ascorbic acid was used a standard.

Table 1.3.6: IC_{50} of extracts calculated by performing DPPH assay, n=3, mean ± SEM				
	Plant	**Plant parts**	**Methanolic extract**	**Aqueous extract**
1.	***Butea monosperma (Bm)***	Leaf	303.5±1.02	362.2±5.30
		Bark	392.5±5.23	333.7±1.56
2.	***Saraca asoca (Sa)***	Leaf	207.6±1.23	351.0±1.48
		Bark	348.4±2.36	381.0±8.47
3.	***Melia azedarach (Ma)***	Leaf	553.5±1.56	410.9±9.56
		Bark	294.5±4.56	459.2±4.78
4.	***Solanum virginianum (Sv)***	Unripe fruit	172.6±4.56	423.0±9.45
		Ripe fruit	216.9±2.56	219.9±8.93
		Leaf	166.3±4.23	156.7±1.05
Ascorbic Acid (Standard)			152.5±4.56	

Table 1.3.7: Protein denaturation inhibition activity of plant parts: Inhibition of protein denaturation % for 1 mg/ml, n=3, mean ± SEM, NA: No activity				
	Plant	**Plant parts**	**Methanolic extract**	**Aqueous extract**
1.	***Butea monosperma (Bm)***	Leaf	NA	74.80±0.002
		Bark	22.64±0.097	65.50±0.008
2.	***Saraca asoca (Sa)***	Leaf	NA	NA
		Bark	NA	NA
3.	***Melia azedarach (Ma)***	Leaf	NA	30.00±0.040
		Bark	NA	35.72±0.016
4.	***Solanum virginianum (Sv)***	Unripe fruit	68.38±0.019	78.30±0.008
		Ripe fruit	64.85±0.005	89.55±0.027
		Leaf	86.80±0.004	83.97±0.005

In the phytochemical screening, methanolic and aqueous plant parts extracts underwent qualitative and quantitative analysis of secondary metabolites. Flavonoids, steroids, phenolic

compounds, and saponins were detected in all extracts. However, anthraquinones and reducing sugars were absent in all extracts, while quinones and terpenoids were specifically present in aqueous extracts. Tannins were predominantly found in methanolic extracts. Phenols, flavonoids, and saponins were consistently present across all extracts (Table 1.3.3A and 1.3.3B).

Alkaloids were absent in the leaf and bark extracts of *Sa*, while cardiac glycosides were exclusively observed in the fruit extracts of *Sv*. Quinones were absent in *Sv* and carbohydrates and reducing sugars were not detected in the extracts of unripe fruits (UNRF) and ripe fruits (RF). Amino acids were present in the aqueous extracts of UNRF and RF but absent in the methanolic extracts of UNRF and RF. Cardiac glycosides were absent in all leaf extracts, while anthocyanins were absent only in the methanolic extract of UNRF.

Table 1.3.4 and 1.3.5 depict the TPC and TFC in plant extracts. In *Bm*, aquesous bark extract showed higher phenols (97.43±0.015 mg GAE/g) and flavonoids (37.33±0.054 mg QE/g) when compared to other *Bm* extracts. In the quantitative estimation of phenols and flavonoids, the total phenol content of various plant parts of *Sv* ranged from 38 to 234 mg GAE/g. Notably, *Sv* leaf extract exhibited the highest total phenol content compared to other extracts. Conversely, the methanolic and aqueous extracts of *Sv* UNRF ($p \leq 0.001$) and RF showed significantly lower concentrations of total phenols compared to leaf extracts (Figure 1.3.3).

Furthermore, *Sv* leaf extracts displayed higher total flavonoid content than fruit extracts. Specifically, the methanolic extract of *Sv* leaf exhibited a significantly higher concentration of flavonoids than UNRF ($p \leq 0.01$) and RF ($p \leq 0.001$) extracts (Figure 1.3.4). However, there was no significant difference between the extracts of UNRF and RF. Interestingly, the aqueous

extract of *Sv* RF demonstrated very low total flavonoid content compared to other extracts (Table 1.3.4).

The DPPH test was performed to assess the antioxidant activity of the extracts. The results revealed that the extract of *Melia azedarach* exhibited the highest antioxidant activity compared to *Azadirachta indica* (Nahak & Sahu 2010). Methanolic extracts demonstrated the highest activity. The free radical scavenging potential of all extracts were evaluated perfoming DPPH assay exhibited a dose-dependent trend (Figure 1.3.5). Comparatively, the aqueous extract of *Sv* unripe fruit exhibited lower antioxidant activity than that of ripe fruit and leaf across different concentrations. *Sv* leaf extract demonstrated higher antioxidant activity than other extracts, with significance ($p \leq 0.05$) observed in the case of the aqueous extract but not in the methanolic extract when compared with the standard ascorbic acid (Table 1.3.6).

Both preliminary phytochemical studies and antioxidant activity assays determined that *Sv* leaf extracts surpassed all other plant parts in terms of activity. The leaf extract exhibited the highest antioxidant activity among the various extracts tested. This superior activity could be attributed to the leaf extract's significantly higher total phenolics and flavonoid content than other extracts. These phenolic and flavonoid compounds likely play a crucial role in enhancing the antioxidant potential of *Solanum virginianum* extracts.

The anti-inflammatory activity determination was performed through denaturation capacity of extract using egg albumin method.Minimal activity was observed in *Bm* bark MeOH extracts, registering at 22.64±0.097%. Aspirin served as the standard, displaying 90.16±0.030% inhibitory activity.Among all extracts, those from Sa did not exhibit protein denaturation inhibition activity, whereas *Sv* leaf extracts demonstrated the maximum inhibition activity of 86.80±0.002% for methanolic extract and 83.97±0.003% for aqueous extract (Table

1.3.7). In the case of *Sv* RF extracts, there was no significant difference in inhibition activity, while in *Sv* UNRF, the aqueous extract showed higher activity than the methanolic extract.

1.4 Discussion

The distribution of phenolics and flavonoids are extensive throughout the plant kingdom, making them the predominant secondary metabolites found in plants (Anullika et al.,2016). Flavonoids are a diverse category of secondary metabolites that are classified as phenolic chemicals and are found throughout plants. Plant polyphenols have garnered growing interest because of their powerful antioxidant qualities and significant role in preventing cancer (Hussain et al.,2019). In recent years, there has been a significant focus on the discovery and advancement of phenolic compounds or extracts derived from various plant sources within the realm of health and medical research (Dai, 2010;Sun and Shahrajabian,2023). The choice of solvent has a significant impact on both the quantity and the rate of polyphenol extraction (Xu.,2007; Osorio-Tobón 2020). In present study water is selected being a universal solvent and also it is non toxic to cells and methanol is considered based on having all phytocompouds extracts while performing hot extraction (Truong et al.,2019). Previous studies support that methanol provides more efficacy in the extraction of polyphenols with lower molecular weights (Prior et al.,2001).

In present study, *Ma* methanolic extracts were showing higher TPC and TFC with compare to aquous extracts.In same way, *Sv* plant part methanolic extracts were showing higher TPC and TFC compare to aquous extracts.

The antioxidant activity of methanolic extracts from the leaves and bark of *Bm* demonstrates a dose-dependent response. Previous studies have highlighted significant antioxidant properties in acetone and methanol extraction extracts of *Bm* (Salar et al.,2011;

Antony and Farid,2022). In the present study,a strong correlation between phenolic content and antioxidant activity has been observed in all extracts, as reported by Salar et al. (2011).

This chapter summarised that the plants were selected based on their folklore and medicinal significance. Careful consideration was given to ensure diverse species with rich cultural and healing value. This meticulous selection aimed to conserve traditional knowledge and exploit the therapeutic potential of these plants for various purposes. While the anticancer potential of these plants remains underexplored, their historical use in traditional medicine suggests promising anticancer properties. Further studies is required to investigate and authenticate these potential benefits, potentially paving the way for developing novel treatments or therapies explained in remaining chapters.

According to the studies performed in this chapter,can be summarized that,in case of overall screening of four selected plant extracts, Sv leaf extract stands out as a promising natural anti-oxidant,boasting a high concentration of phenols and flavonoids compared to other extracts.