



EVALUATION OF THE NUTRACEUTICAL, ANTI-LEISHMANIA AND ANTICANCER POTENTIAL OF CITRUS LIMON PEEL EXTRACTS

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Abstract Citrus belongs to the family Rutaceae and comprises different plants and fruits. The Citrus limon peel consists of rich nutrient values, i.e., essential oil and compounds, which can be used as anticancer and antimicrobial agents. The present study has explored the concept of nutraceutical, antileishmanial, and anticancer activities of Citrus limon peel. The samples of local and hybrid Citrus limon were collected from the district of Mardan. The lemon peel was extracted and dried at room temperature. After drying, the lemon peel was ground to obtain fine powder. Powders of both types of lemon peel were soaked in methanol at room temperature for 15-21 days. Powdered extracts were analyzed for the presence of different active ingredients, i.e., alkaloids, steroids, flavonoids, and tannins. The result of digestibility revealed that hybrid lemon was high in dry matter, Ash, crude proteins, fiber, and lower fat. The preliminary phytochemical study revealed that alkaloids, steroids, flavonoids, and tannins were found in both local and hybrid lemon. The findings of EDX represent that the CK was 65.82 % and 67.51 % of the atomic weights for the CK, while 32.54% and 30.62 had been taken in the OK. The FTIR results showed that functional groups are present in both local and hybrid lemon peels. The alkane and amines are present in local and hybrid lemon peels, while ketones and tertiary alcohols are present in local lemon peels, and carboxylic acid and amine groups are present in hybrid lemon peels. The MTT assay showed that the methanolic extracts of hybrid and local Citrus limon peel were more efficacious than aqueous and chloroform extracts in suppressing promastigotes *L. major* and *L. tropica*, while the aqueous extracts showed the lowest activity against promastigotes *L. major* and *L. tropica* in both cases. Overall, the activity of berberine and hybrid Citrus limon peel was much higher than the activity of local Citrus limon peel. The anticancer potential of different solvent-based lemon peel extracts against the HepG2 cell line was evaluated after 48 hours. The extracts of lemon peel in four different solvents, including methanol, ethanol, ethyl acetate, and chloroform, were compared. In the case of lemon peel chloroform extract, the highest inhibition (65.93 percent) was seen at a concentration of 100ug/ml, but in the case of lemon peel ethyl acetate extract, it was 61.75 percent at a concentration of 200ug/ml. On the other hand, it is quite evident that hybrid lemon peel extract dissolved in various solvents (methanol, ethanol, ethyl acetate, and chloroform) showed significantly reduced cytotoxicity against HeLa cancer cells in comparison to wild-type lemon peel extract. The study concludes that C. lemon extract can be used for better treatment of different anticancer and antileishmaniasis assays.

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Introduction

Medicinal plants are very important natural substances that also cause biological processes in the human body and can be important for developing new kinds of medications. The World Health Organization maintains that medicinal plants can be used for various chemical, synthetic, and semi-synthetic

medications as well as for therapeutic purposes. The value of naturally occurring antibacterial compounds has increased recently (Marzoog et al., 2015). Citrus fruits are among the most important medicinal plants in the Rutaceae family, which includes 140 genera and 1300 species. According to the researchers, lemon blossom, peel, leaves, juice, root, stem, and other citrus-based extracts can be employed and found to be

beneficial in many cancer treatments as well as having a variety of antibacterial properties for various bacterial strains. The alkaloids found in citrus fruits provide them with antifungal, anticancer, antibacterial, and antiviral properties (Alohi et al., 2018). Citrus fruit has a better nutritional content and a higher ratio of elemental bioactive components, such as vitamin C, essential oil, phenols, limonoids, carotenoids, etc., according to medical studies. These compounds can be employed in a variety of ways and have greater health advantages (Duarte et al., 2016). In global markets, *Citrus maxima*, *Citrus limetta*, *Citrus limon*, *Citrus aurantifolia*, and *Citrus reticulata* are among the most often utilized and consumed citrus species (Narayanankutty et al., 2022). In nutraceutical research, Citrus limon, or lemon, has gained attention due to its quantity of bioactive components. The antibacterial, anti-inflammatory, and antioxidant qualities of lemon peel are derived from a combination of phytochemicals, including vitamin C, essential oils, and flavonoids (including eriocitrin and hesperidin), which are often considered waste in citrus processing (Li et al., 2019). Fruit peels are the most common "residue" from the citrus business, which is the largest fruit sector in the world. Citrus peels are employed in industry to extract bioactives (flavonoids, essential oils, and d-limonene) and to make some by-products (cattle feed, molasses, ethanol, and fiber), although they are not profitable. Juice, citrus, and other vending companies continue to squander enormous volumes of peels. With 100 million tons produced annually, citrus fruits account for the greatest portion of global fruit production, with roughly 34 percent of them being utilized to generate juice. Peels are the most common by-product (residue) of citrus juice and other citrus processing businesses, and a significant amount of them are discarded globally. The cellulose, hemicellulose, and pectin-rich dry citrus peels can be utilized as a substrate for fermentation. Cattle feed, molasses, flavonoids, essential oils, and d-limonene are made from citrus waste products (Nair et al., 2018).

The parasite that causes leishmaniasis, which typically inhabits contaminated sand flies, is the cause of the parasitic disease (Dutta et al., 2007; Sarwar et al., 2025). Numerous Leishmaniasis parasite species can cause cutaneous leishmaniasis, a zoonotic parasitic illness. Compound and actual therapy methods, such as copper sulfate, destructive water, or hot metals, have also been employed in areas where endemic Leishmaniasis is present. However, these methods have been callous, and the scraping that results from them can sometimes be worse than the sore itself (Doudi, 2011). Pentavalent antimonials, sodium stibogluconate (pentostam), meglumine antimoniate (glucantime), and amphotericin B deoxycholate are among the essential medications for Leishmaniasis that have been used for many years. However, growing information about treatment dissatisfaction has been revealed (Ahangarkani et al.,

2019). Anti-leishmanial drugs made from natural sources have not been sufficiently studied. Up until now, expensive, synthetic drugs have been the sole treatments for leishmaniasis. The *Leishmania* genus is responsible for the parasite- and vector-borne disease known as leishmaniasis. This protozoan is a major public health concern, causing high rates of mortality and morbidity in many Mediterranean, Asian, African, and Latin American locations. This disease affects more than 12 million people globally, in 98 tropical and subtropical nations. Visceral leishmaniasis, the initial symptom of the disease, is caused by *L. infantum*, whereas cutaneous leishmaniasis is caused by *L. major*. These parasites come in two different varieties. The non-motile intracellular amastigote is the second form, while the flagellated extracellular promastigote is the first (Bouabdallah et al., 2022). Up to 12 million individuals worldwide are afflicted with leishmaniasis, a tropical parasitic illness. Limitations of current chemotherapies include parasite resistance, toxicity, and high cost (Maaroufi et al., 2021). The supporting plants provide a wealth of raw materials for basic therapeutic advantages and different aspects of the underlying condition. *Citrus lemonade* study has its roots in the widespread detection of bitter lemonades in citrus, the knowledge of their origins, and the development of methods for extracting their juices. Citrus lemon inherent properties have demonstrated their potential to improve human health by acting as antiviral, cholesterol-lowering, and anticancer agents. From describing their interest in citrus harshness to their possible application as significant advocates for promoting human prosperity and well-being, this survey details the evolution of citrus lemonade research (Abubakar et al., 2015). The majority of research on lemon's anti-cancer properties has concentrated on two areas: in vitro experiments to determine the action process, such as enzyme inhibition, anti-cancer and anti-proliferative activity, and attenuation of cancer cells. According to certain research, PMFs have been shown to limit the growth of human leukemic cell lines (Manthey et al., 2011). The studies of *lemon* showed phosphorylation and the development of human mammary cancer, and also cytolysis by killing (Alshatwi et al., 2011). The "mandarin orange," or *C. reticulata*, is typically consumed raw or added to fruit salads. In traditional Chinese medicine, its dried fruit peels are frequently used as a treatment for asthma, bronchitis, and indigestion. The largest fruit in the genus, *C. maxima* (*Kambili naragam*), has not yet been evaluated for its anticancer properties. The anticancer potential of citrus peels, which are processed for industrial products or "wasted" globally, has not been investigated. The anticancer properties of fruit peel extracts and essential oils from *Citrus medica* round (*Mathala naragam*), *C. medica* oblong (*Mathala naragam*), *C. sinensis* (Orange), *C. maxima* (*Kambili naragam*), *C. limon* (Lemon), and *C. reticulata*

(Mandarin orange) were examined in this study using both in vitro and in vivo assays (Nair et al., 2018).

This study is important for novice researchers who want to investigate the many antibacterial, antifungal, and insecticidal properties of citrus fruit. The investigation's results will contribute to the body of literature already in existence, which is important for empirical findings in the citrus fruit family. From an antibacterial, antifungal, and insecticidal standpoint, the current research suggests many actions that will be beneficial for the study's new course. The current study aims to investigate the anti-leishmanial, anti-cancer, and nutraceutical properties of *Citrus limon* peel.

Materials and methods

Collection

The samples of *Citrus limon* (local and hybrid) were collected from District Mardan and were washed and dried. The lemon peel was extracted and dried at room temperature. After drying, the lemon peel was ground to obtain fine powder (Mahmoud et al., 2016). The collected specimens were assembled in the Microbiology Laboratories of Abasyn University Peshawar.

Preparation of Extract

The samples of *Citrus lemon* (local and hybrid) were collected from district Mardan, and lemon peel was dried at room temperature and ground to obtain the fine powder. Powdered were soaked in methanol at room temperature for 15-21 days. Whatman No.1 filter paper was used to filter the extract and dried by rotary evaporator under vacuum at 40⁰ and stored in a refrigerator at 4⁰C for further studies (Puri et al., 2015).

Preliminary Phytochemical Screening:

Powdered extracts were analyzed for the presence of different active ingredients, i.e., alkaloids, steroids, flavonoids, and tannins, etc. (Saxena et al., 2014).

Physiochemical analysis

Different types of Physiochemical analysis were performed in Central Resource Laboratory (CRL), University of Peshawar (UOP).

FTIR

A Fourier Transform Infrared (FTIR) spectrophotometer was used for finding the types of chemical bonds present in compounds and to evaluate different solvent extracts of each plant material. A potassium bromide (100mg) pellet was mixed with 10mg of the dried extract powder and loaded into the FTIR spectrometer, with a resolution of 4 cm⁻¹ and a Scan range from 400 to 4000 cm⁻¹ (Ashokkumar et al., 2014).

Ultraviolet Spectroscopy

For characterization and confirmation of silver NPs, ultraviolet light in the visible spectra was used at different time intervals and was noted accordingly. UV light in the range 400-450nm wavelength with a sharp peak confirmed the presence of synthesized silver nanoparticles, according to the protocol used by Singh et al. The element silver in synthesized

nanoparticles was confirmed with the help of Energy Dispersive X-Ray (EDX) analysis (Laraib et al., 2023).

Scanning Electron Microscope (SEM) Analysis

SEM analysis was carried out to determine the size of *C. limon* peel extract. For this purpose, the sample was coated on the carbon grid, and excess solution was removed with the help of blotting paper, then was allowed to dry under the lamp for 6 minutes (Devika et al., 2014).

Transmission Electron Microscopy (TEM)

Further characterization of the extract was performed by using TEM analysis to determine the morphology and size of the extract. The extract was drop-coated on a copper grid (carbon-coated) and placed in the holder. Transmission EM images were observed, and the morphology and size of the extract were determined according to Singh et al method.

Anti-Leishmaniasis and Anticancer Activity

All the extracts of the *Citrus limon* peel were performed using the well diffusion method. Different types of biological activities were performed, such as cytotoxic, antileishmanial, anticancer, etc. bioassay using standard procedures and protocols (Andreza et al., 2017; Ajikumar et al., 2018; Elham et al., 2020; Ullah et al., 2024). Human cervical cancer cells (HeLa) were grown in DMEM supplemented with 10% FBS, 0.25% sodium bicarbonate, and antibiotic antimycotic solution. Cells were cultured in 5% CO₂ at 37°C in a humid environment. The viability of cells was determined prior to the experiments using the protocol. In this work, HeLa cells with more than 95% cell viability were employed. For 48 hours, HeLa cells were treated to various quantities of lemon peel extract dissolved in various solvents. Following the exposures, cells were tested for cytotoxicity using the 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Because lemon peel extracts were not totally soluble in aqueous solutions, stock solutions of all extracts were made in dimethyl sulfoxide (DMSO) and diluted in culture medium to achieve the necessary concentrations. The DMSO concentration in the culture media was less than 0.1 percent, and this medium was employed as a control. The percentage of viable cells was determined using the MTT test as specified. Briefly, 1×10⁴ cells were allowed to adhere on 96-well culture plates in a 37°C CO₂ incubator for 48 hours. After exposure, 10 ul of MTT (5 mg/ml in PBS) was added to 100 ul of cell suspension per well, and the plates were incubated for 4 hours. The supernatants were then discarded, and 200 ul of DMSO was added to each well and gently mixed. Using a Multiwell Microplate Reader, the produced colour was read. Likewise, untreated sets were run under identical experimental conditions as a control.

Results and Discussion

Physicochemical Potential or Digestibility Feature

Citrus juices' abundance of antioxidant components makes them seem like healthful foods to customers. Although there are many studies on citrus juices' antioxidant activity, little is known about how these activities relate to their physicochemical characteristics. Some samples showed a correlation between physicochemical factors and nutritional parameters, including color, fluorescence, and the amounts of 5-hydroxymethylfurfural and furfural (Álvarez et al., 2014). The samples of *Citrus limon* (local and hybrid) were collected from the district of Mardan and used for the experimental study Human as ancient periods fruits as consumption for their nutritive value. We evaluated citrus physicochemical and digestibility properties. These results show the digestibility features and nutritional values of lemon peels as mentioned in Figure 1. It was found that dry matter and fats were higher in local lemon peel than hybrid, while ash content and crude protein were found higher in the hybrid lemon as compared to local lemon peel. The estimation of Nitrogen Free Extract (NFE) was found higher in local lemon (68.65%) and lower in hybrid lemon peel, i.e., 53.725.

Similar results have been found in the (Ezeabara and Okeke, 2019) peels of *C. aurantifolia* (12.0+0.4, 8.30+0.4), *C. limon* (9.01+0.04, 9.37+0.02), and *C. sinensis* (13.89+0.02, 7.86+0.02), all of which had high proportions of fat and crude fiber, respectively. Additionally, the leaf of *C. reticulata* has a high fat content (11.10+0.05%). The leaves of *C. aurantifolia* (14.11+0.02%), *C. limon* (14.15+0.02%), and *C. paradisi* (13.01+0.01%) had extremely high ash concentrations. Additionally, it was high in the stem bark of *C. reticulata* (15.18+0.02%), *C. sinensis* (17.01+0.01%), and the roots of *C. grandis* (12.72+0.04%). Significantly elevated levels of ash were also found in the roots of *C. paradisi* (10.14+0.01%) and leaves of *C. grandis* (10.18+0.02%). Therefore, the results indicated that citrus plant leaves and peels may be included in plant food protein, suggesting that they could be utilized as readily available protein sources for animal production. For the health and productivity of the animals as well as the profitability of the ranch, beef cattle diets must contain enough protein.

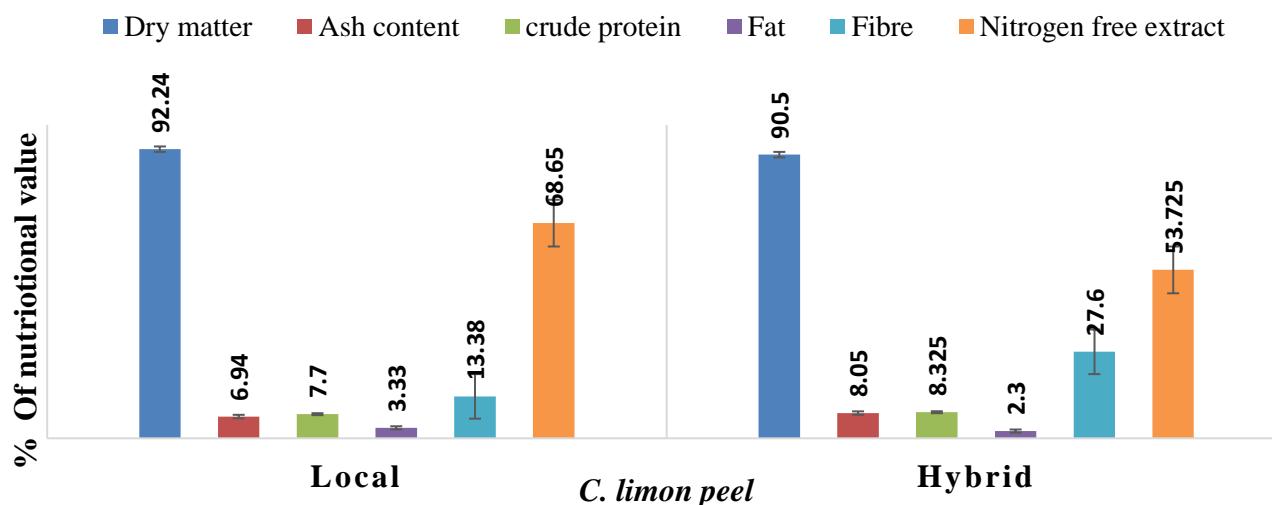


Figure 1: Digestibility features of local and hybrid *C. Limon* peel

Preliminary Phytochemical Screening

Phytochemicals are important phytoconstituents responsible for several biological properties such as antimicrobial, anticancer, antioxidant, etc. It is believed that citrus has a unique diversity of phytochemicals. Phytochemical screening was performed using prescribed methods. Result has been shown the different phytochemical constituents in plant extracts, as shown in Table 1. The first step taken to inform researchers about the existence of phytochemical substances in the tested plants is phytochemical screening, also known as a preliminary test. The plant's main metabolites are proteins, carbohydrates, amino acids, vitamins, and fats. The plant contains phenolic and phenol group compounds, glycosides, anthraquinones, alkaloids, flavonoids, tannins, and saponins as secondary metabolites (Khan et al., 2018). It has been reported that the extracts included alkaloids and steroids, according to

phytochemical screening. Both the ethanolic albedo extract and flavedo extracts had substantial levels of alkaloids. All extracts, with the exception of the ethanolic seed extract, had substantial levels of steroids. Except for the ethanolic seed extract, tannins were only weakly present in the other extracts and significantly detected in the flavedo extracts. Except for the hydroethanolic albedo extract, flavonoids were only weakly present in all extracts. While large amounts of saponins were found in the hydroethanolic flavedo extract, none were found in the ethanolic seed extract. The hydroethanolic seed extract and both albedo extracts included cardiac glycosides, while the hydroethanolic albedo extract and both flavedo extracts contained mild amounts of terpenoids (Oikeh et al., 2013).

Table 1: Results of Phytochemical screening

Phytochemicals	Local Lemon	Hybrid Lemon
Steroids	++	++
Flavonoids	++	++
Alkaloids	++	++
Tannins	++	++

Energy Dispersive X-ray (EDX) for hybrid and local lemon

Table 2 shows the EDX analysis of the hybrid lemon. The findings of four samples were based on the results of weight percentage and atomic percentage has been calculated. The above image shows the higher atomic percentage for OK and CK, while percentages of other particles were found lower. Only these two showed the highest ratio of the nanoparticles. The image below shows the higher atomic percentage for OK and CK, while percentages of other particles were found lower. Only these two showed the highest ratio of the

nanoparticles. It has been observed in Figure 2A-2H. Table 2 shows the EDX analysis of hybrid lemon samples 3 and 4. The findings were based on four samples, and the percentage weight and atomic percentage have been calculated. The results suggested that the CK has shown 60.25% and 68.76% of the atomic weights for the CK, while 31.76% and 30.12% have been taken in the OK. Table 2 shows the findings of EDX analysis, which was done on the extracts of local lemon. The findings were based on four samples of percentage weight and atomic percentage have been calculated. It has been observed in Figure 3A-3H. The table shows the findings of EDX analysis, which has been done on the extracts of local lemon. The findings were based on four samples, and the percentage weight and atomic percentage have been calculated. The CK showed 65.82% and 67.51% of the atomic weights for the CK, while 32.54 and 30.62% were taken in the OK.

Table 2: EDX for Hybrid and Local Lemon

Elements	Sample 1		Sample 2		Sample 3		Sample 4	
	Weight%	Atomic %	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %
Hybrid lemon CK	51.66	59.11	50.55	57.75	52.86	60.27	61.27	68.76
Local lemon CK	57.72	65.82	59.28	67.51	51.46	58.90	51.71	59.14
Hybrid lemon OK	38.41	32.99	37.22	31.93	37.12	31.76	35.74	30.12
Local lemon OK	38.01	32.54	35.82	30.62	35.70	30.67	37.12	

Fourier Transform Infrared Spectrum

Figure 4 shows the different functional groups in both local and hybrid lemon peel. It shows various function group, so they have rich chemical diversity. Our result is quite similar to (Indulekha et al., 2017) FTIR spectra give information about the presence of cellulose, hemicellulose, lignin, and pectin.

Ultraviolet Spectroscopy

When combined with chemometric methods such as principal component analysis (PCA) and canonical discriminant analysis, UV-VIS (ultraviolet-visible) spectroscopy in absorbance mode. With the right adjustments, colorimetric assays are flexible methods that can be used with a variety of substrates and extracts. But over time, some adjustments might provide false results, as seen with the nitrite-aluminum assay, which is frequently used in the literature to measure flavonoids (Mare et al., 2023). The figure 5 shows the UV spectrum for both local and hybrid lemon peel. The wavelengths of the lemon peel lie in the range of 200 nm.

Anti-Leishmanial Activities

Evaluation of anti-leishmaniasis impacts of lemon extracts and berberine was performed by the MTT test; it was observed that different concentrations of lemon, especially berberine, altogether ($P < 0.05$) inhibited the growth of promastigotes *L. major* and *L. tropica* in light of a portion subordinate reaction. In both cases, methanolic extracts of hybrid and local *Citrus limon* were more efficacious than aqueous and chloroform extracts in suppressing promastigotes *L. major* and *L. tropica*. The aqueous extract showed the lowest activity against promastigotes *L. major* and *L. tropica*. Overall, the activity of hybrid *C. limon* peel against promastigotes *L. major* and *L. tropica* was much higher than the activity of local *Citrus limon* peel. Berberine was far more effective against promastigote *L. major* and *L. tropica* than crude concentrates of *C. limon*. The IC_{50} values for different extracts of *C. limon* peel and MA (Meglumine antimoniate Glucantime) as a control drug against promastigote types of *L. limon* and *L. tropica* were higher than berberine in both cases.

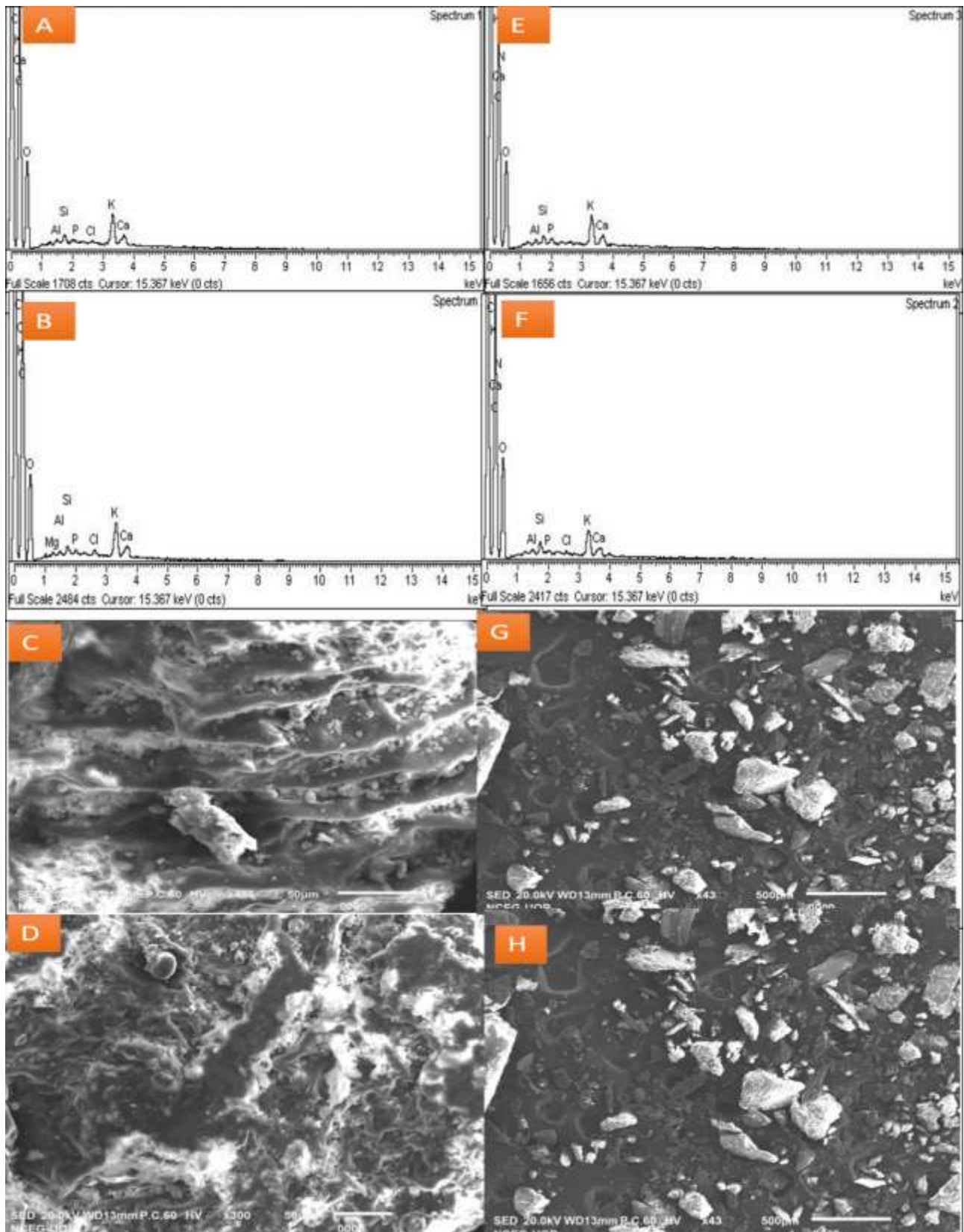


Figure 2. EDX and SEM study of Hybrid Lemon A: EDX Spectrum 1for sample 1 of Hybrid Lemon, B: EDX Spectrum 2 for sample 2 of Hybrid Lemon, C: SEM image of “K” for sample 1 using EDX analysis, D: SEM image of “K” for sample 2 using EDX analysis, E: EDX Spectrum for Sample 3 in Hybrid Lemon, F: EDX Spectrum for Sample 4 in Hybrid Lemon, G; SEM image of “K” for sample 3 using EDX analysis, H: SEM image of “K” for sample 4 using EDX analysis

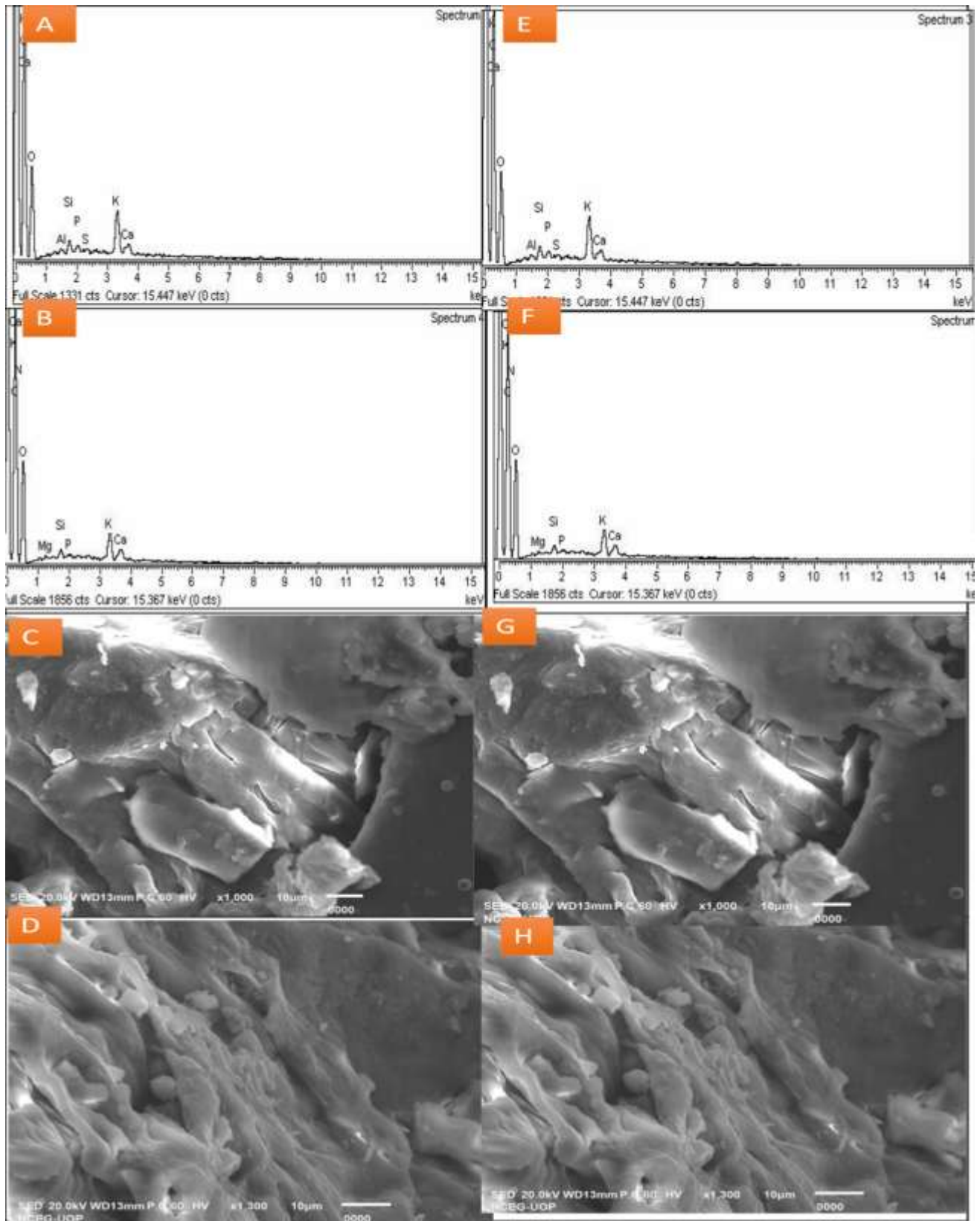
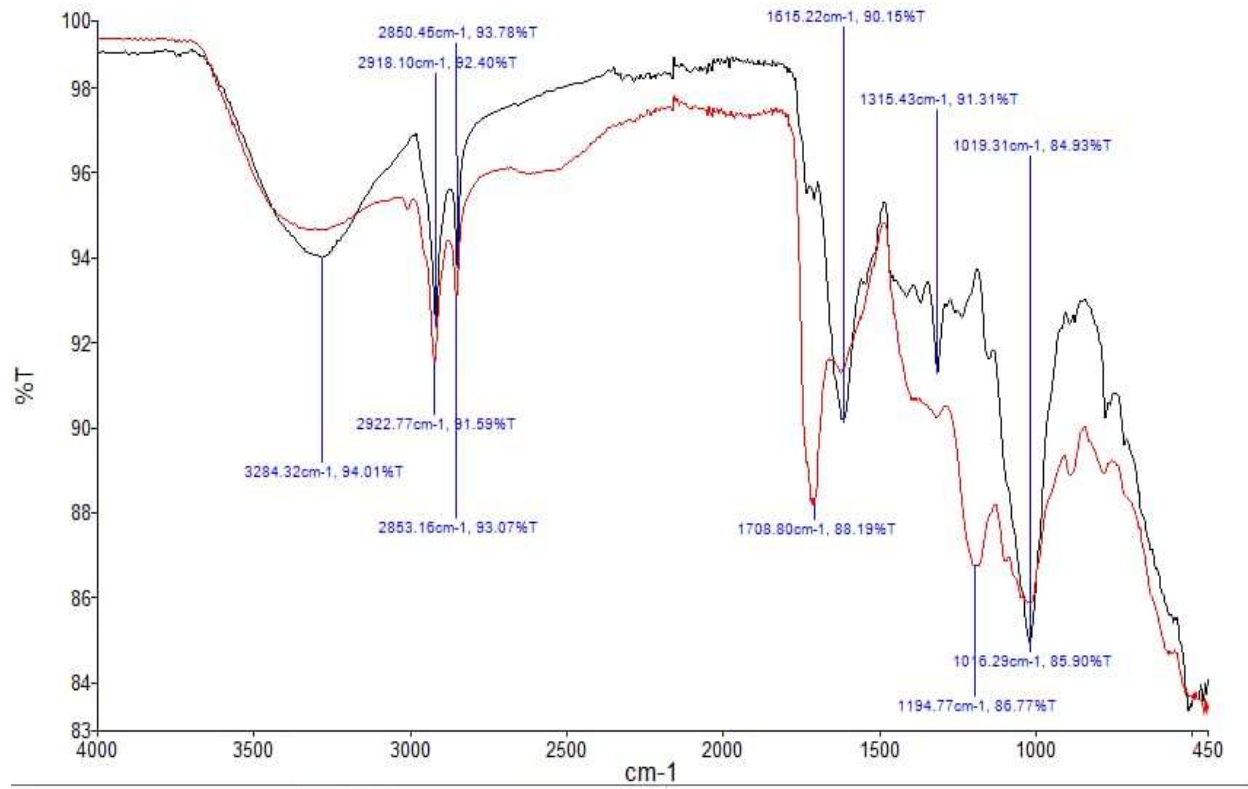
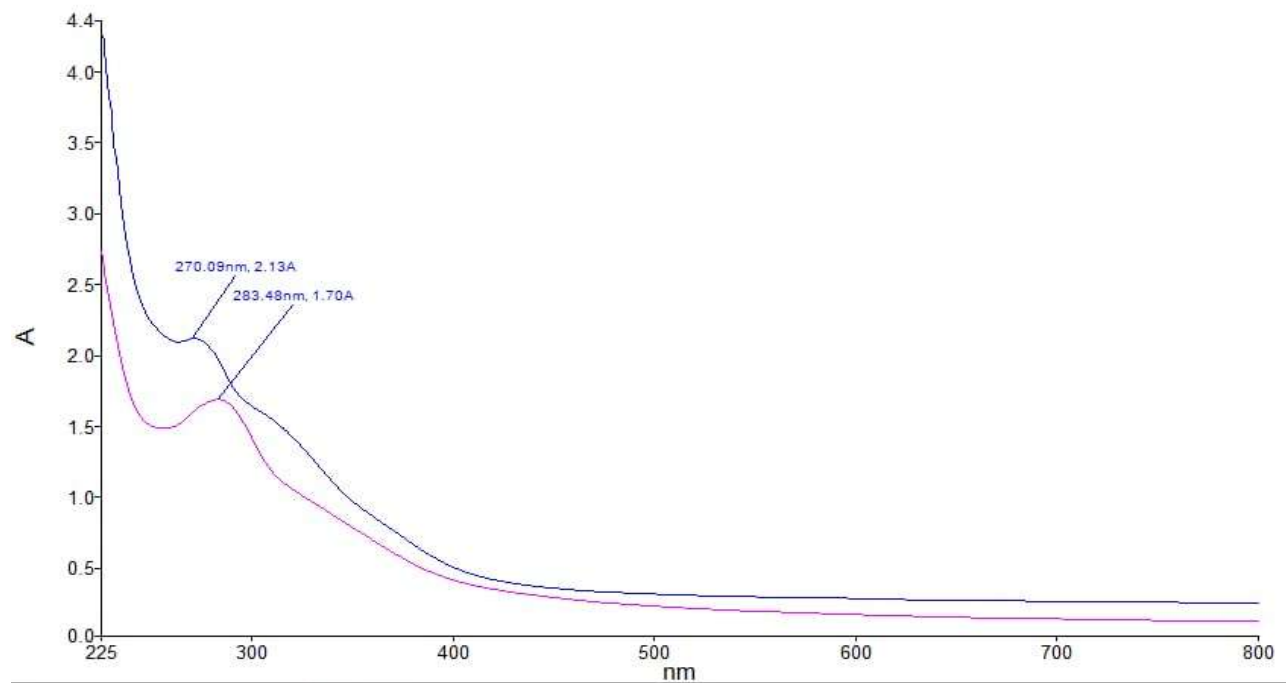


Figure 3. EDX and SEM study of Local Lemon A: EDX Spectrum 1 for Sample 1 in Local Lemon, B: EDX Spectrum 1 for Sample 2 in Local Lemon, C: SEM image of “K” for sample 1 using EDX analysis, D: SEM image of “K” using EDX analysis, E: EDX Spectrum for Sample 3 in Local Lemon, F: EDX Spectrum for Sample 3 in Local Lemon, G: SEM image of “K” for sample 3 using EDX analysis. H: SEM image of “K” for sample 4 using EDX analysis



Name	Description
Hybrid	Sample 017 By Administrator Date Tuesday,...
Local	Sample 016 By Administrator Date Tuesday,...

Figure 4. FTIR Spectra for Hybrid and Local Lemon Peel



Name	Description
Hybrid	
Local	

Figure 5. UV Spectra for Local and Hybrid Lemon Peel

Table 3: Results of Anti-Leishmania activities

	Hybrid		Local	
	<i>L. major</i>	<i>L. tropica</i>	<i>L. major</i>	<i>L. tropica</i>
Methanolic extract	12.8±1.17	16.1±1.15	10.1±1.11	10.55±1.02
Aqueous extract	22.3±2.51	26.6±2.51	19.87±1.98	13.73±1.41
Chloroform extract	19.6±2.08	23.3±2.15	15.10±1.99	12.13±1.53
Berberine	2.1±0.05	2.9±0.05	1.74±0.04	0.93±0.05
MA	8.2±1.15	11.6±0.05	4.67±0.59	7.22±0.05

Anti-cancer Activity

It has been reported that people who eat a lot of fruits and vegetables have a lower risk of developing degenerative illnesses, cardiovascular disease, hypertension, cataracts, strokes, and, most importantly, malignancies, according to a body of evidence from experimental and epidemiological research. The antioxidant properties of the individual or combined bioactive ingredients found in fruits and vegetables, as well as other biochemical and physical traits of the known and unidentified bioactive ingredients, are thought to be primarily responsible for this protective impact. Vitamin C, β -carotene, flavonoids, limonoids, folic acid, and dietary fiber are among the bioactive substances believed to be contained in citrus fruits. Consuming a lot of citrus fruits may lower the chance of developing degenerative illnesses (Silalahi, 2002). With 14 million cases worldwide and 8.2 million deaths in 2012 alone, cancer is the second most common cause of death in the developed world, after aging-related cardiovascular disease. The huge socioeconomic expenses of this cancer epidemic have a significant social impact. To combat cancer, new medications and therapeutic approaches are being developed with the dual goals of curing and preventing the illness. A lot of degenerative diseases have been prevented or treated with natural therapies in recent decades.

The Citrus anticancer potential has been evaluated using standard methodology. After 48 hours, the anticancer potential of different solvent-based lemon peel extracts against the HepG2 cell line was evaluated. Ethyl acetate-based extracts had considerable anticancer activity when compared to the control. The extract of lemon peel in four different solvents, including methanol, ethanol, ethyl acetate, and chloroform, was compared. In contrast to ethanol and methanol, chloroform and, in the case of lemon peel, chloroform extract, the highest inhibition (65.93 percent) was seen at a concentration of 100ug/ml, but in the case of lemon peel ethyl acetate extract, it was 61.75 percent at a concentration of 200ug/ml. The lemon peel extracts derived from methanol and ethanol did not demonstrate substantial cytotoxic action. The strongest cytotoxic activity in the case of methanol extract was 77.38 percent at a concentration 200ug/ml as per observed in Figure 6.

In the previous study by (Ajikumar et al., 2018) examined the anticancer activities from the extracts of

lemon peel. The study reported the biological potential of lemon peels and tested the anticancer potential from *citrus medica* adopted from in vitro assays and in vivo cancer models. The findings of the study argued that the extracts of lemon peel have been found significant for treating the DLA cells in the MTT assay. The study further elaborated that lemon peel is both volatile and non-volatile in nature (*polymethoxy flavones*) due to its anticancer constituents. The study has recommended that a large amount of wasted lemon peel can be used for the treatment of anticancer activities. Restorative plants, as a result of the pervasiveness of this infection, compound medications' inability to completely control it, an increase in the quantity of reports on drug resistance, and disconnected examination as an afterthought impact of engineered drugs. As a rule, the utilization of therapeutic spices for the treatment of different illnesses has a long history. In view of Iran's varied environment and verdure, we can possibly identify the dynamic herbal ingredients in various native plants of the nation and extract them to produce them on a modern scale. Citrus natural items are widely consumed worldwide as new produce, juice, and, most often, the strip is discarded as waste, which contains a wide combination of secondary components with critical malignant growth avoidance specialist development in association with various parts of the regular item (Manthey et al., 2001). As a result, a great deal of strip is conveyed every year. Citrus strip, the primary waste, is a valuable source of molasses, gelatin, and limonene and is regularly dried, mixed with dried pulps, and sold as dairy cattle feed (Bora et al., 2020)

The ratio is higher in developing countries than developed countries. Literature has argued that the extracts of lemon peel have been found significant for treating the DLA cells in the MTT assay. Different assessments have recognized the presence of polyphenols, supplements, minerals, dietary fibres, essential oils, and carotenoid content, which makes citrus a health-benefit propelling natural product. To this regard a couple examples about the usage of citrus regular items as supportive fixes can be cited: oranges to fix scurvy (Sica, 2006), grapefruits as experts fit to lower beat and to dial back calcium channel blockers (Magiorkinis et al., 2011), citrus flavonoids as practical in vivo agents prepared to change hepatic lipid assimilation (Cha et al., 2001), pressed orange to

prevent and adjust inflammatory processes (Coelho et al., 2001), kumquat strip polyphenolics as effective cell support trained professionals (Sadek., 2009), grape fruit juice having against genotoxic impacts and a couple of others. Essential oils extracted from citrus are also thought to have anticancer properties. The gannan navel orange (*C. sinensis*) has the ability to inhibit the growth of human lung and prostate cancer cells. Another plant with anti-neoplastic properties against colorectal cancer cells is *Citrus medica*. In vitro, it has been discovered that the *C. limon* essential

oil-nanoemulsion causes A549 cells to undergo apoptosis. The ability of *C. paradisi*, *C. limon*, and *C. aurantifolia* to inhibit different cancer cell lines. The most efficient and able to induce apoptosis of these was *C. paradisi*. Using the peel waste of commercially used *Citrus limetta*, which was gathered from juice stores, as the source of essential oil for the analysis is what makes this work unique. Additionally, we examined the potential and constituent chemicals (Narayanankutty et al., 2022).

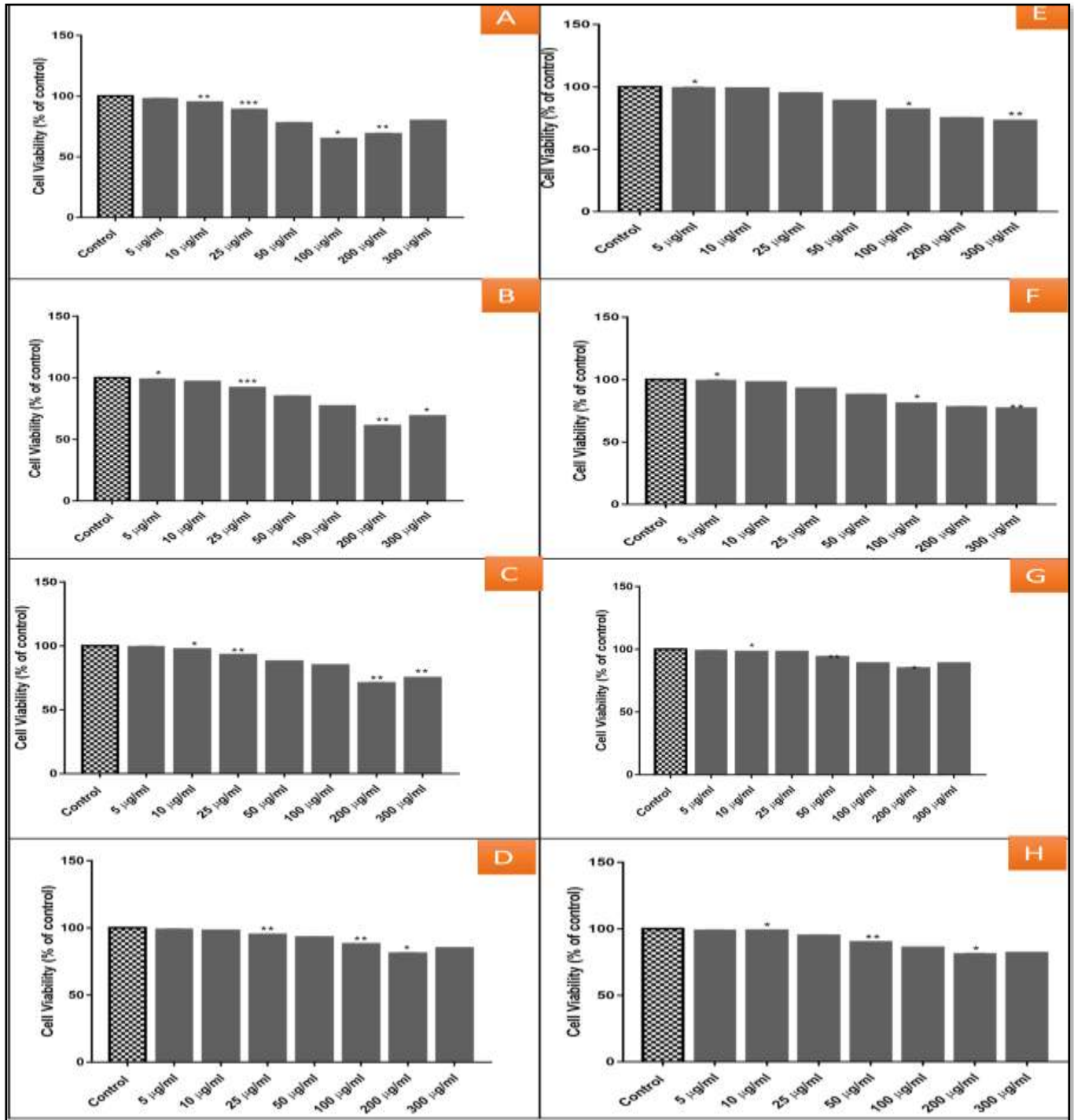


Figure 6. Anticancer Potential of Citrus extract. Triplicate tests were run. Symbols *, **, and *** signify * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ relative to the control group A: After 48 hours, chloroform-dissolved lemon peel extract was tested on HeLa cervical cancer cells for cytotoxicity., B: After an incubation period of 48 hours, an extract of lemon peel dissolved in ethyl acetate was placed to cytotoxicity assay against HeLa cervical cancer cells. C: Cytotoxicity assay of methanol-based lemon peel extract against HeLa cervical cancer cell line after 48 hours of incubation. D: After 48 hours of incubation, ethanol-based lemon peel extract was tested for cytotoxicity against HeLa cervical cancer cells. E: After 48 hours, chloroform-dissolved hybrid lemon peel extract was tested on HeLa cervical cancer cells for cytotoxicity. F: After 48 hours, chloroform-dissolved hybrid lemon peel extract was tested on HeLa cervical cancer cells for cytotoxicity. ,G: After 48 hours of incubation, ethanol-based hybrid lemon peel extract was tested for cytotoxicity against HeLa cervical cancer cells. H: Cytotoxicity assay of methanol-based hybrid lemon peel extract against HeLa cervical cancer cell line after 48 hours of incubation.

Conclusion

Medicinal plants are highly significant in the community and consist of multiple chemical substances which result in different physiological actions in the body. The citrus fruits have anticancer and antileishmanial activities due to the composition of Alkaloids. According to the FTIR results, functional groups are present in both local and hybrid lemon peels. The alkane and amines are present in local and hybrid lemon peels, while ketones and tertiary alcohols are present in local lemon peels, and carboxylic acid and amine groups are present in hybrid lemon peels. In our research, local *Citrus limon* peel showed the highest activity against promastigote *L. major* and *L. tropica* for antileishmanial activity as compared to hybrid limon. The current study further proposes that the anticancer activity of local limon peel is significant as compared to hybrid limon peel. Based on the current study, methanol has significant inhibition for anti-leishmanial activity, while chloroform and ethyl acetate have anticancer activity of local and hybrid limon peel. Overall, the study concludes that local limon peel is more significant as compared to the hybrid limon and therefore, can be used to cure leishmaniasis and cancer.

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Statements and Declarations

Authors' contributions

AU., B., FS: Conceptualization, SK., FAK., IAK; methodology, investigation, data curation, formal analysis, B., KB., AA., writing – original draft, AU., FS., SK., QA., BG., writing – review and editing, visualization (including tables and figures), and project administration. SA and QA: Contributed through general discussion and review of the final manuscript.

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The authors declare that they have no conflict of interest.

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Declaration of Generative AI and AI-assisted Technologies in the Writing Process

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