



# PPHARMACOLOGICAL EVALUATION AND ANTI CANCEROUS PROPERTIES FOUND IN NORTHERN INDIA IN HIBISCUS ROSA SINENSIS, WITHANIA SOMNIFERA, MENTHA PIPERITA, AND MURRAYA. KOENIGII

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## Abstract

Cancer continues to be a global health challenge, necessitating the search for effective and accessible treatment options. In this context, traditional medicinal plants have garnered significant attention due to their potential therapeutic properties. As the environment play humongous role in the genetic makeover of an individual be it be flora or fauna. Within same species of plants a specific phytochemical can be present or absent which is different in different region of the world. In the same manner drugs originated from the people's native area where they are living will suit them better as compare to those originated from elsewhere. In future, medication will not be provided just randomly that single medicine for a particular disorder for whole population but personalized medications will be provided which will suit only that particular individual having that particular genome. The present abstract focuses on the pharmacological evaluation and anticancer potential of four prominent plant species native to Northern India: *Hibiscus rosa sinensis*, *Withania somnifera*, *Mentha piperita*, and *Murraya koenigii*. Hence these kind of study and collection of data is crucial in order to bring novel drugs into the market (drug discovery and development).The selected medicinal plants from Northern India exhibit promising pharmacological profiles with notable anticancer properties. While further research, including clinical trials, is essential to validate their efficacy and safety in human subjects, these plants hold significant promise as potential sources of novel anticancer agents and supportive therapies. The exploration of traditional medicinal knowledge alongside modern scientific techniques presents a valuable avenue for advancing cancer treatment strategies.

**Keywords:** Plant extraction, Phytochemicals, UV-VIS spectrophotometer, TLC, Phytochemical screening, Pharmacological activities, Antioxidant activity.

## Introduction

Cancer is a hereditary illness that causes uncontrolled cell division. It is caused by gene alterations that enhance the rate of cell proliferation while decreasing apoptosis (Erenpreisa and Cragg, 2007). Despite the disease's high notoriety, treating it has been an uphill battle with only somewhat effective results. The main drawbacks of chemotherapy include cancer recurrence, drug resistance, and harmful effects on tissues that aren't being treated, which might limit the use of anticancer medications and reduce patient quality of life. Plant origin medicines/products have been utilized since prehistoric era among African and Asian population for the treatment of various maladies which includes illnesses like diabetes, cancer, infectious

one and others (Syam et al., 2011). According to WHO (world health organisation) some of the developing nation still have dependency on plant kingdom for medicines and they make use of natural compounds for treating purposes (Rajeswara Rao et al., 2007). About 47% of anti-infectious and anti-carcinogenic drugs available in market are originated naturally under clinical trials and these numbers of drugs are raising continuously (Newman et al., 2012). Plants produce secondary metabolites such as alkaloids, flavonoids, phenolic compounds, saponins, tannins and terpenoids are primarily responsible for having anti-cancerous activities which will ultimately lead to the development of new therapeutics. Larger amount of anticancer drugs are originated by plants as it is believed that these natural compounds are safer as there is no side effects, widely available, effective, tolerable, low cost and it is of great source of research and development of new chemotherapeutics (Gullett et al., 2010), (Cragg G. M et al., 2005). More than 30% of all pharmaceuticals (and their analogues and derivatives) on the market today are derived from plants, and natural goods will continue to have a significant influence on human medicine. The majority of synthetic bioactive substances share structural similarities with the plant phytochemicals from which they were initially extracted (Sevindik et al., 2018). Plant-based medicines play a significant part in the prevention and treatment of illness in many underdeveloped nations (Salehi et al., 2018). The therapeutic effects of plants are largely attributed to phytochemical compounds. Among the most significant medications with a herbal origin are immunomodulators, antibiotics, anti-inflammatory pharmaceuticals, and anti-cancer medications (Petrovska et al., 2012).

Medicinal plants are a term used to describe herbal remedies that have been employed for treating various human ailments (Xiao et al., 2016, Farzaei et al., 2016). These healing plants can be viewed as a rich source of components for the creation of pharmaceuticals (Jaberian et al., 2013). Over the past two decades, the anticancer properties of natural polyphenols have attracted significant attention in various research laboratories; the most common cancers among them were lung, colorectal, liver, breast, and stomach (WHO). Natural polyphenols' anticancer properties have gained attention in several labs during the past 20 years. Polyphenols are possible candidates for the development of anticancer medications in the meanwhile. Compounds with at least one aromatic ring and one or more hydroxyl functional groups are referred to as polyphenols. Natural polyphenols are a broad category of secondary metabolites found in plants, ranging in size from tiny molecules to highly polymerized substances (Manach et al., 2011).

For this investigation, we used herbs with anticancer qualities such *Murraya koenigii*, *Withania somnifera*, *Mentha piperita*, and *Hibiscus rosa sinensis*. Along with anti-cancer properties *Hibiscus rosa-sinensis* also possess anti-inflammatory properties, antipyretic and analgesic effects, anti-diabetes activities and many other pharmacological activities i.e., the anti-nociceptive (acetic acid-induced writhing response and tail flick technique) and anti-inflammatory (carrageenin and dextran produced rat paw edema) effects of the methanolic extract of *Hibiscus rosa-sinensis* leaves (250 and 500 mg/kg bw, orally) were investigated. Roots of *withania somnifera* are used in the treatment of asthma, bronchitis, leucoderma, tuberculosis, liver problems, heart disorders, and arthritis and act as an antibacterial, antitumor, antioxidant, immunomodulatory, and neurotic regenerator and show adaptogenic activity, nootropic effect, hypothyroid activity, herbicidal potential, abortifacient astringent, aphrodisiac, and emmenagogue (Javaid A et al., 2010), (Pant M et al., 2012). Leaves of *withania somnifera* are used in the treatment of ulcers, painful swelling, external pains, syphilis, hemorrhoids, eyesores, boils, and edema and act as aphrodisiac, anti-inflammatory, diuretic, hepatoprotective, anti-arthritic, anti-cancerous, and pesticidal (Gupta M et al., 2016), (Gupta A et al., 2014), (Dalzell NA et al., 1861). Numerous research have demonstrated that mentha species' components with cytotoxic effects may be used to develop anticancer medicines. For instance, *Mentha arvensis*, *Mentha longifolia*, *Mentha spicata*, and *Mentha viridis* methanolic and aqueous extracts showed anti-proliferated results in vitro when compared to the appearances of numerous cancer cells at an absorption of 100 g/mL (Al-Okbi et al., 2015). Potential secondary metabolites from *M. koenigii* might be used to create anticancer drugs. Three extracts of *M. koenigii* leaves—hexane, ethyl acetate, and methanol—were tested for their ability to kill HeLa cell lines in one research. In HeLa cancer cells, the extracts were observed to have a strong cytotoxic effect. These outcomes demonstrated the in vitro antitumor activity of *M. koenigii* (Amna et al., 2019)

## Materials and Methods

### 1. Collection of Plant material:

Fresh leaves of all the plant varieties were collected locally from herbarium garden of IIMT University, Meerut, U.P, India.

The collected plant materials were further identified. Fresh leaves were well washed to get rid of all the dirt, then dried at room temperature in the shade (air dried, no sunshine) for about 3 to 5 days, or until they reached a steady weight. After that the dried leaves were ground to fine powder. The ground materials were stored in a labelled sealed plastic bag in a cold room (15-20°C) until use.

### 2. Preparation of 70% Ethanolic Extract

The powdered leaves of all the plant varieties of about 10 gram were immersed in 100ml of 70% ethanol and kept at room temperature for at least 72 hours. The extracts were double filtered through whatman no.1 filter paper. It is thereafter stored in a refrigerator at 4°C until used.

### 3. Preparation of 90% Methanolic Extract

Same while leaves were immersed in 100ml of 90% methanol and kept at room temperature for at least 72 hours. The extracts were double filtered through whatman no. 1 filter paper. It is thereafter stored in a refrigerator at 4°C until used.

The extracts were used for phytochemical screening and analysis using Thin layer chromatography and UV-VIS spectrophotometer techniques.

#### 3.1. Phytochemical Tests

Phytochemicals are the chemicals that have a plant-based origin; these are also known as secondary metabolic compounds. It is the qualitative tests that are often carried out to show the presence of a plethora of phytochemicals in plant extract.

The ethanolic (70%) and methanolic (90%) extracts from each of the selected plant species were analyzed for saponins, steroid, tannins, flavonoids, terpenoids, inulin, alkaloids (Wagner reagents), phenols, phlobatannins, and naphthaquinone.

#### Procedure

##### 3.1.1 Test for Saponin (Frothing test).

One millilitre (1 ml) plant extract was mixed with 3 ml of distilled water in a test tube. The test-tube was then shaken vigorously for 2 minutes. Formation of honeycomb foam persist on warming indicates the presence of saponins in the plant extract.

##### 3.1.2. Test for Steroids (Salkowski test).

1ml of the plant extract was dissolved in 2-3ml of chloroform ( $\text{CHCl}_3$ ), equal volume of concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ) is then added carefully along side of the test tube to form two layers. Upper layer of the solution turns into the red colour and the sulphuric acid layer showed yellow green fluorescence that indicates the presence of steroids in the plant extract.

##### 3.1.3. Test for Tannins.

2 ml of plant extract was allowed to react with few drops of ferric chloride ( $\text{FeCl}_3$ ). Formation of blue or brownish greenish colour of the solution indicates the presence of the tannins in the plant extract.

##### 3.1.4. Test for Flavonoid (Sodium hydroxide test).

1 ml plant extract was treated with 2 ml of 10% sodium hydroxide solution. Formation of intense yellow colour which becomes colourless on the addition of 1% HCl, indicates the presence of flavonoids.

### 3.1.5. Test for Terpenoids.

1 ml of plant extract was mixed in 2 ml of chloroform ( $\text{CHCl}_3$ ) and 2-3 ml concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ) was carefully added along side of test tube and two layers were formed. Reddish-violet colour was formed that indicates the presence of terpenoids in the plant extract.

### 3.1.6. Test for Napthaquinone (Dam-Karrer test).

Take 1 ml of plant extract and add few drops of 10% KOH, formation of the blue-black colour indicates the presence of napthaquinone in the plant extract.

### 3.1.7. Test for Phenols.

In 1 ml of plant extract add 5 ml of distilled water then add 1-2 drops of 1%  $\text{FeCl}_3$  red-green or purple colour indicates the presence of phenols in the plant extract.

### 3.1.8. Test for Inulin.

In 1 ml of plant extract, alpha naphthol and sulphuric acid ( $\text{H}_2\text{SO}_4$ ) solution was added. Formation of brownish red colour indicates the presence of inulin in the plant extract.

### 3.1.9. Test for alkaloids (Wagner's reagent).

In 2 ml of plant extract, 1 ml of wagner reagent is added. Formation of brown and reddish-brown colour was indicative the presence of alkaloids in the plant extract.

### 3.1.10. Test for Phlobatannins (HCl test).

1 ml of plant extract was boiled with 2% HCl solution and observed for red precipitate that was indicative the presence of phlobatannins in the plant extract.

## 4. Thin Layer Chromatography (TLC)

The primary non-volatile chemicals are separated using the fundamental chromatographic technique known as thin layer chromatography (TLC). The most practical method for producing the plant extract for TLC investigations is to extract the plant material using an alcohol and water combination, such as 70% ethanol and 90% methanol (Khurram *et al.*, 2009). Utilizing TLC, the solvent has a significant impact on the separation of the active phytochemicals. The solvent solution utilized has a significant impact on how the active chemicals migrate and the spot that is produced. Spots are produced as a result of the experiments. The dots are seen via a variety of chemical operations and light imprinting techniques. These spots are quantified by the retention factor (Rf).

### 4.1. Steps for TLC plates preparation:

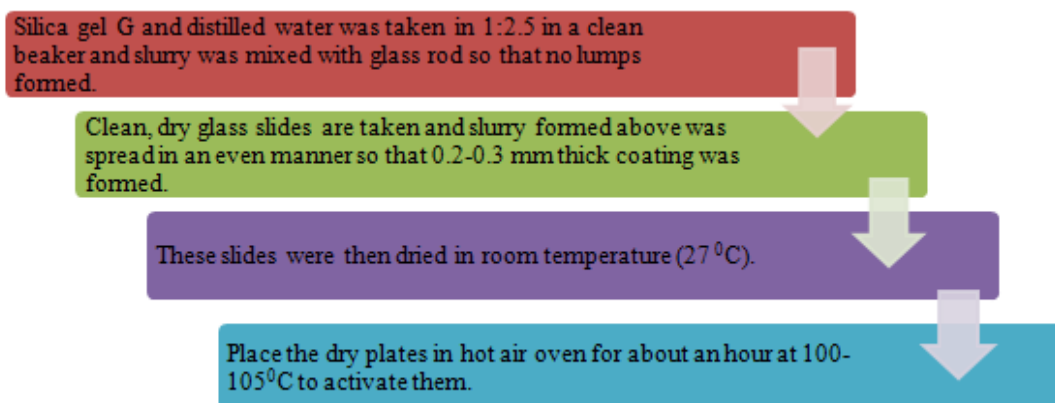


Figure I: Stepwise process of TLC plate preparation

70 % Ethanolic and 90 % methanolic extract of each plant sample was subjected to TLC studies. TLC plate coated with silica gel G. The plate was then marked with the pencil softly 1.5 cm far from the bottom. The material was placed on the TLC plate using a micropipette on the pencil outlined bottom line. The plate was then dried in the fume hood before loading the sample again until a dark spot was achieved. Then the solvent Chloroform: Ethanol (9:1) about 20ml was taken in the chamber. The plate was placed in the chamber cover the top with silver paper. After the run, plates were dried and then used to detect the spots is done.

### Detection of the spot

All of the plates were dried before detecting the spots with a UV transilluminator at 254nm and 365nm. The retention factor (Rf) quantified the mobility of the active substance.

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

### 5. UV-VIS Spectrophotometer

UV-VIS Spectrophotometric analysis was conducted on the ethanolic and methanolic extract of the selected plant species using UV-VIS Spectrophotometer double beam (Shimadzu UV-1800 UV scanning spectrophotometer). The extract was examined under visible and UV light in the wavelength ranging from 200-600 nm for proximate analysis. For UV-VIS spectrophotometer analysis, the filtered extracts (samples) were further diluted to 1:10 with the same solvent. The peaks values were then recorded.

### 6. Result

The result of all phytochemical test was listed below in the Table no. 1

Phytochemical tests	70% Ethanolic extract				90% Methanolic extract			
	<i>Hibiscus rosa sinensis</i>	<i>Withania somnifera</i>	<i>Mentha piperita</i>	<i>Murraya koenigii</i>	<i>Hibiscus rosa sinensis</i>	<i>Withania somnifera</i>	<i>Mentha piperita</i>	<i>Murraya koenigii</i>
Saponin	+	+	+	-	-	-	-	-
Steroid	+	+	+	+	-	-	-	-
Tannin	+	+	+	+	-	-	-	-
Flavonoid	+	+	+	+	-	-	-	-
Terepenoid	+	+	+	+	-	-	-	-
Napthaquione	-	-	-	-	-	-	-	-
Phenol	+	-	-	-	-	-	-	-
Inulin	-	-	-	-	-	-	-	-
Alkaloids	+	+	+	+	-	-	-	-
Phlobatannin	+	+	-	-	-	-	-	-

Table Number 1: Depicts all the phytochemical analysis



### 6.1. Thin Layer chromatography

Thin layer chromatography was performed serially on all plant Species. They are as follows.

#### 6.2. Thin layer Chromatography of *Hibiscus rosa sinensis*

The TLC study of the 70 % ethanolic extract of *Hibiscus rosa sinensis* using a solvent system of chloroform: methanol (9:1) resulted in the visualization of five spots. The Rf (retention factor) values were determined for each spot at both 365 nm and 254 nm wavelengths. The Rf values obtained for the five spots were as follows: Spot 1: Rf value of 0.27, Spot 2: Rf value of 0.44, Spot 3: Rf value of 0.65, Spot 4: Rf value of 0.8, Spot 5: Rf value of 0.98.

The TLC study of the 90 % methanolic extract of *Hibiscus rosa sinensis* using a solvent system of chloroform: methanol (9:1) resulted in the visualization of four spots. The Rf (retention factor) values were determined for each spot at both 365 nm and 254 nm wavelengths.

The Rf values obtained for the four spots were as follows: Spot 1: Rf value of 0.35, Spot 2: Rf value of 0.62, Spot 3: Rf value of 0.9, Spot 4: Rf value of 1.

#### 6.2. Thin layer Chromatography of *Withania somnifera*

The TLC study of the 70 % ethanolic extract of *Withania somnifera* using a solvent system of chloroform: methanol (9:1) resulted in the visualization of two spots. The Rf (retention factor) values were determined for each spot at both 365 nm and 254 nm wavelengths.

The Rf values obtained for the two spots were as follows: Spot 1: Rf value of 0.31, Spot 2: Rf value of 0.75.

The TLC study of the 90% methanolic extract of *Withania somnifera* using a solvent system of chloroform: methanol (9:1) resulted in the visualization of three spots. The Rf (retention factor) values were determined for each spot at both 365 nm and 254 nm wavelengths.

The Rf values obtained for the four spots were as follows: Spot 1: Rf value of 0.36, Spot 2: Rf value of 0.66, Spot 3: Rf value of 0.92.

#### Thin layer Chromatography of *Mentha piperita*

The TLC study of the 70 % ethanolic extract of *Mentha piperita* using a solvent system of chloroform: methanol (9:1) resulted in the visualization of three spots. The Rf (retention factor) values were determined for each spot at both 365 nm and 254 nm wavelengths.

The Rf values obtained for the three spots were as follows: Spot 1: Rf value of 0.28, Spot 2: Rf value of 0.4, Spot 3: Rf value of 0.62.

The TLC study of the 90% methanolic extract of *Mentha piperita* using a solvent system of chloroform: methanol (9:1) resulted in the visualization of three spots. The Rf (retention factor) values were determined for each spot at both 365 nm and 254 nm wavelengths.

The Rf values obtained for the one spot at 365 nm was spot 1: Rf value of 0.85 and at 254 nm three spots were as follows: Spot 1: Rf value of 0.45, Spot 2: Rf value of 0.65, Spot 3: Rf value of 0.85.

#### 6.3. Thin layer Chromatography of *Murraya koenigii*

The TLC study of the 70 % ethanolic extract of *Murraya koenigii* using a solvent system of chloroform: methanol (9:1) resulted in the visualization of three spots. The Rf (retention factor) values were determined for each spot at both 365 nm and 254 nm wavelengths.

The Rf values obtained for the three spots were as follows: Spot 1: Rf value of 0.0311, Spot 2: Rf value of 0.45, Spot 3: Rf value of 0.60.

The TLC study of the 90 % methanolic extract of *Murraya koenigii* using a solvent system of chloroform: methanol (9:1) resulted in the visualization of four spots. The Rf (retention factor) values were determined for each spot at both 365 nm and 254 nm wavelengths.

The Rf values obtained for the four spots at 365 nm were as follows: Spot 1: Rf value of 0.1, Spot 2: Rf value of 0.21, Spot 3: Rf value of 0.31 and Spot 4: Rf value of 0.81. The Rf values obtained for the five spots at 254 nm were as follows: Spot 1: Rf value of 0.1, Spot 2: Rf value of 0.21, Spot 3: Rf value of 0.23, Spot 4: Rf value of 0.31, and Spot 5: Rf value of 0.81.

## 7. UV-VISIBLE SPECTROPHOTOMETER

The UV Visible Spectrophotometer of 70 % ethanolic and 90 % methanolic extract was performed serially on all plant species. They are as follows.

### 7.1. *Hibiscus rosa sinensis*

The UV-VIS analysis of the 70 % ethanolic extract of *Hibiscus rosa sinensis* leaves revealed several peaks in the wavelength range of 200 to 600 nm. These peaks correspond to specific absorption bands and can provide information about the presence of certain functional groups and chromophores in the extract.

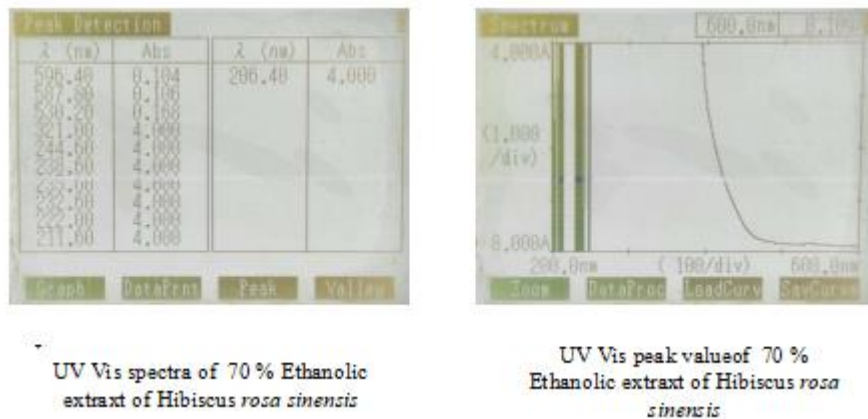


Figure number 2: UV Vis spectra and peak value of 70 % Ethanolic extract

The qualitative UV-VIS profile of 70 % ethanolic extract of *Hibiscus rosa sinensis* was taken at wavelength of 200 nm to 600 nm. The profile showed the peaks at 596, 587 and 530 nm with the absorption 0.104, 0.106 and 0.168 respectively. Figure number 2 shows the absorption spectrum *Hibiscus rosa sinensis* extract and these are almost transparent in the wavelength region of 200-600 nm. In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400 nm is a clear indication of the presence of the unsaturated groups and heteroatoms such as S, N, O. The *Hibiscus rosa sinensis* extract spectrum shows peaks at positions 321, 244, 238, 235, 232, 222, 211, 206 nm which confirms presence of organic chromophores.

The UV-VIS analysis was conducted on the 90 % methanolic extract of *Hibiscus rosa sinensis* to identify the phytoconstituents present in the extract. The UV-VIS spectra were used to detect compounds containing sigma-bond, pi-bond, lone pair of electrons, chromophores, and aromatic rings.

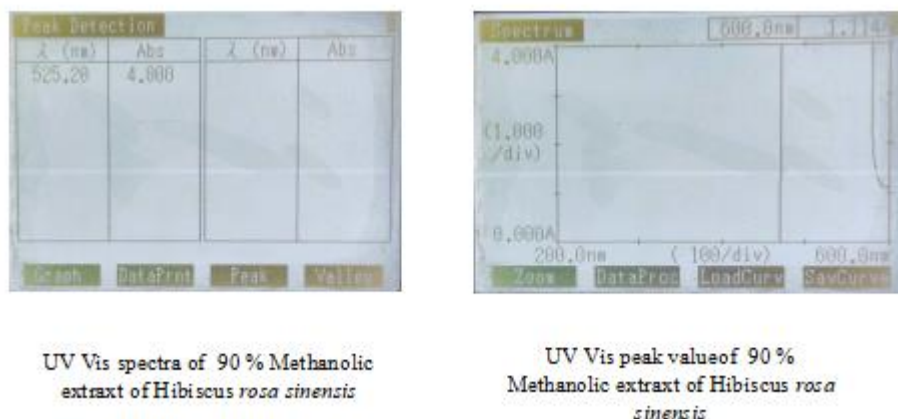


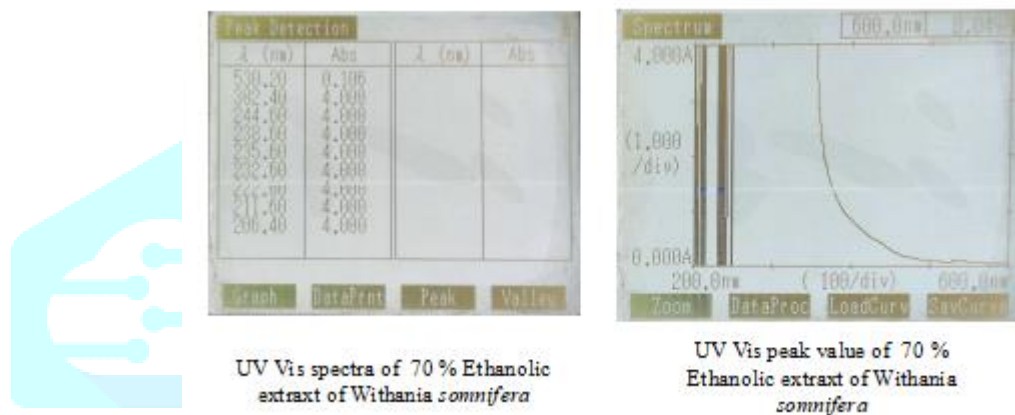
Figure number 3: UV Vis spectra and peak value of 90 % Methanolic extract

The qualitative UV-VIS profile of the methanolic extract of *Hibiscus rosa sinensis* was obtained by measuring the absorbance at wavelengths ranging from 200 nm to 600 nm. The profile displayed peaks at 525 nm with an absorption of 4A. Figure number 3 illustrates the absorption spectrum of the *Hibiscus rosa sinensis* extract. In UV-VIS spectra, the appearance of one or more peaks in the region between 200 nm and 400 nm is typically indicative of the presence of unsaturated groups and heteroatoms such as S, N, and O. However, in the case of the *Hibiscus rosa sinensis* extract, no peaks were observed in this region, suggesting the absence of organic chromophores.

It is important to note that the absence of peaks in the UV-VIS spectra does not necessarily imply the absence of phytoconstituents in the extract. Other compounds may be present that do not exhibit strong absorbance in the measured wavelength range or that do not possess the specific functional groups detected by the UV-VIS analysis.

## 7.2. *Withania somnifera*

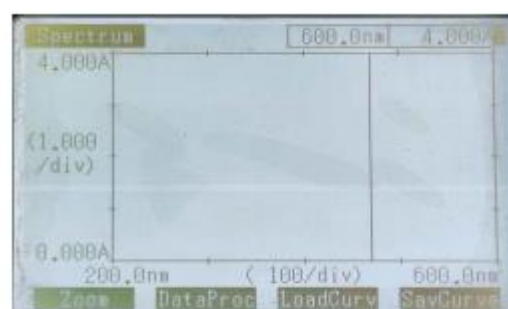
The UV-VIS analysis of the 70 % ethanolic extract of *Withania somnifera* leaves revealed several peaks in the wavelength range of 200 to 600 nm. These peaks correspond to specific absorption bands and can provide information about the presence of certain functional groups and chromophores in the extract.



**Figure number 4: UV Vis spectra and peak value of 70 % Ethanolic extract**

The qualitative UV-VIS profile of ethanolic extract of *Withania somnifera* was taken at wavelength of 200 nm to 600 nm. The profile showed the peaks at 530 nm with the absorption 0.106. Figure number 4 shows the absorption spectrum of *Withania somnifera* extract and these are almost transparent in the wavelength region of 200-600 nm. In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400 nm is a clear indication of the presence of the unsaturated groups and heteroatoms such as S, N, O. The *Withania somnifera* extract spectrum shows peaks at positions 302, 244, 238, 235, 232, 222, 211, 206 nm which confirms presence of organic chromophores.

The UV-VIS analysis was conducted on the 90 % methanolic extract of *Withania somnifera* to identify the phytoconstituents present in the extract. The UV-VIS spectra were used to detect compounds containing sigma-bond, pi-bond, lone pair of electrons, chromophores, and aromatic rings.



**Figure number 5: UV Vis spectra of 90 % Methanolic extract**

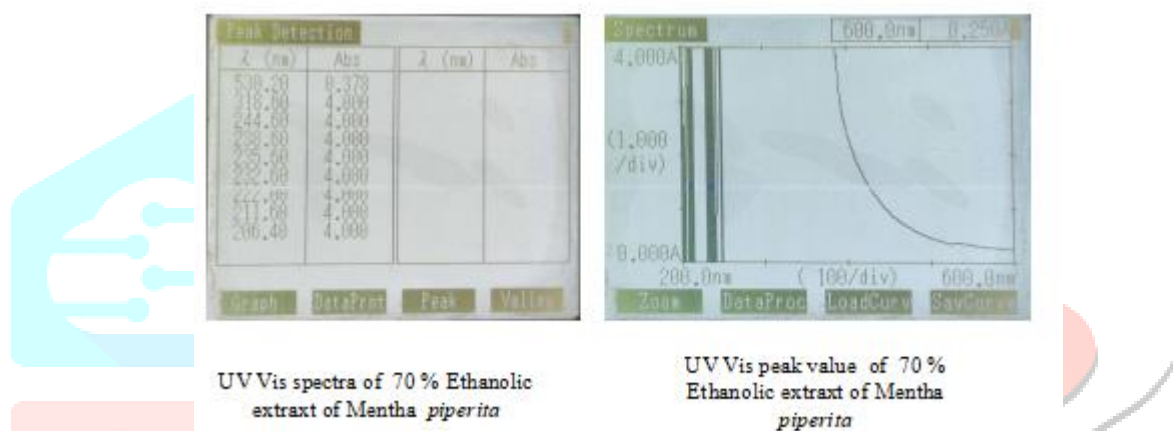


The qualitative UV-VIS profile of the methanolic extract of *Withania somnifera* was obtained by measuring the absorbance at wavelengths ranging from 200 nm to 600 nm. However, no peaks were observed in the profile. Figure number 5 displays the absorption spectrum of the *Withania somnifera* extract. In UV-VIS spectra, the appearance of one or more peaks in the region between 200 nm and 400 nm is typically indicative of the presence of unsaturated groups and heteroatoms such as S, N, and O. However, in the case of the *Withania somnifera* extract, no peaks were observed in this region, suggesting the absence of organic chromophores.

It is important to note that the absence of peaks in the UV-VIS spectra does not necessarily imply the absence of phytoconstituents in the extract. Other compounds may be present that do not exhibit strong absorbance in the measured wavelength range or that do not possess the specific functional groups detected by the UV-VIS analysis.

### 7.3. *Mentha piperita*

The UV-VIS analysis of the 70% ethanolic extract of *Mentha piperita* leaves revealed several peaks in the wavelength range of 200 to 600 nm. These peaks correspond to specific absorption bands and can provide information about the presence of certain functional groups and chromophores in the extract.



**Figure number 6: UV Vis spectra and peak value of 70 % Ethanolic extract**

The qualitative UV-VIS profile of ethanolic extract of *Mentha piperita* was taken at wavelength of 200 nm to 600 nm. The profile showed the peaks at 530 nm with the absorption 0.378. Figure number 6 shows the absorption spectrum of *Mentha piperita* extract and these are almost transparent in the wavelength region of 200-600 nm. Absorption bands observed.

In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400 nm is a clear indication of the presence of the unsaturated groups and heteroatoms such as S, N, O. The *Mentha piperita* extract spectrum shows peaks at positions 318, 244, 238, 235, 232, 222, 211, 206 nm which confirms presence of organic chromophores.

The UV-VIS analysis was conducted on the methanolic extract of *Mentha piperita* to identify the phytoconstituents present in the extract. The analysis aimed to detect compounds that contain sigma-bond, pi-bond, lone pair of electrons, chromophores, and aromatic rings.

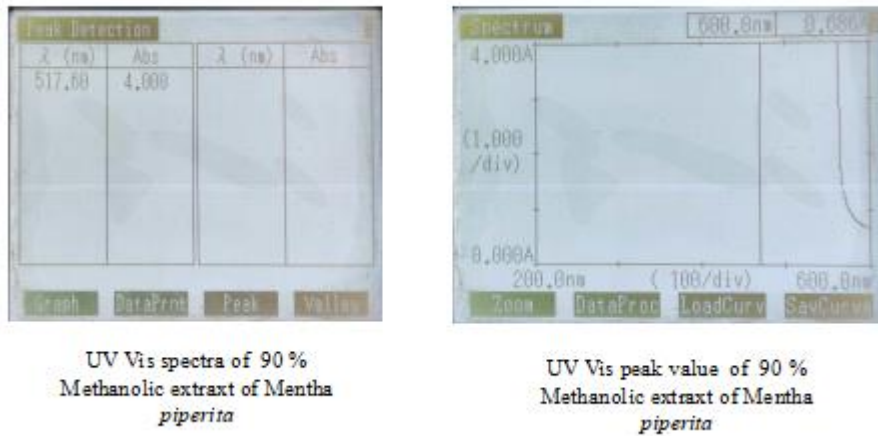


Figure number 7: UV Vis spectra and peak value of 90 % Methanolic extract

#### 7.4. *Murraya koenigii*

The UV-VIS analysis of the ethanolic extract of *Murraya koenigii* leaves revealed several peaks in the wavelength range of 200 to 600 nm. These peaks correspond to specific absorption bands and can provide information about the presence of certain functional groups and chromophores in the extract.

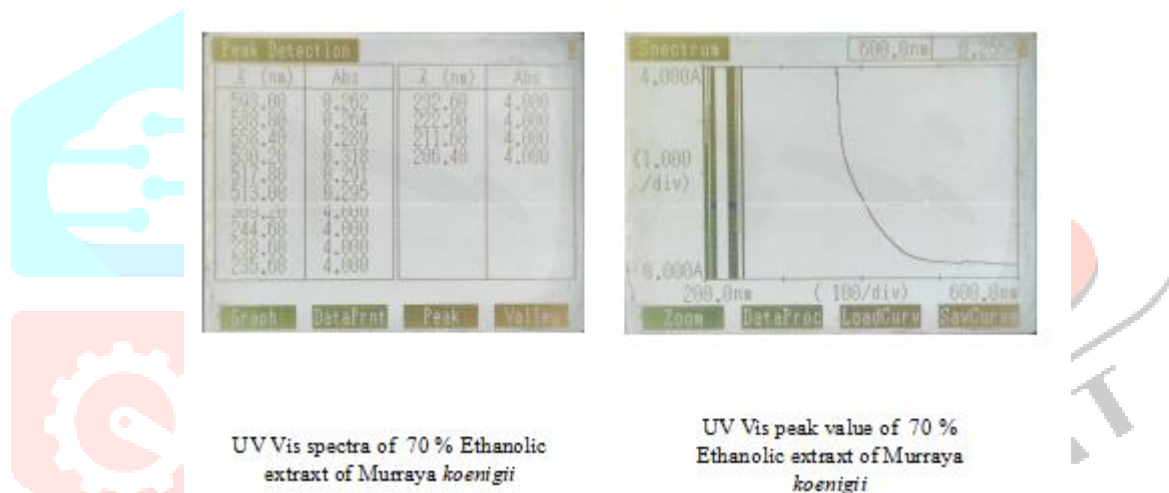
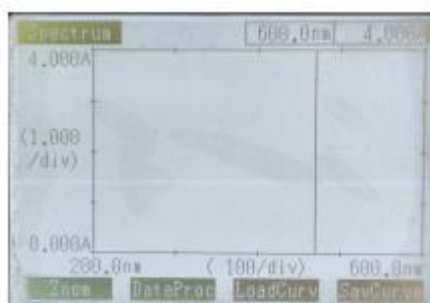


Figure number 8: UV Vis spectra and peak value of 70 % Ethanolic extract

The qualitative UV-VIS profile of ethanolic extract of *Murraya koenigii* was taken at wavelength of 200 nm to 600 nm. The profile showed the peaks at 593, 588, 558, 530, 517 and 513 nm with the absorption 0.262, 0.264, 0.289, 0.318, 0.291 and 0.295 respectively. Figure number 8 shows the absorption spectrum of *Murraya koenigii* extract and these are almost transparent in the wavelength region of 200-600 nm. In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400 nm is a clear indication of the presence of the unsaturated groups and heteroatoms such as S, N, O. The *Murraya koenigii* extract spectrum shows peaks at positions 309, 244, 238, 235, 232, 222, 211, 206 nm which confirms presence of organic chromophores.

The UV-VIS analysis performed for the identification of the phytoconstituents present in the methanolic extract of *Murraya koenigii*. The UV-VIS spectra were performed to identify the compounds containing sigma-bond, pi-bond and lone pair of electrons, chromophores and aromatic rings.



UV Vis spectra of 90 % Methanolic extract of *Murraya koenigii*

### Figure number 9: UV Vis spectra of 90 % Methanolic extract

The qualitative UV-VIS profile of the 90 % methanolic extract of *Murraya koenigii* was obtained by measuring the absorbance at wavelengths ranging from 200 nm to 600 nm. However, in this profile, no distinct peaks were observed. Figure number represents the absorption spectrum of the *M. koenigii* extract. Typically, the appearance of one or more peaks in the region between 200 nm and 400 nm indicates the presence of unsaturated groups and heteroatoms such as S, N, and O. However, in the case of the *Murraya koenigii* extract, no peaks were detected, suggesting the absence of organic chromophores.

It is important to note that the absence of peaks in the UV-VIS spectra does not necessarily imply the absence of phytoconstituents in the extract. Other compounds may be present that do not exhibit strong absorbance in the measured wavelength range or that do not possess the specific functional groups detected by the UV-VIS analysis.

## 8. Discussions

Cancer is a pathological condition in which cells divide uncontrollably and evade programmed cell death. While various treatments such as chemotherapy, hormone therapy, immunotherapy, photodynamic therapy, radiation therapy, targeted therapy, stem cell transplant, surgery, and hyperthermia are available, they often come with a multitude of side effects due to their toxic nature. Consequently, researchers are exploring the use of plants to develop non-toxic medications that can effectively treat cancer and other diseases.

Ayurveda, one of the oldest traditional systems of medicine, holds untapped wisdom that is crucial in today's context. By combining ancient knowledge with modern approaches, we can discover ultimate drugs that not only cure but also minimize side effects, while remaining affordable for all. Therefore, the study of phytochemicals in different medicinal plants across various regions is vital, as climatic conditions significantly influence the genetic makeup of flora and fauna. Phytochemical variations in different regions can serve as valuable data for the development of anti-cancer and other therapeutic drugs.

For instance, when conducting phytochemical screening of ethanolic extracts of *Hibiscus rosa sinensis*, a study (Shilpi Patel *et al.*, 2018) conducted in Indore, Madhya Pradesh reported the absence of saponin, whereas our study found saponin in the ethanolic leaves extract. Similarly, a study (S. Fathaunnisha *et al.*, 2020) in Chennai, Tamil Nadu on *Withania somnifera* showed the absence of alkaloid and the presence of phenol, whereas our study revealed the presence of alkaloid and absence of phenol. In the case of *Mentha piperita*, a study (Sachin *et al.*, 2016) conducted in Jaysingpur, Maharashtra reported the absence of saponin, tannin, and flavonoid, and the presence of phenol, while our study found the presence of saponin, tannin, flavonoids, and the absence of phenol. Furthermore, a study (Rashmi *et al.*, 2016) in Dehradun, Uttarakhand on *Murraya koenigii* indicated the absence of flavonoid and the presence of phenol, while our study showed the presence of flavonoid and absence of phenol.

Regarding the methanolic extract, a study (Udita *et al.*, 2015) in Agra, Uttar Pradesh on *Hibiscus rosa sinensis* reported the presence of saponin and alkaloids, whereas our study found these constituents to be absent. Similarly, a study (Anubha *et al.*, 2013) in Roorkee on *Withania somnifera* reported the presence of alkaloid, saponin, flavonoid, tannin, and terpenoid, which were not found in our study. In the case of *Mentha piperita*, a study (Bipin *et al.*, 2019) conducted in Kathmandu reported the presence of flavonoid, alkaloid, and phenols, whereas our study did not detect these phytochemicals. Additionally, a study (Pujan *et al.*, 2019) in Ahmedabad, Gujarat on *Murraya koenigii* revealed the presence of phenol, flavonoid, saponin, terpenoids, steroid, and tannin, which were absent in our study.

## 9. Conclusion

During phytochemical screening of *Hibiscus rosa sinensis*, *Withania somnifera*, *Mentha piperita*, and *Murraya koenigii* in northern India, it was observed that the 70% ethanolic extract contained a wide range of phytochemicals such as tannin, phenol, alkaloid, terpenoid, steroid, saponin, flavonoid, and phlobatannins. However, none of these phytochemicals were found in the 90% methanolic extract of the same plants.

The presence of these phytochemicals in the ethanolic extract indicates that it possesses higher potency and exhibits various pharmacological activities. Therefore, the ethanolic extract of *Hibiscus rosa sinensis*, *Withania somnifera*, *Mentha piperita*, and *Murraya koenigii* from northern India holds promise for the production and development of medicines.

### Data availability statement:

The above data used to support the findings of this study are included within the article.

**Conflict of Interest:** The Authors declare that they don't have any Conflict of Interest.

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