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Use of Diaion HP20 Resin to Achieve High Rutin Containing *Moringa oleifera* Extract: Its Solubility and Anti-Bacterial Properties and Possible Applications in Nebulizer Formulation for COVID Patients

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Abstract

Rutin with several pharmacological properties has been reported to be an effective inhibitor for SARS-COV-2 viral protease. Due to lack of specific drugs available for treatment of covid infection, hunt is on for possible herbal supplements that will impact multiplication of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and also provide immunity to people against such infections. We report a simple and cost-effective method for extraction of rutin from *Moringa oleifera*, a plant with high nutritive value due to its rich contents in minerals, vitamins and other essential phytochemicals. The solubility of rutin in water was achieved using rutin-arginine mixture (identified as a good water-soluble version of rutin) and tested for its antibacterial activity against *Klebsiella pneumoniae* and *E. coli* by MIC studies and compared with rutin solubilized in methanol. Our results demonstrate easy scale of the rutin extraction process and such a process could be applicable to extraction of rutin from other medicinal plants as well. We demonstrate that rutin purified through column chromatography has 20% higher solubility in water and maintains its anti-bacterial properties against *Klebsiella pneumoniae* and *E. coli*. Our findings reveal new possibilities of using resin column chromatography for concentrating rutin from plant extracts. Also, potential use of water soluble rutin is envisaged for development of nebulizers for treatment of asthma, hyperglycemia, and pneumonia, seen in COVID-19 affected patients.

Keywords: Rutin, *Moringa oleifera*, COVID-19, protease, inhibitor, asthma

1. Introduction

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside), a nutritional food component (Harborne, 1986), is a flavonol, abundantly found in plants, such as passion flower, buckwheat, tea, apple, apricots, cherries, grapes, plums and oranges (Enogieru et al., 2018). Rutin has antibacterial and antiprotozoal properties including pharmacological activities of being an effective antitumor, anti-inflammatory and an anti-viral agent, to name a few. Rutin is known to protect plants against ultraviolet radiation or plant pathogens and is also used to prevent the side effects of some diseases such as cancer treatments, diabetes, and hypercholesteremia (Mehta, Murillo, Naithani & Peng, 2010; Yang et al., 2016). Rutin was estimated in *Asparagus officinailis* by Motoki et al. (2019) where they demonstrate the maximum rutin presence in plant parts and storage roots.

Based on molecular modelling studies, rutin has been proposed to be an effective anti-covid agent (Wu et al., 2020). It is well established by several studies that the SARS-CoV-2 virus enters its target cells through angiotensin-converting enzyme 2 (ACE2) (Anand et al., 2003) and rutin has been shown to bind to ACE2 receptor effectively, making it an interesting molecule as an anti-covid effector. Rutin is able to associate in the active site of 3CLpro, interacting with the catalytic dyad (His41/Cys145), hence has been suggested to be an effective drug for control of covid (Rizzuti et al., 2021). 3CLpro is a viral protease that is highly conserved among the different members of the coronavirus family with no similarity with human host–cell proteases. Since this protease plays a crucial role in multiplication of SARS-CoV-2, this protease is an attractive target for drug discovery researchers. The 51

phytochemicals from the traditional medicine used for treating flu in Saudi Arabia was analyzed for interaction with the covid protease and rutin was found to have the maximum binding score (Al-Zahrani, 2020) making it an attractive target for further studies. Very recently, rutin has also been shown to affect the activity of RdRp, the polymerase that is essential for replication of the covid-2 virus (da Silva et al., 2020). Additionally, rutin was suggested as a potential anti-SARS-CoV-2 Mpro following a virtual screening of 2030 natural compounds (Xu et al., 2020; Mittal et al., 2021).

Rutin inhibits clumping of platelets and is known for its anti-oxidant and anti-inflammatory nature. Its use as an anti-gingivitis agent in toothpaste is also explored. Due to its low aqueous solubility and in turn low bioavailability, use of rutin in topical applications is limited. The aim of this article is to give an overview of the various extraction methods employed for the extraction of rutin, as well as the recent findings of its potential uses in covid treatment.

Also, recent conflicting reports of Eldalawy (2013) showed that 931 mg/100g *Ruta graveolens* plant is achieved which reflects a yield of merely 1% rutin while Molnar, Jakovljevic, and Jokic (2018) report rutin extraction of 1.8%/100 g of *Ruta graveolens* (14). The rutin extracted from the leaves of *E. pulcherrima* using 80% methanol is reported with no data on quantification (Shlini, Clare, & Immaculate, 2020). Rutin has been quantified in thirty four plants of Pakistan recently where highest amounts of rutin was found in seeds of *Sophora secundiflora* (8.4%) and leaves of *Mangifera indica* (5.2%) (Shafi & Ikrum, 2016). Other reports include ~3% rutin in Buckwheat (Kreft, Strukelj, Gaberscik & Kreft, 2002), followed by Japanese Pagoda tree (61.76 mg/g) and Rue with 9.68 mg/g (Lachman, Orsák, Pivec & Faustusová, 2000). The content of flavonoid expressed as rutin equivalents, varied from 14.9±0.4 to 60.9±2.2 mg rutin equivalent/g extract of *Torilis leptophylla* (Saeed, Khan & Shabbir, 2012). By HPLC, the results showed that soxhlet extraction with 50% ethanol gave rutin content of 10.21% (w/w) (Chaisawangwong & Gritsanapan, 2008). The rutin content in *C. spinosa*, a plant known for its medicinal properties, showed 2.76% in leaves and less than in buds and fruits (Musallam, Duwayri, Shibli, & Alali, 2012) while Chua (2013) describes all the methods employed for extraction of rutin from plant sources. The presence of rutin has been reported in many plant species, but only a limited number of plants such as *Fagopyrum esculentum* and Cappris species, especially, *C. spinosa* are identified as the biggest plant sources of rutin (Kianersi et al., 2020).

Moringa oleifera Lam., also known as Drumstick tree, is a medicinal plant that is indigenous to the western and sub-Himalayan tracts, including India, Pakistan, Asia, Africa, and Arabia and is known for presence of natural antioxidants such as ascorbic acid, flavonoids, phenolics, and carotenoids (Siddhuraju & Becker, 2004). Isolation of rutin from *Moringa oleifera* has been attempted by several workers for the past several years and different types of solvents have been evaluated for the efficient extraction of rutin from various plant parts. In this article, we describe a simple and cost-effective method of higher extraction of rutin from Moringa leaves and discuss the possible implications of such extraction procedures on rutin extraction from other plants.

The major limitation in inclusion of rutin in foods and food supplements is its low water solubility (0.125 g/L). Interestingly, a recent report by Sancineto et al. (2021) show that arginine improves the solubility of rutin dramatically, and such an improved soluble rutin demonstrates higher anti-SARS-COV-2 protease activity, but not its antiviral activity in Vero cells infected with SARS-COV-2 virus. This indicates that arginine not only improves the solubility of rutin but also enhances its biological activity (Acquaviva et al., 2009).

2. Method

2.1 Materials

Rutin hydrate (purity: > 94%) was procured from Sigma Aldrich, USA. Diaion HP20 resin was procured from IPSUM Life Sciences LLP (Mumbai, India). The bacterial strains *Klebsiella pneumoniae* (MCC 2451) and *Escherichia coli* (MCC 2412) were procured from National center for microbial resource (NCMR), Pune, India.

2.2 Plant Material

Moringa oleifera leaves were procured from Amrita Herbs, Andhra Pradesh, India. The identity of the plant was confirmed and documented by Dr. P. Sanathan, a taxonomist at Durva Herbal Centre, Chennai, Tamilnadu, India.

2.3 Regular Rutin Extraction Process (Process A)

The grounded 500g dried leaves were extracted with 6 volumes of a suitable solvent at 75-80°C under reflux conditions for 3 hrs. Total 3 extraction cycles were performed. All the three extraction cycle liquids were mixed and filtered through Whatman filter paper no 1. Later, the liquid extract was concentrated on Rotary evaporator to get methanol free extract (750 mL).

2.4 Enrichment of Rutin Using Resin (Process B)

Diaion HP20 is an effective resin that acts as an adsorbent to separate traces heavy metal ions and is useful for the purification of peptides, proteins, fermentation products and polyphenols. Due to its large pores, it is used as an adsorbent for open column chromatography. The pre-treatment of resin was carried out with treatment of 95% ethanol for 24 h and then washed with deionized water to remove ethanol. Then resin was soaked in 1.0 M hydrochloric acid for 3 h, and washed with deionized water till the pH was 7.0.

The column chromatography of *M. oleifera* extract was carried out using HP20 Diaion resin (bed volume 1 L) on a glass column. The concentrated 750 mL *M. oleifera* liquid extract was loaded on resin for adsorption. After adsorption, the elution was initiated with 1 bed volume of water and then using acetone until get colorless fraction. The collected acetone fractions were then concentrated and dried on rotary evaporator (Rota evaporator, Buchi, India) at 50 °C.

2.5 Rutin Analysis by HPLC

Method of Mustabha et al. (2011) and Habib et al. (2016) was employed with minor modifications for carrying our rutin analysis by RP-HPLC.

2.6 Chromatographic Conditions

The HPLC was carried out on an Inertsil ODS, 3V C18 column (4.6 × 250 mm, 5 µm) using Waters Corporation HPLC system that consisted of quaternary pump (Alliance e2695), PDA detector (Waters 2998) with online degasser, an auto-sampler and column oven. The mobile phase consisted of a mixture of water: methanol: trifluoracetic acid (60:40:0.1% v/v). The injection volume was 20µl with flow rate 1.2 mL/min. HPLC chromatograms were recorded at 256 nm. The elution was carried out at ambient temperature (27 ± 1 °C). The sample integration was done using Chromeleon (version 7) software.

2.6.1 Standard Preparation

The stock solution of standard was prepared by adding 5 mg of reference standard Rutin hydrate (Sigma Aldrich, USA) into a 50 ml volumetric flask. Then 30 mL methanol was added & sonicated to dissolve and volume was made up to 50 ml with methanol to achieve 100 µg/ml concentration.

2.6.2 Sample Preparation

Around 250 mg of the extract was added in a 50 ml of volumetric flask containing 30 ml of methanol (diluent), and sonicated for 20 minutes. After making up the volume to 50 ml with methanol, the sample was mixed thoroughly by inversion, cooled to room temperature and filtered through 0.45 µm filter paper and 20 µL was injected into the HPLC system.

2.7 Growth Conditions of the Bacterial Strains

Both the strains grown aerobically in soybean casein digest medium (SCDB) at 37 °C in shaking incubator adjusted at 150 rpm for 16 hours.

2.8 Minimum Inhibitory Concentration (MIC) Determination

The MIC value for the compounds against the bacterial strains was determined by broth microdilution method. The stock solution of rutin hydrate (14 mg/ml) was prepared in methanol and the stock solution of L-arginine (28 mg/mL) was prepared in sterile water. The mixture was prepared by grinding rutin hydrate and L-arginine in 1:2 ratio (14 mg rutin hydrate and 28 mg L-arginine) in a pestle-mortar. The resultant mixture was dissolved in water by heating at 80 °C for 2-3 min. The stock solutions of the compounds was diluted 5 times in sterile SCDB (Stock II) and used for MIC determination. This stock II was diluted two fold serially in the wells of 96 micro titer plate and 100 µL of bacterial culture (~10^6cfu/ml) was added in each well. The concentrations of the compound ranging from 1400 µg/ml to 5.46 µg/ml were used to determine the MIC values. The wells with no compounds (only diluent) were used as control for 100% growth. The microplate was incubated at 37 °C for 24 h in shaking incubator adjusted at 150 rpm. After incubation, the absorbance was recorded at 600 nm using Multiskan Skyhigh microplate reader (Thermo Fisher Scientific, Massachusetts, USA). The MIC is the lowest concentration of the compound that completely inhibited the bacterial growth.

2.9 Antibiotic Susceptibility of Bacterial Strains

The antibiotic susceptibility of the bacterial strains was studied by using Kirby-Bauer disk diffusion susceptibility test protocol. In short, the bacterial colonies were suspended in 2 mL sterile saline solution and turbidity of the solution was adjusted at 0.5 McFarland Standard. The resulting suspension was spread on SCDA and antibiotic discs were placed using sterile forceps. The plates after incubation at 37 °C overnight were observed for the zone

of inhibition and the diameters of zone of inhibition measured and recorded. The Method section describes in detail how the study was conducted, including conceptual and operational definitions of the variables used in the study, Different types of studies will rely on different methodologies; however, a complete description of the methods used enables the reader to evaluate the appropriateness of your methods and the reliability and the validity of your results, It also permits experienced investigators to replicate the study, If your manuscript is an update of an ongoing or earlier study and the method has been published in detail elsewhere, you may refer the reader to that source and simply give a brief synopsis of the method in this section.

3. Results and Discussion

Table 1 summarizes the earlier reports of all the studies carried out to date on isolation of rutin from *Moringa oleifera* leaves. In our initial trials, we attempted extraction of rutin using different solvents namely water, acetone, ethanol and methanol. It is evident from Table 1 that the extraction of rutin from *Moringa oleifera* leaves was highly variable. Although, the ethyl acetate defatted material followed by methanolic extraction showed the highest rutin yield of 4.4%, since the resultant extract was sticky and could not be dried, we opted for the next best extraction method which was 70% methanol that yielded 16-18% extract with 3.3% rutin content. Our observations support the report of ethanol and methanol being the most ideal solvent for rutin extraction in general for several plants (Stalikas, 2007).

Figure 1 A shows the regular process employed for rutin extraction from Moringa leaves using methanol as the solvent based on our initial extraction experiments as detailed in Table 1 while Figure 1B depicts the modified methodology developed in our laboratory on rutin extraction that employs use of a Diaion HP20 resin for rutin enrichment. To the best of our knowledge, this is the first report of highest yield of rutin from *Moringa oleifera* leaves.

Table 1. Rutin content from *Moringa oleifera* leaves using different solvents

S.N.	Extraction solvent	Yield	Rutin content/g extract
1	Water	17.4%	1.57%
2	50% methanol	25%	2.37%
3	70% ethanol	14%	2.65%
4	70% methanol	16-18%	3.3%
4	50% methanol (maceration)	25%	2.37%
5	100% methanol	20%	2.70%
6	Defatting with ethyl acetate solvent followed by methanolic extraction (sticky material)	12%	4.4%
7	Alkaline water (pH 11-12)	18%	1.37%
8	7% β-cyclodextrin in water followed by 70% methanolic extraction	3.2%	2.82%

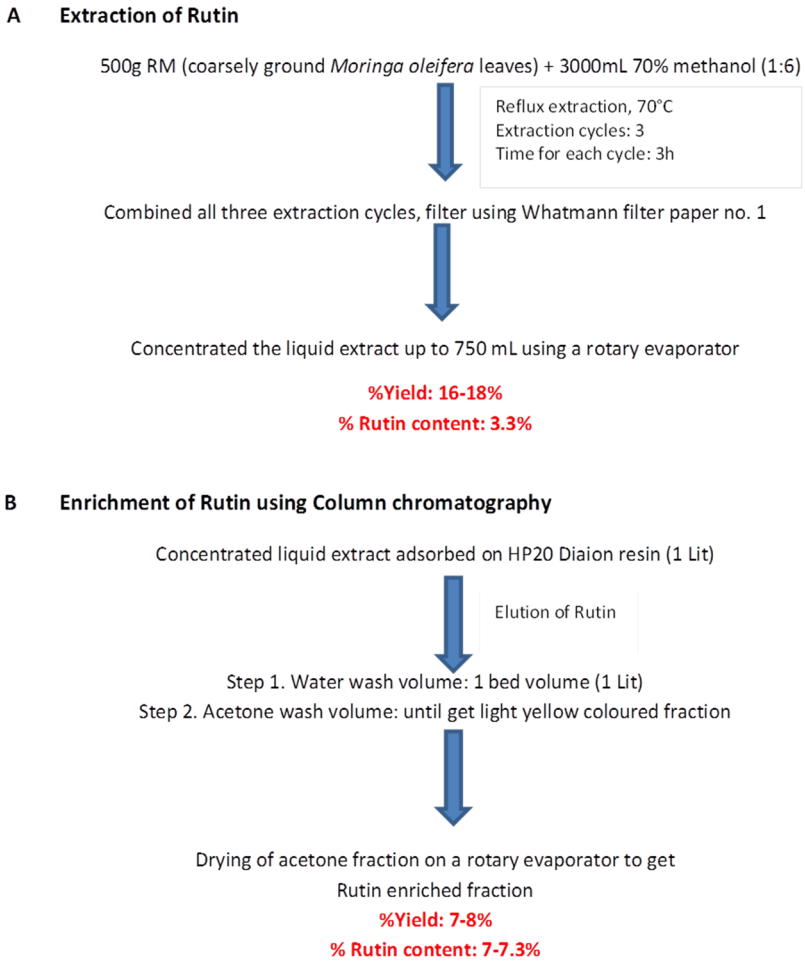

Figure 1. Flow chart depicting process flow for making *Moringa oleifera* leaf extract. Process A shows methanolic extraction process while process B shows the processing of methanolic extract on resin chromatography

The rutin content achieved from *Moringa oleifera* leaves is highly variable based on the solvent, time and extraction temperature employed for its isolation (Table 2). The values ranged from 0.006% (Mohamed et al., 2021a), 0.014% (Cuellar-Nuñez et al., 2018), 0.039% (Bajpai, Pande, Tewari, & Prakash, 2005), 0.065% (Mohamed, Ibrahim, Abdel-Azima, & El-Missiry, 2021b), 0.072 % (Zhu, Yin, & Yang, 2021), 0.085% (Valdez-Solana et al., 2015), 0.12% (Dessalegn & Rupasinghe, 2021), 0.183 % and 0.189 % (Amaglo et al., 2010; Lin et al., 2021), 0.61% (Bennour et al., 2019) with methanol and an yield of rutin of 2.25% (Khudaer, Hassn, AL-Sammarrae, & Ibraheem, 2016) using 70% methanol. With ethanol, the rutin yields were also variable and ranged from 0.0345 (El-Fadl et al., 2020), 0.097% (Ahmed, Jahan, Jahan, & Hossain, 2021), 4.5% (Fombang, Nobossé, Mbofung, & Singh, 2020) and 6.2% (Vongsak et al., 2013). Upon the use of mixtures of ethanol and methanol, there was hardly any improvement of rutin extraction (4.43%) (Sancineto et al., 2021).

Table 2. Rutin Content in *Moringa oleifera* leaf extract using various extraction solvents

S.N.	Solvent used	% Rutin Content	Reference
1	Methanol	0.006	Mohamed et al. 2021a [30]
2	Methanol	0.014	Cuellar-Nuñez et al 2017 [31]
3	Methanol	0.039	Bajpai et al. 2005[32]
4	80% Methanol	0.065	Mohamed et al. 2021b [33]
5	Ethyl acetate	0.31	Mohamed et al. 2021b [33]
6	Butanol	1.03	Mohamed et al. 2021b [33]
7	Methanol	0.072	Zhu et al. 2021 [34]
8	Methanol	0.085	Valdez-Solana et al. 2015 [35]
9	Methanol	0.120	Dessalegn et al. 2021 [36]
10	70% methanol	0.183	Amaglo et al. 2010 [37]
11	Ethanol	0.189	Lin et al. 2020 [38]
12	70% Methanol	0.61	Bennour et al. 2019 [39]
13	70% Methanol	2.25	Khudaer et al. (2016) [40]
14	Ethanol	0.034	El-Fadl et al. 2020 [41]
15	Ethanol	0.097	Ahmed et al 2021 [42]
16	70% ethanol/ 80% methanol	4.43	Siddhuraju and Becker, 2003 [25]
17	Ethanol	4.5	Fombang et al. (2020) [43]
18	70% ethanol	6.2 (isoquercetin equivalent)	Vongsak et al., 2013 [44]
19	Water + 1% acetic acid	6.03	Oyeleye et al. 2019 [46]

Use of non-polar solvents such as ethyl acetate that are known to improve extraction of non-polar compounds (Yewale et al., 2021), however, the rutin yield was merely 0.31% with ethyl acetate while with butanol also the yield was merely 1% (Mohamed, Ibrahim, Abdel-Azima, & El-Missiry, 2021b) while acidic water yielded 6% rutin (Oyeleye et al., 2019).

The HPLC chromatograms of rutin extracted with water, methanol and resin purified from *Moringa oleifera* leaves is given in supplementary Figures (Figures. S2, S3 and S4 respectively). The HPLC chromatogram of the standard rutin is shown in supplementary Figure S1.

We see that the rutin isolated using the resin column chromatography showed 20% higher solubility than the rutin obtained by plain methanol extraction from *Moringa oleifera* leaves (Figure 2) with no change in the absorption spectra of the isolated and concentrated rutin (data not shown). This indicates that the column chromatography does remove some inhibitors from the methanolic extract of rutin and render the rutin more soluble in water.

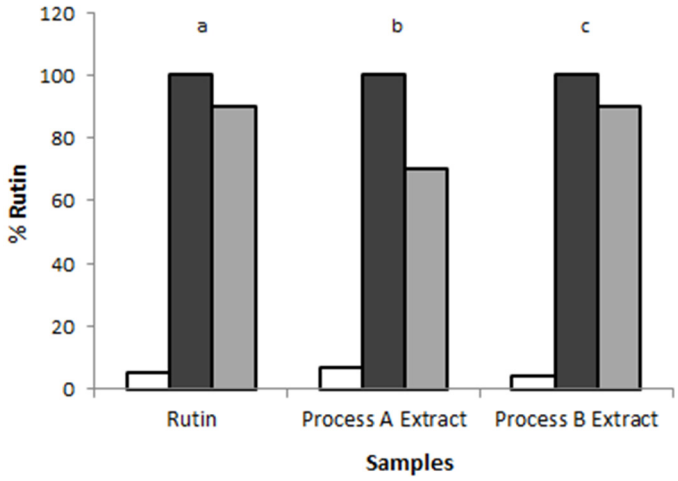

Figure 2. Solubility pattern of Rutin. Panel a shows standard rutin, panel b shows rutin from methanolic extract from *Moringa oleifera* leaf (Process A) and panel C shows rutin extracted from *Moringa oleifera* leaf purified using resin chromatography (Process B)

Our results on higher water soluble property of the rutin purified using column chromatography supports observations of Sancineto et al. (2021) who reason that the addition of two molar equivalents of L-arginine makes rutin more soluble through an acid-base interaction and by a pi cation interaction affecting the formation of rutin/rutin intermolecular interactions (Sancineto et al., 2021). Since basic amino acids such as arginine and lysine are capable of delivering large molecules into bacterial cells gaining access to the cell interior (Madani et al., 2011), we hypothesized that rutin being a small molecule might be hindered to show its affect in bacterial killing due to its hydrophobic nature, which could be addressed by co-administration with arginine. Hence, we tested the antibacterial activity of rutin with arginine at 1:2 ratio and rutin solubilized in methanol.

Rutin is shown to have activity against *Staphylococcus aureus* (Amin, Khurram, Khattak, & Khan, 2015) with an MIC of 280 µg/ml while the MIC's of rutin against *K. pneumoniae* ATCC700603 and *E. coli* ATCC25922 has been shown to be 1024 µg/mL and 512 µg/mL respectively (Wang et al., 2021). We examined the MIC of rutin in methanol and rutin-Arg against the bacterial strains *Klebsiella pneumoniae* (MCC 2451) and *Escherichia coli* (MCC 2412), which are resistant to majority of the tested antibiotics (supplementary Table S1) and the results indicate that the MIC's of both rutin in methanol and rutin+arginine are similar indicating that L-arginine solubilizes rutin and also maintains the antibacterial properties of rutin (Figure 3).

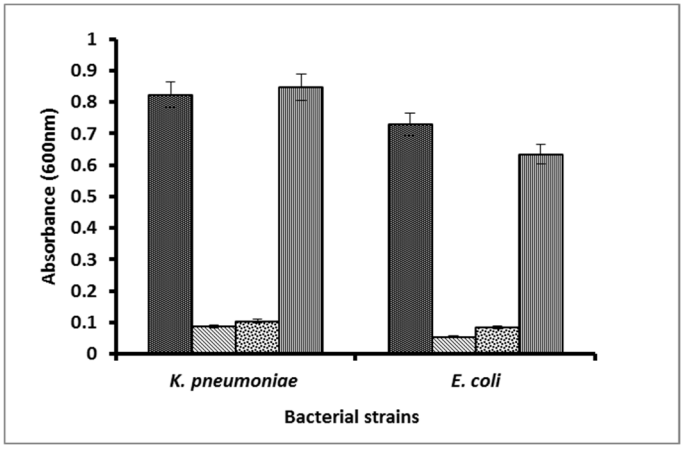

Figure 3. Effect of rutin on growth of *Klebsiella pneumonia* and *E. coli* by MIC studies. All experiments were done in triplicates. Values are mean ±SD, n=3. ****P<0.001, when compared to cell control

The emergence of multidrug resistance (MDR) and extensive drug resistance (XDR) in *Klebsiella pneumoniae* strains and *E. coli* has posed great threats to human health and flavonoids such as rutin has been shown to have anti-bacterial activity against these bacteria, hence the disclosure of maintenance of antibacterial properties in water: arginine mixture assumes critical importance.

The rutin extraction process from other plant sources have been studied extensively. It is clear from literature reports that irrespective of the methods simple or laborious, the rutin yield is highly variable. Sofic et al. (2010) have described rutin extraction from 50 medicinal plants and observed *Ruta graveolens* leaves to have maximum content of rutin (8.6g/100g raw material) followed by buckwheat leaves (5.6%) and least content of rutin in lemon balm leaves (0.25%). Similarly, Mostafa (2017) has estimated rutin content in 25 Egyptian medicinal plants and the results indicated that quantitative estimation of rutin in the tested samples ranged from 4.4 to 158 mg/g extract. Kraujalis and coworkers (Kraujalis, Venskutonis, Ibáñez, & Herrero, 2015) have reported 1.3% rutin from *Amaranthus paniculata* leaves using ultrasound assisted extraction with methanol. Since such results purely depend on the amount of extract yield per gram of raw material and the type of solvent used for extraction, we believe the rutin estimation results not very lucid for interpretation. It is tempting to speculate that our resin column chromatographic method is an attractive method to concentrate rutin in the plant extracts and appears scalable (Kraujalis, Venskutonis, Ibáñez, & Herrero, 2015).

Moringa oleifera, also called as Miracle Tree, is rich in proteins, phenolic acids, flavonoids, glucosinolates and Isothyocinates (Brunelli et al., 2010). Since this plant grows both in severe drought and mild frost conditions, it is widely cultivated for either nutritional or commercial purposes (Amaglo et al., 2010). Uses of *Moringa oleifera* for asthma, hyperglycemia, and pneumonia, make it an attractive herbal agent to use in human diseases such as the recent COVID-19 pandemic. Moringa leaves also have a low calorific value, hence is an ideal diet component of the obese. Due to its high iron, zinc content, its use in anemic conditions is well-known (Gopalakrishnan, Doriyaa, & Kumar, 2016). Also, U.S. Food and Drug Administration (FDA) approved Quercetin and its conjugates for human use. Both quercetin and rutin are used as ingredients in numerous herbal remedies and have been extensively studied for their multiple pharmacological activities, including antiviral, antibacterial, and anti-inflammatory properties (Latos-Brozio & Masek, 2019), hence alternate methods that aid in higher availability of active constituents would be beneficial to drug manufacturers.

Cho and Lee (2015) have reported rutin content as high as 31% from buckwheat grains. However, on close look at the process followed, we realize that the actual rutin content in buckwheat grains was 3.61% which increases after steam treatment that is 4.99%. Further extraction was performed using ultrasonic agitation for enrichment of rutin to 31%. We believe such steam treatment and ultrasonic agitation is not feasible in manufacturing scale, hence our method of enrichment of rutin to 7% using two steps process utilizing a simple resin chromatography appears feasible and cost-effective.

Extraction by maceration or using Soxhlet apparatus and/or by reflux using solvents is the most commonly used methods for the extraction of polyphenols from herbal plant materials. Some of the modern extraction methods for extractions of polyphenols such as rutin include microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), accelerated solvent extraction (ASE), ultrafiltration (UF) (Ajila et al., 2011; Azwanida, 2015) with solvents such as methanol, ethanol, acetone, diethyl ether and ethyl acetate. Other factors, such as pH, temperature, sample to solvent volume ratio, and the number and time intervals of individual extraction steps, also play an important role in the extraction procedure. Our current procedure of simple reflux of *Moringa oleifera* leaf with methanol followed by resin chromatography appears an interesting proposition that can be replicated in other herbal plants.

Infections caused by antibiotic-resistant gram-negative bacteria *E. coli* and *K. pneumoniae* are increasing in frequency in hospitals in the United States (Wiener et al., 1999; Zanichelli et al., 2019). Gram-negative bacteria in particular multidrug-resistant bacteria such as *K. pneumoniae* and *Escherichia coli*, are more often responsible for nosocomial pneumonia seen in COVID-19- infected patients (García-Meniño et al., 2021; Arcari et al., 2021; Dayoub et al., 2021). Hence, it is always beneficial if the drug is delivered directly to the lungs for faster recovery of affected patients. To deliver a drug by nebulization, the drug must first be dispersed in a liquid (usually aqueous) medium (O'Riordan, 2002). While some drugs readily dissolve in water, some require co-solvents such as ethanol or propylene glycol for solubilization. With the current observations of effective solubility and function of rutin-arginine mixture, it is tempting to speculate the possibility of development of a nebulizer solution of rutin and arginine in water in place of strategies of delivery of water-insoluble drugs, as liposomes (Tulbah & Lee, 2021). Highest hepatoprotective effects was seen in animals with hepatic injury due to oxidative stress by the co-administration of rutin and L-arginine (Acquaviva et al., 2009; Costa et al., 2014), hence use of arginine in the

nebulizer formulation of rutin appears feasible. Also, L-arginine preserves the integrity of the intestinal epithelium (Wang et al., 2021) and acts as an additional source of energy for cell growth since it is converted to ornithine and ammonia with the production of 1 mol ATP/mol arginine consumed acting as an additional energy for cell growth (Tonon & Lonvaud-Funel, 2000). We are in the process of developing such formulations of rutin with arginine and other excipients to optimize drug solubility and stability and these aspects will be disclosed elsewhere.

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Competing Interests Statement

The authors declare that there are no competing or potential conflicts of interest.

References

Acquaviva, R., Lanteri, R., Destri, G. L., Caltabiano, R., Vanella, L., … Giacomo, C. D. (2009). Beneficial effects of rutin and L-arginine co-administration in a rat model of liver ischemia-reperfusion injury. *Am J Physiol Liver Physiol, 296*, G664-G670. https://doi.org/10.1152/ajpgi.90609.2008

Ahmed, K. S., Jahan, I. A., Jahan, F., & Hossain, H. (2021). Antioxidant activities and simultaneous HPLC-DAD profiling of polyphenolic compounds from *Moringa oleifera* Lam. leaves grown in Bangladesh. *Food Res., 5*, 401-408. https://doi.org/10.26656/fr.2017.5(1).410

Ajila, C. M., Brar, S. K., Verma, M., Tyagi, R. D., Godbout, S., …. Valéro, J. R. (2011). Extraction and analysis of polyphenols: recent trends. *Crit Rev Biotechnol., 3*, 227-249. https://doi.org/10.3109/07388551.2010.513677

Al-Zahrani, A. A. (2020). Rutin as a promising inhibitor of main protease and other protein targets of COVID-19: in silico study. *Nat Prod Commun., 15*, 1-4. https://doi.org/10.1177/1934578X20953951

Amaglo, N. K., Bennett, R. N., Lo Curto, R. B., Rosa Eduardo, A. S., Turco, V. L., … Timpo, G. M. (2010). Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana. *Food Chem., 122*, 1047-1054. https://doi.org/10.1016/j.foodchem.2010.03.073

Amin, M. U., Khurram, M., Khattak, B., & Khan, J. (2015). Antibiotic additive and synergistic action of rutin, morin and quercetin against methicillin resistant Staphylococcus aureus. *BMC Complement Altern Med., 15*, 59. https://doi.org/10.1186/s12906-015-0580-0

Anand, K., Ziebuhr, J., Wadhwani, P., Mesters, J. R., & Hilgenfeld, R. (2003). Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. *Science, 300*, 1763-1767. https://doi.org/10.1126/science.1085658

Arcari, G., Raponi, G., Sacco, F., Bibbolino, G., Di Lella, F. M., … Carattoli, A. (2021). Klebsiella pneumoniae infections in COVID-19 patients: a 2-month retrospective analysis in an Italian hospital. *Int J Antimicrob Agents., 57*, 106245. https://doi.org/10.1016/j.ijantimicag.2020.106245

Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, Principle, strength and limitation. *Med Aromat Plants., 4*, 196. https://doi.org/10.4172/2167-0412.1000196

Bajpai, M., Pande, A., Tewari, S. K., & Prakash, D. (2005). Phenolic contents and antioxidant activity of some food and medicinal plants. *Int J Food Sci Nutr., 56*, 287-291. https://doi.org/10.1080/09637480500146606

Bennour, N., Mighri, H., Eljani, H., Zammouri, T., & Akrout, A. (2019). Effect of solvent evaporation method on phenolic compounds and the antioxidant activity of *Moringa oleifera* cultivated in Southern Tunisia. *South Afr J Bot., 129*, 181-190. https://doi.org/10.1016/j.sajb.2019.05.005

Brunelli, D., Tavecchio, M., Falcioni, C., Frapolli, R., Erba, E., …. D'Incalci, M. (2010). The isothiocyanate produced from glucomoringin inhibits NF-kB and reduces myeloma growth in nude mice in vivo. *Biochem Pharmacol, 79*, 1141-1148. https://doi.org/10.1016/j.bcp.2009.12.008

Chaisawangwong, W., & Gritsanapan, W. (2008). Quercetin and rutin contents in Siamese neem flower extracts prepared by different extraction methods. *Planta Med., 74*, PC15. https://doi.org/10.1055/s-0028-1084533

Cho, I. J., & Lee, S. (2015). Extraction of rutin from Tartary buckwheat milling fractions and evaluation of its thermal stability in an instant fried noodle system. *Food Chem., 176*, 40-44. https://doi.org/10.1016/j.foodchem.2014.12.020

Chua, L. S. (2013). A review on plant-based rutin extraction methods and its pharmacological activities. *J*

Ethnopharmacol, 150, 805-817. https://doi.org/10.1016/j.jep.2013.10.036

Costa, K. A., Soares, A. D. N., Wanner, S. P., Santos, Rd. G. Cd., Fernandes, S. O. A., …. Cardoso, V. N. (2014). L-arginine supplementation prevents increases in intestinal permeability and bacterial translocation in male swiss mice subjected to physical exercise under environmental heat stress. *J Nutr., 144*, 218-223. https://doi.org/10.3945/jn.113.183186

Cuellar-Nuñez, L., Luzardo-Ocampo, I., Campos-Vega, R., Gallegos-Corona, M.A., de Mejía, E. G., & Loarca-Piña, G. (2018). Physicochemical and nutraceutical properties of Moringa *(Moringa oleifera*) leaves and their effects in an in vivo AOM/DSS-induced colorectal carcinogenesis model. *Food Res Int., 105*, 159-168. https://doi.org/10.1016/j.foodres.2017.11.004

da Silva, F. M. A., da Silva, K. P. A., de Oliveira, L. P. M., Costa, E. V., Koolen, H. H. F., …. de Souza, A. D. L. (2020). Flavonoid glycosides and their putative human metabolites as potential inhibitors of the SARS-CoV-2 main protease (Mpro) and RNA-dependent RNA polymerase (RdRp). *Mem Inst Oswaldo Cruz., 115*, e200207. https://doi.org/10.1590/0074-02760200207

Dayoub, Z., Battah, B., Ani, L. A., Aljebeh, M., Darwisha, A., … Khani, F. Al. (2021). Multi drug resistant Escherichia coli superinfection in patient with COVID-19. *J Biosci Med. 9*, 1-7. https://doi.org/10.4236/jbm.2021.99001

Dessalegn, E., & Rupasinghe, H. P. V. (2021). Phenolic compounds and in vitro antioxidant activity of Moringa stenopetala grown in South Ethiopia. *Int J Food Prop. 24*, 1681-1692. https://doi.org/10.1080/10942912.2021.1990943

Eldalawy, R. (2017). Quantitative estimation of rutin in Rue (Ruta graveolens L.) cultivated in Iraq with the evaluation of its antioxidant activity. *Asian J Pharm Clin Res., 10*, 353-355. https://doi.org/10.22159/ajpcr.2017.v10i2.15726

El-Fadl, S. R. A, Osman, A., Al-Zohairy, A. M., Dahab, A. A., & El Kheir, Z. A. A. (2020). Assessment of total phenolic, flavonoid content, antioxidant potential and HPLC profile of three Moringa species leaf extracts. *Sci J Flowers Ornam Plants, 7*, 53-70. https://doi.org/10.21608/sjfop.2020.91397

Enogieru, A. B., Haylett, W., Hiss, D. C., Bardien, S., & Ekpo, O. E. (2018). Rutin as a potent antioxidant: Implications for neurodegenerative disorders. *Oxid Med Cell Longev., 2018*, 6241017. https://doi.org/10.1155/2018/6241017

Fombang, E. N., Nobossé, P., Mbofung, C. M., & Singh, D. (2020). Optimizing extraction of antioxidants from roasted *Moringa oleifera* Lam. leaves using response surface methodology. *J Food Process Pres., 44*, e14482. https://doi.org/10.1111/jfpp.14482

García-Meniño, I., Forcelledo, L., Rosete, Y., García-Prieto, E., … Fernández, J. (2021). Spread of OXA-48-producing *Klebsiella pneumoniae* among COVID-19-infected patients: The storm after the storm. *J Infect Public Health, 14*, 50-52. https://doi.org/10.1016/j.jiph.2020.11.001

Gopalakrishnan, L., Doriyaa, K., & Kumar, D. S. (2016). Moringa oleifera: A Review on nutritive importance and its medicinal application. *Food Sci Hum Wellness, 5*, 49-56. https://doi.org/10.1016/j.fshw.2016.04.001

Habib, H. I., Omar, S. K., & Mohamed, H. S. (2016). Estimation of rutin and ascorbic acid in some Libyan herbal plants by RP-HPLC. *Med Aromat Plants., 5*, 255-259. https://doi.org/10.4172/2167-0412.1000255

Harborne, J. B. (1986). Nature, distribution and function of plant flavonoids. *Prog Clin Biol Res., 213*, 15-24.

Khudaer, N. B., Hassn, Z. Y. M., AL-Sammarrae, K. W., & Ibraheem, N. K. (2016). Purification and identification of total flavonoids extracted from *Moringa oleifera* leaves in Iraq. *J Biotechnol Res Cen., 10*, 73-80. https://doi.org/10.24126/jobrc.2016.10.2.487

Kianersi, F., Abdollahi, M. R., Mirzaie-asl, A., Dastan, D., & Rasheed, F. (2020). Identification and tissue-specific expression of rutin biosynthetic pathway genes in Capparis spinosa elicited with salicylic acid and methyl jasmonate. *Sci Rep., 10*, 8884. https://doi.org/10.1038/s41598-020-65815-2

Kraujalis, P., Venskutonis, P. R., Ibáñez, E., & Herrero, M. (2015). Optimization of rutin isolation from Amaranthus paniculatus leaves by high pressure extraction and fractionation techniques. *J Supercrit Fluids., 104*, 234-242. https://doi.org/10.1016/j.supflu.2015.06.022

Kreft, S., Strukelj, B., Gaberscik, A., & Kreft, I. (2002). Rutin in buckwheat herbs grown at different UV-B radiation levels: comparison of two UV spectrophotometric and an HPLC method. *Exp Bot., 53*, 1801-1804.

https://doi.org/10.1093/jxb/erf032

Lachman, J., Orsák, M., Pivec, V., & Faustusová, E. (2000). Content of rutin in selected plants sources. *Scientia Agric Bohem., 31*, 89-99.

Latos-Brozio, M., & Masek, A. (2019). Structure-activity relationships analysis of monomeric and polymeric polyphenols (quercetin, rutin and catechin) obtained by various polymerization methods. *Chem Biodivers., 16*, 1-14. https://doi.org/10.1002/cbdv.201900426

Lin, X., Wu, L.F., Wang, X., Yao, L., & Wang, L. (2021). Ultrasonic-assisted extraction for flavonoid compounds content and antioxidant activities of India *Moringa oleifera* L. leaves: Simultaneous optimization, HPLC characterization and comparison with Other methods. *J Appl Res Med Aromat Plants., 20*, 100284. https://doi.org/10.1016/j.jarmap.2020.100284

Madani, F., Lindberg, S., Langel, U., Futaki, S., & Gräslund, A. (2011). Mechanisms of cellular uptake of cell-penetrating peptides. *J Biophys., 2011*, 414729. https://doi.org/10.1155/2011/414729

Mehta, R. G., Murillo, G., Naithani, R., & Peng, X. (2010). Cancer chemoprevention by natural products: how far have we come? *Pharm Res., 12*, 950-961. https://doi.org/10.1007/s11095-010-0085-y

Mittal, L., Kumari, A., Srivastava, M., Singh, M., & Asthana, S. (2021). Identification of potential molecules against COVID-19 main protease through structure-guided virtual screening approach. *J Biomol Struct Dyn., 39*, 3662-3680. https://doi.org/10.1080/07391102.2020.1768151

Mohamed, S. H., Youssef, A. F. A., Issa, M., Salam, H. S. A., & EL-Ansary, A. (2021a). Validated HPLC method for quantitative analysis of gallic acid and rutin in leaves of *Moringa oleifera* grown in Egypt. *Egypt J Chem., 64*, 1583-1591. https://doi.org/10.21608/ejchem.2020.46603.2949

Mohamed, M. A., Ibrahim, M. T., Abdel-Azima, N. S., & El-Missiry, M. M. (2021b). Chemical and biological studies on *Moringa oleifera* L. cultivated in Egypt. *Egypt Pharm J., 20*, 33-41. https://doi.org/10.4103/epj.epj_37_20

Molnar, M., Jakovljevic, M., & Jokic, S. (2018). Optimization of the process conditions for teh extraction of rutin from Ruta graveolens L. by choline chloride based deep eutectic solvents. *Solvent Extr Res Dev Jpn., 25*, 109-116. https://doi.org/10.15261/serdj.25.109

Mostafa, N. M. (2017). Comparative analysis of rutin content in some Egyptian plants. A validated RP-HPLC-DAD approach. *European J Med Plants, 19*, 1-8. https://doi.org/10.9734/EJMP/2017/33760

Motoki, S., Tang, T., Taguchi, T., Kato, A., & Ikeura, H., (2019). Distribution of rutin and protodioscin in different tissue parts of Asparagus (Asparagus officinalis L.). *HortScience, 54*, 1921-1924. https://doi.org/10.21273/HORTSCI14131-19

Musallam, I., Duwayri, M., Shibli, R., & Alali, F. (2012). Investigation of rutin content in different plant parts of wild Caper (Capparis spinosa L.) populations from Jordan. *Res J Med Plants., 6*, 27-36. https://doi.org/10.3923/rjmp.2012.27.36

Mustabha, S.M., Athar, M.T., Kamal, Y.T., Baboota, S., Ali J., & Ahmad, S. (2011). Fast analysis and validation of rutin in anti-psoriatic ayurvedic formulation by HPLC. *J Liq Chromatogr Relat Technol., 34*, 446-455. https://doi.org/10.1080/10826076.2011.555687

O'Riordan, T. G. (2002). Formulations and nebulizer performance. *Respir Care, 47*, 1305-1312.

Oyeleye, S. I., Olasehinde, T. A., Ademosun, A. O., Akinyemi, A. J., & Oboh, G. (2019). Horseradish (*Moringa oleifera*) seed and leaf inclusive diets modulates activities of enzymes linked with hypertension, and lipid metabolites in high-fat fed rats. *Pharma Nutrition, 7*, 100141. https://doi.org/10.1016/j.phanu.2018.100141

Rizzuti, B., Grande, F., Conforti, F., Jimenez-Alesanco, A., Ceballos-Laita, L., … Velazquez-Campoy, A. (2021) Rutin is a low micromolar inhibitor of SARS-CoV-2 main protease 3CLpro: Implications for drug design of Quercetin analogs. *Biomedicines, 9*, 375. https://doi.org/10.3390/biomedicines9040375

Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. *BMC Complement Altern Med., 12*, 221. https://doi.org/10.1186/1472-6882-12-221

Sancineto, L., Ostacolo, C., Ortega-Alarcon, D., Jimenez-Alesanco, A., Ceballos-Laita, L., …. Santi, C. (2021). L-arginine improves solubility and anti SARS-CoV-2 Mpro activity of rutin but not the antiviral activity in cells. *Molecules, 26*, 6062. https://doi.org/10.3390/molecules26196062

Shafi, N., & Ikram, M. (1982). Quantitative Survey of rutin-containing plants. *Part 1. Int J Crude Drug Res., 20*, 183-186. https://doi.org/10.3109/13880208209083302

Shlini, P., Clare, H. M., & Immaculate, V. T. M. (2020). Isolation and purification of rutin from Euphorbia pulcherrima. *Int J Pharm Sci Rev Res., 64*, 45-49. https://doi.org/10.47583/ijpsrr.2020.v64i01.008

Siddhuraju, P., & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J Agric Food Chem., 51*, 2144-2155. https://doi.org/10.1021/jf020444

Sofic, E., Copra-Janicijevic, A., Salihovic, M., Tahirovic, I., & Kroyer, G. (2010). Screening of medicinal plant extracts for quercetin-3-rutinoside (rutin) in Bosnia and Herzegovina. *Med Plants - Int J Phytomed Relat Ind., 2*, 97-102. https://doi.org/10.5958/j.0975-4261.2.2.015

Stalikas, C. D. (2007). Extraction, separation, and detection methods for phenolic acids and flavonoids. *J Sep Sci. 30*, 3268-3295. https://doi.org/10.1002/jssc.200700261

Tonon, T., & Lonvaud-Funel, A. (2000). Metabolism of arginine and its positive effect on growth and revival of Oenococcus oeni. *J Appl Microbiol., 89*, 526-531. https://doi.org/10.1046/j.1365-2672.2000.01142.x

Tulbah, A. S., & Lee, W-H. (2021). Physicochemical characteristics and in vitro toxicity/anti-SARS-CoV-2 activity of Favipiravir solid lipid nanoparticles (SLNs). *Pharmaceuticals, 14*, 1059. https://doi.org/10.3390/ph14101059

Valdez-Solana, M. A., Mejía-García, V. Y., Téllez-Valencia, A., García-Arenas, G., Salas-Pacheco, J., ... Sierra-Campos, E. (2015). Nutritional content and elemental and phytochemical analyses of *Moringa oleifera* grown in Mexico. *J Chem., 2015*, 860381. https://doi.org/10.1155/2015/860381

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., & Gritsanapan, W. (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. *Ind Crop Prod., 44*, 566-571. https://doi.org/10.1016/j.indcrop.2012.09.021

Wang, Z., Ding, Z., Li, Z., Ding, Y., Jiang, F., & Liu, J. (2021). Antioxidant and antibacterial study of 10 flavonoids revealed rutin as a potential antibiofilm agent in *Klebsiella pneumoniae* strains isolated from hospitalized patients. *Microb Pathog, 159*, 10512. https://doi.org/10.1016/j.micpath.2021.105121

Wiener, J., Quinn, J. P., Bradford, P. A., Goering, R. V., Nathan, C., … Weinstein, R. A. (1999). Multiple antibiotic-resistant Klebsiella and Escherichia coli in nursing homes. *JAMA, 281*, 517-523. https://doi.org/10.1001/jama.281.6.517

Wu, C., Liu, Y., Yang, Y., Zhang, P., Zhong, W., ... Li, H. (2020). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharm Sin B., 10*, 766 -788. https://doi.org/10.1016/j.apsb.2020.02.008

Xu, Z., Yang, L., Zhang, X., Zhang, Q., Yang, Z., … Liu W. (2020). Discovery of potential flavonoid inhibitors against COVID-19 3CL proteinase based on virtual screening strategy. *Front Mol Biosci., 7*, 556481. https://doi.org/10.3389/fmolb.2020.556481

Yang, W., Xu, X., Li, Y., Wang, Y., Li, M., … Chu, Z. (2016). Rutin-mediated priming of plant resistance to three bacterial pathogens initiating the early SA signal pathway. *PLoS One, 11*, e0146910. https://doi.org/10.1371/journal.pone.0146910

Yewale, S., Farash, Z., Kulkarni, S., Palghadmal, S., Athawale, N., … Padmanabhan, S. (2021). Effect of solvent polarity on extraction yield of total flavonoids with special emphasis to glabridin from Glycyrrhiza glabra roots. *FABAD J Pharm Sci., 46*, 219-230. https://doi.org/10.55262/fabadeczacilik.1078751

Zanichelli, V., Huttner, A., Harbarth, S., Kronenberg, A., & Huttner, B. (2019). Antimicrobial resistance trends in Escherichia coli, *Klebsiella pneumoniae* and *Proteus mirabilis* urinary isolates from Switzerland: retrospective analysis of data from a national surveillance network over an 8-year period (2009-2016). *Swiss Med Wkly., 149*, w20110. https://doi.org/10.4414/smw.2019.20110

Zhu, Y., Yin, Q., & Yang, Y. (2021). A comparative study of HPLC-DAD and UPLC-UV methods for simultaneous determination of 11 polyphenols in *Moringa oleifera* leaves. *Trop J Pharm Res., 20*, 2371-2379. https://doi.org/10.4314/tjpr.v20i11.20

Appendix

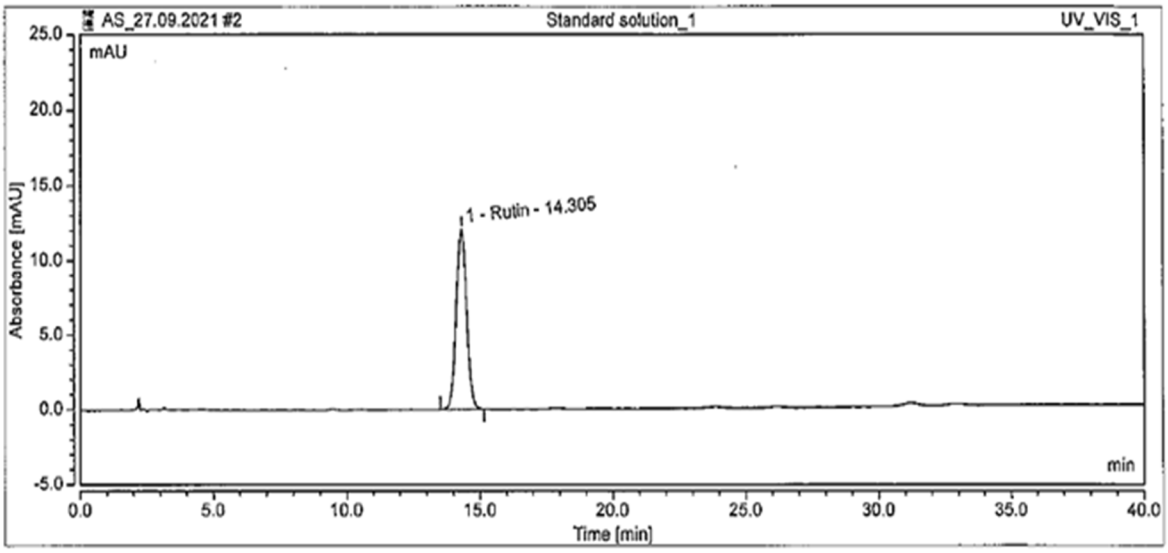

Figure S1. HPLC chromatogram of standard rutin in methanol at 256 nm. The x-axis is in units of time (min) while y-axis of the chromatogram is a measure of the intensity of absorbance (in units of mAU, or milli-Absorbance Units). The retention time of rutin was observed at 14.305 min (See experimental section for details of chromatographic protocol)

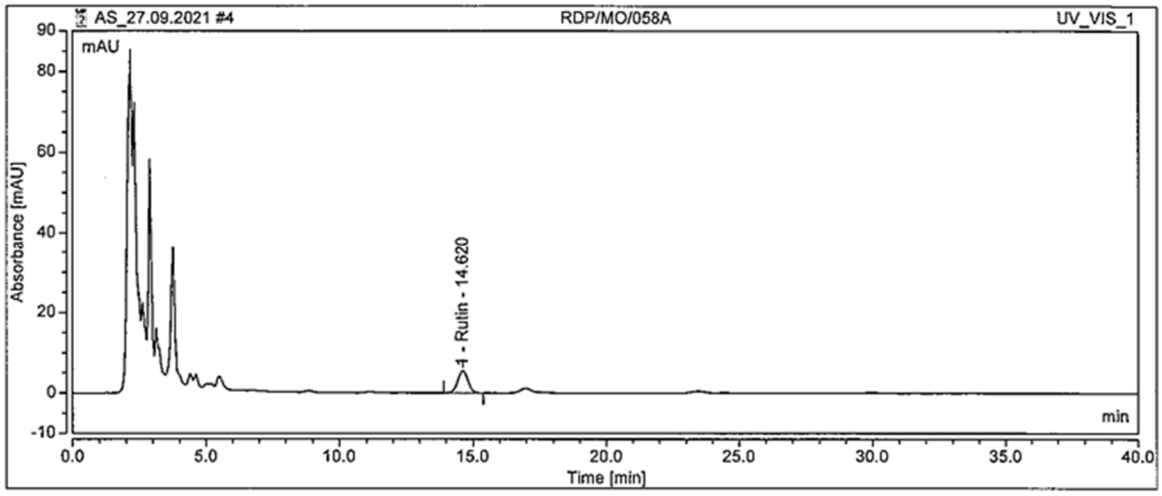

Figure S2. HPLC chromatogram of rutin extracted from water from *Moringa oleifera* leaves at 256 nm. Note the low absorbance of the 5 mAU indicating low levels of rutin extracted using water as the extraction solvent

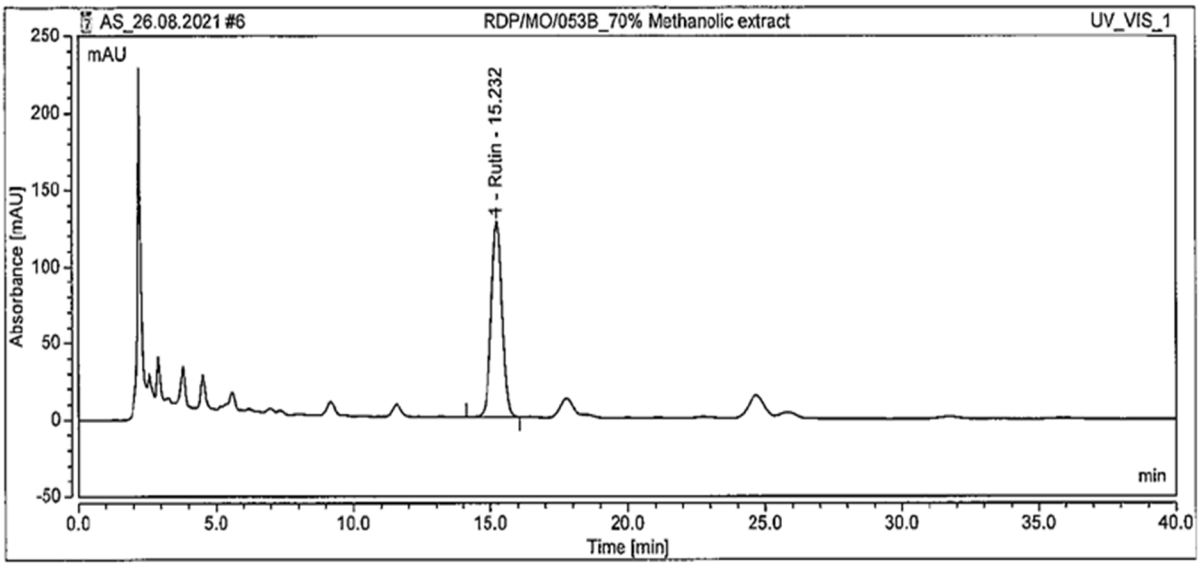

Figure S3. HPLC chromatogram of rutin extracted using methanol (Process A) from *Moringa oleifera* leaves at 256 nm. The absorbance of the 130 mAU was seen with the 100 ppm solution of the rutin extracted using methanol as the extraction solvent. (See Process A of Figure 1 for details)

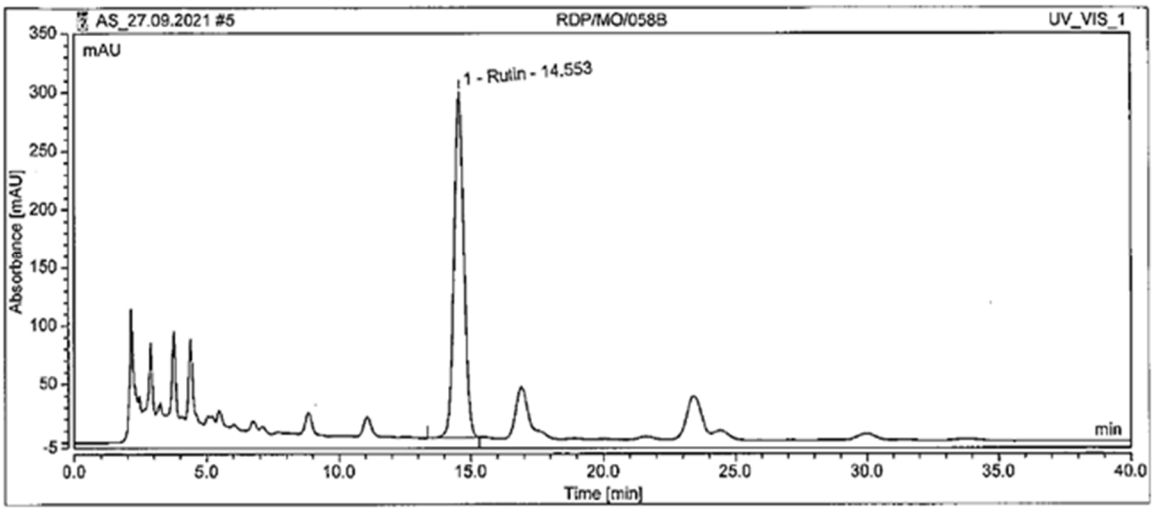

Figure S4. HPLC chromatogram of rutin extracted using resin chromatography (Process B) from *Moringa oleifera* leaves at 256 nm. The absorbance of the 320 mAU was seen with the 100 ppm solution of the rutin extracted using methanol as the extraction solvent. (See Process B of Figure 1 for details) indicating its higher purity than the methanolic extract obtained from Process A

Supplementary Table S1: Antibiotic susceptibility of *K. pneumoniae* and *E. coli*

Sr. No.	Antibiotic	MCC 2452		MCC 2412	
		DZI (mm)	Susceptibility	DZI (mm)	Susceptibility
1	Fusidic acid (10 µg)	-	Resistant	-	Resistant
2	Trimethoprim (5 µg)	14	Intermediate	26	Sensitive
3	Nalidixic acid (30 µg)	08	Resistant	21	Sensitive
4	Vancomycin (30 µg)	-	Resistant	07	Resistant
5	Rifampicin (5 µg)	06	Resistant	09	Resistant
6	Kanamycin (30 µg)	06	Resistant	16	Intermediate
7	Azithromycin (15 µg)	-	Resistant	13	Resistant
8	Tobramycin (10 µg)	13	Intermediate	15	Sensitive
9	Gentamycin (10 µg)	13	Intermediate	16	Sensitive
10	Moxifloxacin (5 µg)	11	Resistant	23	Sensitive
11	Streptomycin (10 µg)	14	Intermediate	09	Resistant
12	Tetracycline (30 µg)	09	Resistant	21	Sensitive
13	Penicillin-G (10 µg)	-	Resistant	-	Resistant
14	Carbenicillin (100 µg)	-	Resistant	21	Intermediate
15	Doripenem (10 µg)	31	Sensitive	32	Sensitive
16	Ampicillin (10 µg)	-	Resistant	21	Sensitive

DZI: Diameter of zone of inhibition.