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Phytochemical Profiling of Medicinal Plants Extracts and Their Antioxidant and Anticancer Potentialities Against Human Liver Cancer (Hep G2) Cell Lines

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Abstract*. Although Pakistan is stacked with enormous varieties of medicinal plants, only a little proportion of these plants has been evaluated for their medicinal and therapeutic properties. Herein, four indigenous medicinal plants Citrus sinenesis, Citrus paradiasii, Moringa olifera, and Hibiscus rosa-sinenesis were collected and subjected to phytochemical analyses to scrutinize the presence of secondary metabolites. Qualitative analysis showed the presence of an array of secondary metabolites in the selected plants, which were further corroborated by high-performance liquid chromatography. Results revealed the presence of 33.24, 21.04, 15.2 ppm gallic acid in methanol, ethyl acetate and n-hexane fraction of C. sinensis peels extract, respectively. C. paradaissi peels consist of 24.06, and 18.24 ppm of gallic acid and caffeic acid, respectively, in methanol and chloroform fractions, whereas its methanolic seeds extract contain caffeic acid as a major component (10.63 ppm). H. rosa-sinenesis has shown p-coumaric acid, caffeic acid, and gallic acid at 35.26, 15.04, and 11.4 ppm, respectively. M. olifera contained 3.24 ppm gallic acid in pods extract while stems and leaves extract contain a very low amount. Anticancer profile evinced that Citrus sinensis extract showed the highest percent inhibition (142.746%) of human liver cancer (Hep G2) cell lines followed by H. rosa-sinensis (132.49%), C. paradaisii (82.39%) and M. olifera (68.0%). The determined IC_{50} values for antioxidant activity were C. sinenesis (IC_{50}=0.49 mM), C. paradaisii (IC_{50}=0.43 mM), M. olifera (IC_{50}=0.42 mM) and H. rosa-sinensis (IC_{50}=0.41 mM). Conclusively, the selected plants could be an effective alternative and deliverable chemical therapeutic to the pharmaceutical industry due to their excellent biological effects.*

Keywords: *Medicinal plants, Phytoconstituents, Flavonoids, Antioxidant activity, Anticancer, Cytotoxicity*

1. Introduction

Fruits and vegetables are important for human life because their persistent utilization reduces the risk of many incurable diseases. With the advancements in technologies making life easier and facile, there is an increasing trend in the risk of stress-related diseases. Therefore, it is essential to take a healthy and natural diet. To cover this need, worldwide, intake of fruits, vegetables, plant-based foods, and nutrient-rich plant products has been focused [1-5]. Nevertheless, the utilization of proper diet (a complex of vegetable, fruits, and nutritional juices) is not enough to prevent chronic diseases because a specific diet only provides more than 25,000 bioactive constituents. Among these, many are related to modification causing in processes of these chronic diseases. Thus, it is of paramount significance to develop bio-drugs based on food and dietary products for the betterment of human health, which is only possible through a complete understanding of the complex relationship of food, vegetables, fruits, plant-products

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to cure diseases. According to the recommendation of nutritionist and biologist, food-based drugs have synergic and additive effects, which are necessary to cover health benefits and maintain human health. Therefore, it is a better approach by researchers and pharmacists around the world to design and develop food-based strategies to derive bio-drugs for health improvement. Mostly, food constitutes, and bioactive reagents are obtained from plants by extraction, known as phytoconstituents. These phytochemicals are reduced products of plants acting as antioxidants and produced as defense tools in response to the environmental stress and conditions in which the plants exist (Figure S1). As anti-oxidants, these bio-active constituents act as an inhibitor of free radicals like reactive oxygen and nitrogen species, which accelerate the chronic diseases [6]. From the beginning of life, natural products have been serving as excellent sources of novel drugs, which have proven as an inspiration and lead compounds for the synthesis of natural as well as non-natural compounds. It is important to introduce new pharmacological sources to scientific and social communityy [7]. Natural products are always acting as a supplier of bioactive scaffolds reservoir that has demonstrated significant avenues for the treatment of human diseasess [8]. Increasing interest in the use of medicinal plants and plant-derived drugs is due to their emerging effects in the maintenance of human health. It has been observed that the drugs derived from natural products are less toxic and free from adverse effects [9]. Keeping all beneficial properties in mind, the local flora of Pakistan was explored to identify their constituents to derive drugs and provide cost-effective, cheap, and promising remedies to treat diseases.

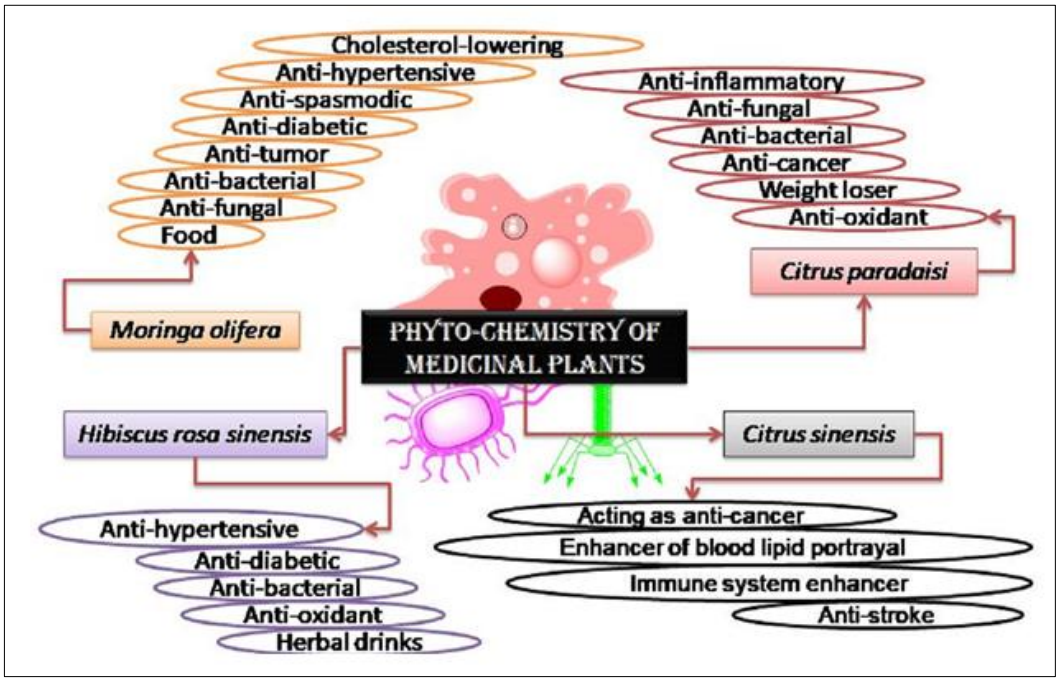

Figure 1. Biological applications of selected plants *(Citurs sinenesis, Citrus paradaisiis, Moringa olifera, Hibiscus rosa-sinenesis*

Citrus belongs to the family *Rutaceae* and is the most admired and favored fruit crop. The undeveloped peels of *Citrus sinensis* are capable of acting as a chemotherapeutic mean. Hesperidin, a *C. sinensis* flavonoid, exhibits potential stability on the bone condition and their metabolism. It is demonstrated that many types of cancers like colorectal, stomach, and esophageal can be retarded by *C. sinensis* fruit. *Citrus sinensis* fruit also enhances the blood lipid profile and anti-stroke action [10]. *Moringa olifera*, locally called as drumstick-tree or horseradish tree, and its different parts are used as food since ancient times. Traditional medicine systems used this plant to cure different diseases like ulcers, wound information, heart disorders, cancer, obesity, liver problems [11]. Different parts of the plant have been evaluated for their potential as therapeutics and observed to exhibit anti-hypertensive, anti-tumor, anti-inflammatory, anti-ulcer, anti-spasmodic, cholesterol-lowering, anti-diabetic, anti-

bacterial, and anti-fungal activities [12]. *Citrus paradisi* (commonly called grapefruit) is the second most worldwide used citrus crop [13]. Natural bioactive flavonoids reveal the antioxidant action and these flavonoids are present in higher amounts in the peels of *C. sinensis.* It has been utilized in various therapies as weight loss and immune system enhancement [14]. Traditionally, it has used as an antibacterial, anti-fungal, anti-inflammatory, antioxidant, and anticancer agent in the folk medicine system. Moreover, with health awareness and rising demand for anti-aging products, grapefruit has been used to clear oil from skin and acne issues [13]. A plant belongs to the genus *Hibiscus* is famous for its use in traditional medicines known as *Hiboscus sabdariffa* L. It has been used as food and herbal medicines, as well as, in herbal drinks, hot and cold drinks. Its extracts find use as antibacterial, anti-diabetic, anti-oxidant, and antihypertensive agents (Figure 1) [15].

A large variety of phytochemicals is present in nature, and many of these phytochemicals possess medicinal and health beneficial properties. Many components are present in plant extract that manifest the physiological and medicinal actions. Many biological properties are also exhibited by these components such as anti-fungal, anti-apoptosis, anti-inflammation, anti-microbial, anti-urease, anti-carcinogen, anti-bacterial, anti-oxidant, and anti-aging etc. [16]. Parasitic infections, which generated due to viruses, fungi, bacteria, and others, were treated for thousands of years by medicinal plants and their products. Recently, a number of synthetic and semi-synthetic drugs have been synthesized using these plants [16]. Different parts of these plants like flowers, seeds, leaves, roots, barks, and fruits have been exploited to cure various diseases and disorders. The oils extracted from medicinal plants have also been tested for their activity against pests, bacteria, fungi, insects and other microorganisms [17]. Therefore, in this study, indigenous medicinal plants *C. sinenesis, C. paradiasii, M. olifera,* and *H. rosa-sinenesis* were subjected to phytochemical analyses to scrutinize their bioactive constituents. Moreover, anticancer and antioxidant activities were also investigated to appraise their therapeutic potentialities.

2. Material and methods

2.1 Plant materials and preparation of extracts

All selected plants *C. sinenesis*, *C. paradaisii, H. rosa-sinensis,* and *M. olifera* were collected locally from different regions of Faisalabad. The selected plants were identified by Dr. Fouzia Shakoor, Department of Botany, The Government College Women University, Faisalabad**.** The collected plants were dried under shade and ground to a fine powder. The powdered material of the plant was extracted with water, chloroform, methanol, petroleum, and ethyl acetate. For each plant extract, powdered plant material was soaked in respective solvents for 24 h to obtain all polar and non-polar compounds. The extracts were evaporated under reduced pressure by a rotary evaporator.

2.2 Qualitative and phytochemical analysis by HPLC

Preliminary analysis of prepared plant extracts was carried out by performing different tests (Mayer's Test, Wagner's test, Ninhydrine, Molisch's test, Benedict's test, Spot test, Saponification test, Borntrager's test, Legal's test, Ferric chloride test, Gelatin test, Lead acetate test, Alkaline test, Magnesium & Hydrochloric acid test, Libermann-Burchard's test, Millon's test, Biuret test, Shinoda test, Keller-Kiliani Test, and Salkowski's test) [18, 19] to confirm the presence of secondary metabolites in the selected plants (Table 1). For quantitative analysis, the prepared extracts of selected plant species were subjected to HPLC to estimate the number of different phytochemicals. To perform a quantitative analysis of different solvent fractions of selected plants, their solutions were prepared. About 0.1 g of each extract (petroleum, chlororform, ethyl acetate, water, and methanol) was taken in a conical flask (100 mL). Then about 5 mL HCl and 10 mL distilled water was added, followed by the addition of 15 mL methanol. After mixing all, the mixture was put into an oven for 1.5 h and then subjected to HPLC for analysis. For HPLC analysis, sample preparation was done according to the method described in literature. The separation of plant samples on gradient HPLC was performed using shim-pack CLC-ODS (C18), 25 cm 4.6 mm, 5-µm column. The chromatographic separation was carried out using a mobile phase gradient: A (H_2O : Acetic acid 94:6, *p*H = 6), B (Acetonitrile 100%) 0-15 min = 15% B, 15-30

min = 45% B, 30-45 min= 100% B with 1 mL/min flow rate using UV-visible detector at 280 nm wavelength at room temperature. The identification of each compound was established by comparing the retention time and UV-Vis spectra of the peaks with that obtained by the injection of standards. The quantification was performed by external calibration with standards.

Table 1. Tests performed for the qualitative analysis of secondary metabolites in selected medicinal plants

S. No.	Class of compounds	Tests performed	Observations	References
1	Tannins	2 mL of plant extract was added to 2 mL of distill water and then added 2-3 ferric chloride (5%) drops.	Precipitates of green color	29-30
2.	Flavonoids	1 mL of solution of (10%) lead tetra-acetate was mixed to the 1 mL of plant extract.	Yellow color	30
		From 2% NaOH solution just 2mL was added to the crude extract	Deep Yellow coloration, which disappears on acidification	30
		In the crude extract, some or little fragments of magnesium ribbon were mixed and then drop wise concentrated hydrochloric acid was mixed.	Pink vermilion color	30
		To the plant extract add alcohol and then filter paper strips were dipped in it and then the solution was ammoniated	Change of color of strips to yellow	31
		0.15 mL of 5% $NaNO_2$ and 2 mL of distill water was merged to the plant extract. 0.15 mL of $AlCl_3$ solution (10%) was added after 5 min and then for 5 min allows it to stand. Then mix 2 mL of NaOH solution (4%) to the mixture. To make the total volume up to 5 mL instantly water was mixed, stir and then for 15 min allow it to stand.	Pink color	31
3.	Phenols	The Crude extract was merged with 2 mL of $FeCl_3$ solution (2%).	Black color	29
		To 1 mL of plant extract, 4 mL of Na_2CO_3 and 5 mL of Folin-ciocalteu reagent were added.	Blue color	32
		Few drops of lead acetate solution (10%) were merged to the solution.	White precipitates	33
4	Terpenoids	To 2 mL of plant extract, 2 mL of acetic anhydride and 2-3 drops of concentrated H_2SO_4 was merged.	Deep red coloration	34
		2 mL of chloroform was dissolved in the crude extract and evaporated to dryness. Afterwards this was heated for about 2 min with the addition of 2 mL of concentrated H_2SO_4.	Grayish color	30
5	Saponins	5 mL of distill water was merged to 5 mL of plant extract.	Heat froth appearance	34
		Some drops of olive oil were merged to the 5 mL extract of plant.	Formation of the emulsions	34
		Some drops of sodium bicarbonate were merged to 1 mL extract of plant.	Formation of honey comb like structures	35-36
6	Steroids	To the 2 mL of extract merged 2 mL of chloroform and 2 mL of H_2SO_4 (conc.).	Reddish brown ring at the junction indicated	30
7	Phytosterols	5 mL chloroform, 3 mL acetic anhydride, few drops of dil. Acetic acid and some of the drops of concentrated H_2SO_4 were mixed to the 1 mL extract of plant	Bluish green coloration	37
8	Phlobatannins	1% solution of HCl (2 mL) was merged to 2 mL of the plant extract and then heat.	Red precipitates	34
9	Carbohydrates	10 mL of distill water, 2 mL of concentrated H_2SO_4, 2 drops of ethanolic α-naphthol (20%) were merged to 2 mL of extract of plant.	Reddish violet ring at the junction	30
		Fehling B and Fehling A reagents were taken in same quantities then merged together and 2 mL from this mixture was merged to unrefined extract and boiled mildly.	Brick red precipitates	30
		Unrefined extract was merged to 2 mL of Benedict's reagent and then boiled.	Reddish brown precipitates	30
		Unrefined extract was merged to 2 mL of Benedict's reagent and then boiled.	Dark blue or purple coloration	30
10	Starch	1 mL of iodine solution is merged in extract of 1 mL.	Blue coloration	30
11	Glycosides	To 2 mL of the plant extract, 2mL of acetic acid and 2mL of chloroform was merged.	Color change violet to blue	30

		To 1 mL of plant extract, 1 mL of 5% $FeCl_3$ solution and same quantity of acetic acid is used, and then some drops of H_2SO_4 were added to the mixture.	Greenish blue color	[38]
12	Cardiac glycosides	1-2 drops of $FeCl_3$ (2%) were added to 2 mL of glacial acetic acid and then this mixture was mixed with the unrefined extract. Take another test tube having 2 mL of concentrated H_2SO_4 and then add the above-prepared mixture to this test tube**.**	Brown ring at the inter phase	[30]
13	Coumarins	To 2 mL of the extract, mix 3 mL of 10% solution of sodium hydroxide.	Yellow color	[34]
14	Alkaloids	To 2 mL of extract of plant, mix some drops of Hager's reagent.	Yellow precipitates	[34]
		To 1 mL extract of the plant mix some drops of Dragandrof reagent	Orange brown precipitates	[36]
		2 mL of HCl (1%) was merged to the unrefined extract and heated lightly. Then the reagents added to the mixture were Wagner's and Mayer's reagents	Opaqueness of the end precipitates	[30]
15	Proteins	To 1 mL of extract of the plant, 1 mL of concentrated H_2SO_4 is mixed.	White precipitates	[34]
		To 3 mL of the plant extract, 1 mL of 4% NaOH solution and 1 mL of 1% copper sulphates were mixed.	Violet or pink color	[30]
		Precipitates of white color occurs when the unrefined was merged with 2 mL of Millon's reagent, and these precipitates were lightly heated	Red Precipitates	[30]
		A violet color occurred when 2 mL of Ninhydrin (0.2%) was boiled with unrefined extract	Violet color	[30]
16	Emodin	2 mL of NH_4OH and 3 mL benzene was mixed to 2 mL extract of the plant.	Red color	[34]
17	Anthraquinon	3 mL of benzene, 5 mL of 10% solution of NH_3 was mixed to 3 mL of the plant extract.	Different colors in ammonical layer like pink, violet or red	[36]
18	Anthocyanins	2 mL of HCl (2N) and NH_3 was mixed to 2 mL of extract.	Pinkish red to bluish violet coloration	
		To 1 mL extract of the plant, 1 mL of NaOH (2 N) was mixed and then heated.	Bluish-green colour	[34]
19	Betacyanin	To 1 mL extract of the plant, 1 mL of NaOH (2 N) was mixed and then heated.	Yellow colour	[34]
20	Leucoanthocyanins	5 mL of isoamyl alcohol was mixed to 5 mL of plant extract.	Red color in organic layer	[34]
21	Fixed oils	Two filter papers were taken and then individually the extracts were compressed between these filter papers and let it dry.	Oil strain on filter paper	[36]
22	Gums and mucilage	To 0.1 mL of plant extract, 10 mL of distilled water and 2 mL of pure alcohol were mixed with perpetual stirring.	White precipitates	[39]

2.3 Biological evaluation

2.3.1 Cytotoxicity and cell viability analysis by MTT assay

The human HepG2 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 µg/mL streptomycin and maintained at 37°C with 5% CO_2 in a humidified atmosphere. Cells were treated with extracts/compounds dissolved in DMSO with a final DMSO concentration of 0.05%. DMSO-treated cells were used as a control in all the experiments [20]. Cell viability was determined by MTT assay as described previously. Briefly, HepG2 cells were treated with different concentrations of compounds for 48 h. Following treatment, the MTT reagent was added (500 µg/mL) and cells were further incubated at 37°C for 4 h. Subsequently 150 µL DMSO was added to dissolve formazan crystals and absorbance was measured at 490 nm in a microplate reader (Thermo Scientific). The percentage of cell viability was calculated [21]. Different extracts of the selected plants were evaluated for their effects against human

liver cancer lines. It was found that the extract of *H. rosa sinensis* flowers exhibits a strong activity as compared to the other four (Table 3).

2.3.2 Antioxidant (free radical scavenging) activity using DPPH assay

In order to perform the antioxidant activity, the stock solution of plant extracts having a concentration of 200 mM (0.022 g) was prepared. This solution was diluted to 250 µg, 200 µg, 150 µg, 100 µg, 50 µg, and 25 µg. DPPH solution of 4% was prepared by mixing 0.004 g of DPPH in 100 mL of methanol. Ascorbic acid was used as a control, which was prepared by mixing 0.0017612 g into 100 mL of water having 100-µg concentration. In test tubes, 1 mL of the sample from each diluted solution added and followed by the inclusion of 2 mL of DPPH solution. In the same way, 1 mL of the ascorbic acid was added along with 2 mL of DPPH as control and allowed to stay overnight. The next day, the readings were taken at 517 nm in triplicate by using a Hitachi U-2900 spectrophotometer and calculated their inhibitory concentration by using formula [22].

$$A = \frac{Acontrol - Asample}{Acontrol}\ 100$$

3. Results and discussions

Plants have been widely used for therapeutic purposes to cure diseases at the beginning of human history. According to the WHO report, about 80% population from rural areas depends upon natural products, herbs, and plants for their necessary health care. Unfortunately, not much attention has been given to investigate the medicinal plants for their useful effects, and constituents with medicinal properties as well as nutraceuticals to discover their health benefits. Therefore, some selected plants were considered to explore their phytochemical, pharmaceutical, and biological characteristics. For that, selected plants were analyzed qualitatively to discover the presence of compounds responsible for their health effects. In addition to qualitative analysis by HPLC to discover the percentage of these compounds, antioxidant and anticancer potential were also investigated.

3.1 Preliminary qualitative phytochemical analysis

Preliminary analysis showed the presence of an array of secondary metabolites in the selected plants. Results of qualitative analysis (Table 2) revealed that the targeted plants were rich sources of phytochemicals, particularly flavonoids, phenolic acids, alkaloids, steroids that were responsible for biological activities of plants helping in curing disease, increasing human body immune level and proved as a wound healer. Methanolic extract of *C. paradaisii* seeds has been observed to contain cardiac glycosides, which were not present in peels extract. Moreover, coumarins were found in seed extract while absent in peels extract of *C. paradaisii*. The results of the analysis of *M. olifera* showed that the phenols, phytosterols, and reducing sugars were present in leave extract while absent in pods and stem extract. Whereas extract of pods and stem (*M. olifera*) contain alkaloids, absent in leave extract as observed from Table 2. In comparison, *H. rosa-sinenesus* extracts lack of terpenoids, carbohydrates, cardiac glycosides, proteins, emodines, anthraquinones, fixed oils, and gums while others all present (Table 2). Different fractions (Methanol, chloroform, n-hexane, ethyl acetate) of selected plant species (*C. sinenesis* peels, *C. paradaisii* peels, *H. rosa sinenesis, M. olifera*) were found to contain phytoconstituents in different amounts, which were further evaluated by HPLC analysis (quantitative analysis).

Table 2. Phyto-constituents of selected indigenous plants

S#	Secondary metabolites	*Citrus sinensis* peels			*Citrus paradiasii* Peels			*Citrus paradiasii* seeds	*Moringa olifera* (Methanol)			*Hibiscous rosa sinensis* Flowers
		Me-thanol	Ethylacetate	n-hexane	Methanol	Chloroform	n-hexane	Methanol	Pods	Stems	Leaves	Methanol
1	Tannins	+	+	+	+	-	-	+	+	+	+	+
2	Flavonoids	+	+	+	++	++	-	+	+	+	+	+
3	Phenols	-	+	+	+	+	-	+	-	-	+	+
4	Terpenoids	+	+	+	+	-	-	+	-	+	-	+
5	Saponins	+	+	+	-	-	-	+	+	+	+	-
6	Steroids	+	+	+	+	+	-	+	-	+	-	+
7	Phytosterols	-	-	+	-	-	-	+	-	-	+	+
8	Phlobatannins	-	+	-	-	-	-	-	-	-	-	+
9	Carbohydrates	+	+	+	-	-	-	-	-	-	-	-
10	Starch	-	+	-	-	-	-	-	-	-	-	-
11	Glycosides	+	-	-	-	-	-	-	-	+	-	+
12	Cardiac glycosides	+	-	-	-	-	-	+	-	-	-	-
13	Coumarins	+	+	-	-	-	-	+	-	-	-	+
14	Alkaloids	-	-	-	++	+	-	+	+	+	-	+
15	Proteins	+	+	+	-	-	-	-	-	-	-	-
16	Emodines	-	-	-	-	-	-	-	-	-	-	-
17	Anthraquinones	-	-	-	+	-	-	-	-	-	-	-
18	Anthocyanins	+	-	-	-	-	-	-	-	-	-	+
19	Betacynins	-	-	-	-	-	-	-	-	-	-	+
20	Leucoanthocyanins	-	-	-	-	-	-	-	-	-	-	+
21	Fixed oils	+	+	+	++	++	-	-	-	-	-	-
22	Gums & Mucilage	-	-	-	-	-	-	-	-	-	-	-
23	Reducing sugars	-	-	-	+	+	-	-	-	+	+	+

3.2 Quantitative phytochemical analysis by HPLC

Quantitative analysis by HPLC was performed to confirm the percentage of flavonoids and phenolic acids and results are shown in Table 3. Previously, a study of HPLC analysis has been done for the estimation of phenolics and flavonoids in the root and stem of *C. sinenesis* [23] but we selected its peels for HPLC analysis. Table 3 results showed that the methanolic extract of *C. sinensis* peels consists of quercetin, gallic acid, caffeic acid in high quantities than other fractions but *C. sinensis* peels lack of vanillic acid in all fractions. The m-coumaric acid and syringic acid were absent in ethyl acetate fraction, while synaptic acid, caffeic acid, m-coumaric acid, p-coumaric acid, chlorogenic acid, and syringic acid were not detected in n-hexane fraction. A previous report on *C. sinenesis* determined chlorogenic acid, quercetin, rutin, and hydroxybenzoic acid in both stem and root extract, whereas mandalic acid in root extract and phloroglucinol was detected only in stem extract [23]. In *M. olifera*, pod, stem, and leave extract to contain quercetin and gallic acid but previously Shervington et al. [24] reported that the *M. olifera* species were influenced by geographical changes strongly and resultantly, its chemical constituents (presence, percentage of occurrence) also varies [24]. HPLC results of *H. rosa-sinensis* showed the detection of quercetin, gallic acid, synaptic acid, caffeic acid, p-coumaric acid, and chlorogenic acid in good percentage. In comparison to the previous report [25] that showed the presence of only flavonoids quercetin, rutin, kaempferol, and myricetin in methanolic extract. From Table 3, it was found that vanillic acid was absent in all plant species but m-coumaric acid and syringic acid were absent in all plants except the methanolic fraction of *C. sinensis* peels. Gallic acid and quercetin were present in all fractions of selected plants except the n-hexane fraction of *C. paradaisii* peels, n-hexane fraction of *C. sinenesis* peels have only quercetin (0.1 ppm) and gallic acid (15.2 ppm). By observing HPLC results (Table 3), it was found that n-hexane fraction of *C. paradaisii* peels lack of any flavonoids and phenolic acids.

Table 3. Results of quantitative phytochemical analysis by HPLC

S#	Secondary metabolites	*Citrus sinensis peels*			*Citrus paradiasii Peels*			*citrus paradaisii seeds*	*Moringa olifera (Methanol)*			*Hibiscous rosa sinensis Flowers*
		Methanol (ppm)	Ethylacetate (ppm)	n-hexane (ppm)	Methanol (ppm)	Chloroform (ppm)	n-hexane (ppm)	Methanol (ppm)	Pods (ppm)	Stems (ppm)	Leaves (ppm)	Methanol (ppm)
1	Quercetin	6.0	3.99	0.1	5.94	4.48	Not	3.48	2.84	1.98	0.549	6.03
2	Flavonoids	+	+	+	++	++	-	+	+	+	+	+
3	Gallic acid	33.24	21.04	15.2	24.06	18.24	-	5.25	3.24	0.11	1.99	11.4
4	Synaptic acid	4.02	1.25	-	2.45	-	-	2.58	-	-	-	5.62
5	Caffeic acid	12.56	8.26	-	10.7	-	-	10.63	-	-	-	15.4
6	Vanillic acid	-	-	-	-	-	-	-	-	-	-	-
7	m-coumaric acid	0.125	-	-	-	-	-	-	-	-	-	-
8	p-coumaric acid	5.29	3.33	-	6.76	-	-	3.26	-	-	-	35.26
9	Chlorogenic acid	6.32	0.236	-	-	-	-	-	-	-	-	6.23
10	Syringic acid	0.325	-	-	-	-	-	-	-	-	-	-

3.3 Anticancer and antioxidant activities

Anticancer activity results (Table 4) revealed that the *M. olifera* showed very low cellular viability against Hep G2 cell lines at a value of 68.0, whereas Lucia et al. (2018) reported 50% cellular viability against MDCK cellular lines [26]. Another report showed that *M. olifera* leaf extract has a significant inhibitory (80%) impact on Hep G2 cells [27]. *H. rosa-sinenesis* showed a cell viability of 132.49%, while a previous report showed its excellent apoptosis induction in breast cancer cell lines [28]. *C. sinensis* was found to exhibit significant cell viability of 142.74% as compared to other plant extracts due to the presence of phenolics and flavonoids, whereas *C. paradaisii* showed a cell viability of 82.39% (Figure 2). Notably, there is no report on the selected citrus species (*C. paradaisii* and *C. sinensis*). Similarly, the selected plant extracts were evaluated for their antioxidant activity by using DPPH as a free radical using ascorbic acid as a standard. Table 5 showed the percentage of inhibition of each selected plant extracts. Among all extracts, *H. rosa-sinenesis* extract has the lowest IC_{50}= 0.41 mM value, which makes it a good scavenger followed by *M. olifera* (IC_{50}=0.42 mM), *C. paradaisii* (IC_{50}=0.43 mM) and *C. sinenesis* (IC_{50}=0.49 mM). This excellent scavenging activity confirms the presence of flavonoids and phenolic acid in an elevated concentration. In comparison to Ayyakkannu, [25] reports, the major factor responsible for excellent antioxidant potential was rutin [25] but in our findings, the main percentage was of p-coumaric acid (Table 3). Antioxidant potential results were in close agreement with HPLC findings, which confirmed that the excellent free radical scavenging potential of *H. rosa-sinensis* was due to a higher concentration of p-coumaric acid and caffeic acid in addition to other flavonoids and phenolic acids.

Table 4. Anti-cancer activity of selected plant extracts against liver cancer cell lines

Absorbance	1	2	3	% viability	% viability	% viability
Control	0.904	0.842	1.034	100	100	100
Citrus sinenesis (MeOH)	1.03	1.21	1.376	113.9381	143.7054	142.74660
Citrus paradiasii peels (MeOH)	0.776	0.962	0.852	85.84070	114.2517	82.3984
Moring olifera	0.706	0.821	0.902	83.50	84.06	68.0
Hibiscus rosa sinensis	1.002	1.051	1.245	120.2	148.42	132.4951

Table 5. Antioxidant activity of selected plants extracts by using DPPH

Test samples	Antioxidant activity (Percentage inhibition)				
Conc.	10 µg	25 µg	50 µg	250 µg	IC_{50} (mM)
Citrus sinenesis (MeOH)	14±0.04	21±0.01	42±0.006	68±0.01	0.49
Citrus paradiasii peels (MeOH)	11±0.008	23±0.004	43±0.02	66±0.01	0.43
Moring olifera	11±0.0005	34±0.0003	54±0.001	62±0.03	0.42
Hibiscus rosa sinensis	15±0.001	36±0.009	67±0.006	61±0.05	0.41

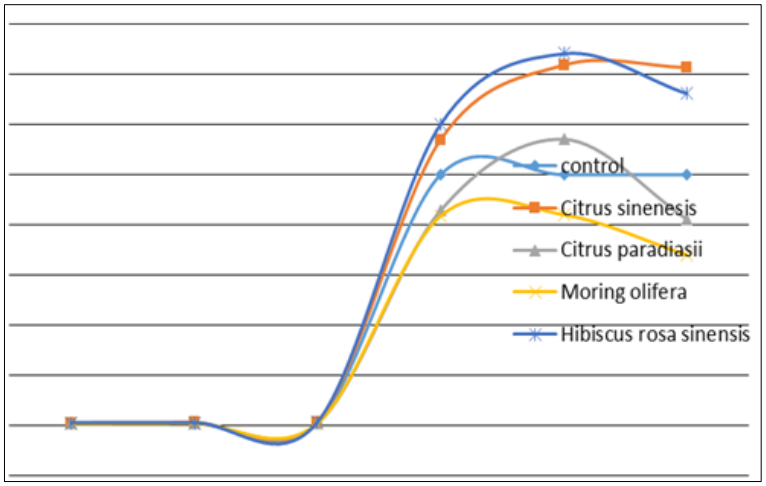

Figure 2. Graph representing the percent viability of cells

4. Conclusions

From the above study, it has been concluded that *Citrus plants (sinensis and paradasii)* are of enormous importance in pharmaceuticals and medicines due to the presence of many important phytoconstituents. The phytochemical analysis of *C. sinensis* peels proves to be very beneficial because of the presence of phytochemicals or bioactive compounds that are useful in various ways. These healing properties were observed due to the presence of tannins, flavonoids, terpenoids, saponins, reducing sugars. *H. rosa sinensis* has been used in various ways like tea, extract to weight loss, its flower water extract used to cure stomach disorders, skin irritations as anti-aging. Due to its much use as nutraceuticals, when it was phytochemically analyzed, it showed the presence of flavonoids, phenolics, alkaloids. Biological evaluation of the selected plants in different concentrations showed good results. *C. sinensis* showed the highest IC_{50} at 0.49 mM, while *H. rosa-sinenesis* plant species showed the lowest IC_{50} at 0.41 mM. These results showed that *H. rosa sinensis* has more activity, *C. sinenesis* has lower activity, while the *C. paradaisii* and *M. olifera* have medium activities.

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