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Evaluation of extraction techniques and solvents for phytochemical screening, antioxidant and antimicrobial activity of mungbean (*Vigna radiata* (L.) R. Wilczek) seeds

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The mung bean (*Vigna radiata* (L.) R. Wilczek), belonging to Leguminoseae family, is major short-seasoned legume grown in tropical and subtropical regions, mainly during the Kharif and summer seasons. Present investigation aimed to check the effects of different extraction techniques and solvents on FTIR spectrophotometric analysis and quantitative profiling of phenols and flavonoids, followed by an assessment of the antioxidant and antimicrobial activity of mung bean seeds. Methanolic extracts of seeds obtained through Soxhlet extraction, maceration and ultrasonication were subjected to solvent-aided fractionation using the separation funnel method. The extractive yield of obtained fractions ranged from 2.86 to 59.85%. The results indicated maximum yield in aqueous fractions, followed by butanol and hexane fractions, while the lowest in ethyl acetate and chloroform fractions. The phenolic content, flavonoid content and FRAP values in the different fractions varied from 16.32±1.67 to 56.82±0.88 mg of GAE/g, 38.21±0.07 to 149.95±0.53 mg QE/g, and 41.54±0.56 to 104.81±0.48 mg Fe (II) E/g, respectively. Phenolics were higher in non-polar fractions, while flavonoids and FRAP values were higher in polar fractions. Pearson correlation displayed a strong correlation between TFC and FRAP assay. The antimicrobial activity revealed *Staphylococcus aureus* as the most susceptible, followed by *Bacillus subtilis* and *Saccharomyces cerevisiae*; conversely, *Pseudomonas* sp. was shown to be most resistant with no inhibition activity. Overall, ethyl acetate fractions from all the extraction techniques presented the best results against tested microbial samples. PCA analysis presented significant discrimination among solvents and extraction techniques. Mung beans can be used as nutraceuticals pharmaceuticals and functional foods.

Keywords: Antimicrobial activity, Fractionation, FTIR, Phytocomponents, Principal component analysis, *Vigna radiate* (L.) R. Wilczek

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Introduction

Humankind has been using dietary plants to improve their well-being over the ages[1]. In fact, many clinical reports have also proved the interlinked connection between health and diet. Accordingly, the scientific community is now focusing on propounding nutritional supplements with functional value, as in addition to nutrition, they are also beneficial against numerous chronic diseases, including cancer, diabetes, coronary infarction, and gastroenteric disorders. All these biological activities were attributed to the presence of biologically active secondary metabolites; thereby, it is critical to characterise such active phytochemicals from plant-based products and check their biological potential[2,3]. Among all these components, polyphenols, including phenolic acids and flavonoids, are the key category of interest for studies because of their evidently proven antioxidant, antimicrobial, anti-inflammatory,anti-mutagenic and other biological properties[4].

The polyphenolic compounds reduce oxidative stress by donating electrons through the hydroxyl group to neutralise the free radicals, the highly reactive species that stabilise themselves by interacting with other physiologically important molecules of the body and cause damage to DNA, proteins and lipids[5,6]. Eventually, such conditions lead to various degenerative pathology, especially cancer, diabetes, cardiovascular, respiratory and neurological dysfunctions[7]. In addition to the antioxidant potential, the polyphenols are thought to be effective in preventing the aforesaid diseases[8]. Thus, currently, there has been a significant hike in prospecting novel,

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cost-effective and sustainable natural antioxidants as an alternative to synthetic ones, as the latter imposes toxic and mutagenic effects[9].

Bacterial infections caused by *Bacillus subtilis*, *Staphylococcus aureus, and Pseudomonas aeruginosa* can be responsible for the fatal foodborne disease and may also be the prime reason for mortality due to microbial infections[10]. Several antibiotic drugs have been developed to address the underlying problems of such infectious diseases, but now most of them are at risk of being rendered obsolete due to the emergence of multiple drug resistance in pathogenic microorganisms, which emanate from the frequent and inappropriate use of antibiotics for human medications, animal growth enhancers, and in food sectors[11,12]. Against this backdrop, polyphenolic compounds, especially phenols and flavonoids found in plants, may have considerable antimicrobial properties in addition to their proven antioxidant capacity[10].

Bioactive compounds derived from plants differ in chemical composition, particularly in the attached functional groups, which determine their chemical properties. The valorisation of these compounds tends to rely on factors such as the employed extraction procedure, given temperature, extraction time, and polarity of the working solvent[13]. In the same way, many studies have noted that biological activities vary depending on the type of extraction used, highlighting the significance of choosing a befitting extraction technique[14]. Therefore, to enhance the therapeutic efficacy of plants, there has been an eloquent upswing in the development of quick, reliable and repeatable procedures for extracting the bioactive principles[15]. In this context, various conventional extraction methods, including maceration, percolation and Soxhlet, have been widely implemented in plant-based research due to their broad utility, adequacy, and user convenience[16].

Soxhlet extraction uses a heated solvent to continually extract phytocomponents from powdered plant material packed in thimble, with the solvent vaporising, condensing, and siphoning back to facilitate extraction. Maceration involves soaking ground plant material into a solvent with intermittent agitation at room temperature[17]. Despite that, conventional methods have some drawbacks, such as poor efficiency, thermal degradation of active metabolites, mass-transfer resistance, lengthy procedure, and a non-greener approach due to extensive solvent usage and electricity consumption that ultimately increases the carbon load[18,19].

The research trend to overcome this neglect is shifting towards more contemporary non-conventional extraction techniques like microwave-assisted extraction, ultrasound-assisted extraction and supercritical fluid extraction[20]. Ultrasonication damages the plant cell walls by propagating ultrasound waves through the liquid environment[21]. These techniques can be used to obtain high-quality extracts in a short period of time as well as with the least energy expenditure and minimal extractive solvent use, suggesting them as green methods[16]. Ultrasonication has also been proven to be a more efficient and cost-effective technique for the extraction of heat-labile components because of the higher diffusion and mass transfer rate, cell wall disruption, solvent penetration, and capillary actions of soundwaves[22,23].

The mung bean (*Vigna radiata* (L.) R. Wilczek), belonging to the Leguminosae family, is amongst the major short-seasoned legumes grown in the tropical and subtropical regions, mainly during the kharif and summer seasons[24]. The mung bean seeds and sprouts are rich in proteins, carbs, minerals, vitamins and roughage and are widely consumed across Asian countries[25]. Aside from nutritional benefits, it can also be used therapeutically to help detoxify, convalescence mental health, heat-stroke alleviation and gastrointestinal distress regulation[26]. Evidence from previous studies has confirmed the presence of active metabolites in mung beans, including phenolics, flavonoids, organic acids, and lipids, which contribute to its biological activities such as antioxidant, antimicrobial, anti-inflammatory, hypotensive, hepatoprotective, and hypoglycaemic activities[27].

To the best of the authors' knowledge, only a few studies have been recorded on comparative analysis of different extraction techniques for the assay of the biological potential of mung beans. Therefore, the present investigation aimed to check the effects of different extraction techniques (Soxhlet extraction, maceration, ultrasonication) and solvents on quantitative profiling of phenols and flavonoids, followed by assessment of antioxidant and antimicrobial activity of mungbean seeds. FTIR fingerprinting was also performed to characterise functional groups in test samples.

Materials and Methods

Plant material

V. radiate (Guj – 4) seeds procured from the commercial market were cultivated at the field in

Deodar (24˚08′49.0″ N; 71˚51′54.4″ E), Gujarat, India, from June to August 2021. At maturity, the seeds were harvested and collected for testing purposes. The collected seeds were assiduously washed with distilled water and oven-dried at 50°C for 24 h. The dried seeds were milled to fine powder by grinder (Bajaj Mixer Grinder, 500W) and stored in evacuated containers at low temperatures until further use.

Extract preparation

Various parameters such as extraction technique, completion time, polarity of solvent, temperature, and size of pulverised particles inevitably influence the extraction of bioactive components from plant samples; for this reason, it is prudent to choose appropriate approaches for extraction[28]. Accordingly, three different extraction techniques, namely Soxhlet extraction (Hot extraction), Maceration (Cold extraction), and Ultrasound-assisted extraction (Sonication), were performed for the extraction of powdered seeds of *V.radiata*followed by liquid-liquid fractionation. The approaches employed are summarised below.

Soxhlet extraction

Arduous Soxhlet extraction was performed using a conventional Soxhlet extractor (Sigma-Aldrich, Germany). The 20 g dried seed powder was extracted with 200 mL of methanol for 42 h at 50°C temperature. Thereafter, isolated extracts were filtered with Whatman filter paper No. 1 and the excess methanol from the filtrate was evaporated with a rotary evaporator (Heidolph, Germany) to obtain a completely dry crude extract. Lastly, the dried crude extract was stored in the refrigerator after noting the percentage yield until further use. Percentage Yield was calculated by following the formula:

$$\text{Yield (\%)} = \frac{W_1 * 100}{W_2}$$

where W_1= Weight of extract after solvent evaporation, W_2= Weight of powdered leaf.

Maceration

The 20 g dry powder of the seed sample was dissolved in 200 mL methanol solvent (1:10). Then, the mixture was kept on an open-air orbital shaker (ThermoFisher Scientific, United States) at room temperature for 72 h at 120 rpm with constant shaking for maceration. Subsequently, the macerated mixture was filtered, dried, and processed as described above.

Ultrasound-assisted extraction (Ultrasonication)

A2 L digital ultrasonic sonicator bath with a regulated timer and thermo-sensitive probe was selected for the experimental task. 20 g powdered seeds were mixed with 200 mL methanol in a glass beaker and placed in an ultrasonicator for 30 min at 40 KHz frequency. Ultrasonic wave-induced overheating of the bath was obviated by water circulation at room temperature. Thereafter, the obtained extract was further processed as delineated in the preceding technique.

Fractionation

An equal portion of the crude extract obtained from Soxhlet, maceration and ultrasound-assisted extracts were separately subjected to liquid-liquid extraction using the separation funnel method. At first, the crude methanol extract was thoroughly dissolved in 50 mL of distilled water and transferred to a separating funnel of 250 mL (Sigma-Aldrich, Germany). In addition, 50 mL of *n*-hexane was added to the funnel, and then it was shaken well for 2 minutes and left undisturbed to settle. Once the content was settled, the lower aqueous layer was removed, and the surplus *n*-hexane fraction was collected in a separate container. The fractionation was repeated 2 times with the same solvent to get a concentrated fraction. Following the same cycle as mentioned above, the leftover aqueous extract was further exposed to partition with solvents from low to high polarity, i.e. chloroform, ethyl acetate, and *n*-butanol. Each fraction was collected in a separate container and evaporated. Lastly, the dried crude extracts of hexane fraction (HF), chloroform fraction (CF), ethyl acetate fraction (EF), butanol fraction (BF) and aqueous fraction (AF) were tagged as HF(A), CF(A), EF(A), BF(A) and AF(A) for fractions obtained from Soxhlet extract. Similar tagging was given to fractions obtained from maceration extract as HF(B), CF(B), EF(B), BF(B) and AF(B) and sonication extract fractions were named HF(C), CF(C), EF(C), BF(C) and AF(C). All the fractions were stored in a cool and dry place until further use.

Total phenol content

The Folin-Ciocalteu method, originally described by Singleton and Rossi (1965), was used with few modifications to determine the total phenol content of fraction samples[29]. The modifications involve reducing both the total reaction volume and the volume of the Folin-ciocalteu reagent. In brief, 500 µL of the

sample (0.5 mg/mL) was transferred to 500 µL of Folin-ciocalteu reagent, followed by dilution with 10 mL of distilled water and incubated immediately at room temperature for 5 min. In addition, 2 mL of sodium carbonate (20% Na_2CO_3) solution was added to the mixture, and a total volume of 25 mL was made by adding distilled water. Thereafter, the solution was left undisturbed in the dark for 30 min at room temperature. Lastly, the absorbance was recorded at 765 nm in a spectrophotometer (Lasany UV-VIS Spectrophotometer Model: LI-294). The calibration curve was plotted using gallic acid as standard (50 – 500 µg/mL) following the previously stated procedure. All the experimental tasks were conducted in triplicates to avoid errors. The total phenolic content of each fraction was expressed as milligrams of gallic acid equivalent/gram of sample (mg of GAE/g of sample) using the equation given below.

$$\text{GAE} = \frac{C * V}{M}$$

where C = Concentration of gallic acid established from the calibration curve in mg/mL; V = Volume of the fraction solution in mL; M = Weight of the extract in g.

Total flavonoid content

The flavonoid concentration in each fraction was quantified by Aluminium chloride ($AlCl_3$) spectrophotometric assay with minor modifications[30]. At first, 500 µL of the sample (0.5 mg/mL) was mixed with 50 µL 10% $AlCl_3$, followed by the addition of 50 µL of 1M potassium acetate (CH_3COOK); finally, distilled water was added to make a total volume of 5 mL and mix well. The mixture was then well shaken and kept in the dark at room temperature for 30 minutes. After incubation, the absorbance of the test solution was recorded at 415 nm in a spectrophotometer (Lasany UV-VIS Spectrophotometer Model: LI-294). The total flavonoid content was computed from the calibration curve constructed from the serial concentrations of standard Quercetin (50–500 µg/mL). Errors in the experiment were avoided by conducting tests in triplicates. The findings were expressed as milligrams of quercetin equivalent/gram of sample (QE/g of sample) reckoned by the equation given below:

$$\text{QE} = \frac{C * V}{M}$$

where C = Concentration of quercetin established from the calibration curve in mg/mL; V = Volume of the fraction solution in mL; M = Weight of the extract in g.

FTIR

FTIR analysis can be used to identify different bonds in the chemical composition and to indicate the types of functional groups present in the plant sample[31]. The dried extracts were crammed into a sample chamber of Fourier Transfer Infrared Spectrophotometer (Agilent Cary 630 FTIR Spectrophotometer, United States) coupled with attenuated total reflectance (ATR), and the absorption spectra were recorded using Agilent Microlab Software, with an average of 8 scans and spectral resolution of 8 cm^{-1} in the frequency range of 4000-650 cm^{-1} at room temperature. The distinctive peak frequencies recorded in the spectrograph were analysed by comparing them with standard references.

Antioxidant activity

Antioxidant activity of the fractions of *V. radiate* obtained from the seed extracts of three different exaction methods was analysed by the FRAP (Ferric reducing antioxidant power) assay with some modifications[32].

FRAP assay

This is the most common and popular method used to check the presence of antioxidant activity in a given plant material. The FRAP reagent was freshly prepared by mixing Sodium acetate buffer (300 mM; pH 3.6) with 10 mM TPTZ in 40 mM HCL and 20 mM $FeCl_3.6H_2O$ in a proportion of 10:1:1, respectively. The prepared mixture was incubated for 30 min at 37°C in a water bath before use. 500 µL of fraction solutions (0.5 mg/mL) were added separately to the 4 mL of FRAP reagent. The mixtures were then diluted with 10 mL distilled water and incubated for 20 minutes at room temperature. The absorbance of reaction mixtures was recorded at 593 nm in a spectrophotometer (Lasany UV-VIS Spectrophotometer Model: LI-294). A similar procedure was followed for standard iron (II) sulphate ($FeSO_4.7H_2O$) (50–500 µg/mL) to construct a calibration curve. The above procedure was repeated 3 times for each sample and standard to avoid errors. The results were expressed as mg Fe (II) equivalent/gram of plant material calculated by the given equation:

$$\text{Fe (II) equivalent/gram of sample} = \frac{C * V}{M}$$

where C = Concentration of Fe (II) established from the calibration curve in mg/mL; V = Volume of the Extract solution in mL; M = Weight of the extract in grams.

Antimicrobial activity

The antimicrobial activity of various fractions was evaluated using the Agar well diffusion method with a few modifications[33].

Culture and maintenance of microorganisms

Pure cultures of all experimental bacteria, i.e., *S. aureus* (gram-positive), *B. subtilis* (gram-positive) and *Pseudomonas sp.* (gram-negative), and fungus *S. cerevisiae* (yeast) were procured from the Department of Microbiology, Gujarat University. The pure bacterial cultures were maintained with subsequent sub-culturing on a nutrient agar medium and fugal cultures on potato dextrose agar medium, and both were stored at 4°C until use.

Agar well diffusion method

The agar well-diffusion method was followed to check the antimicrobial susceptibility of given fractions using nutrient agar medium (Himedia, India) for bacteria and potato dextrose agar (Himedia, India) for fungus. The test microorganisms were cultured overnight at 37°C in nutrient broth, and the turbidity of the suspension culture was adjusted to 0.5 McFarland standards using a spectrophotometer to obtain the desired cell density. The agar medium was poured into petri plates in a sterile condition and allowed to solidify for 30 minutes under ultraviolet light. Each labelled agar medium plate was inoculated with a test microorganism by a streaking sterile cotton swab rolled in the suspension culture to obtain uniform lawn growth over the agar medium. The 5 mm diameter wells were made using a sterile cork borer. The stock solution of each extract was prepared in DMSO with a concentration of 10 mg/mL. Gentamicin (1 mg/mL) and fluconazol (1 mg/mL) were used as positive for antibacterial and antifungal activity, respectively, while DMSO was used as a negative control. 80 µL of the given samples were added to each well separately and allowed to diffuse at room temperature for 1 h. The plates were then kept at 37°C and incubated for 24 h. Later, the results were expressed by measuring the diameters of the growth inhibition zone around wells.

Statistical Analysis

All the experiments were performed in triplicates, and the values were presented as Mean±SE. The results were subjected to a one-way analysis of variance (ANOVA) with Tukey's test to determine the significant difference among extracts at a significance level of 0.05. Additionally, Pearson's correlation between quantified phytocomponents and antioxidant activity was computed using OriginPro®2022b.

Results and Discussion

Extraction yield

Extraction methods, solvent polarity and their extraction efficiency had a direct impact on the percentage yield of different extracts[34]. Nevertheless, the preferred extraction methods should be simple, rapid, ecologically and economically sound, and capable of preserving therapeutically valued components. Therefore, in the current investigation, three different methods (Soxhlet, maceration, and ultrasonication)were employed to extract mung bean seeds.

A nearly equal extractive yield was obtained in the methanol extracts obtained from Soxhlet (4.72%) and maceration (4.91%), followed by ultrasonication (1.49%). These methanolic extracts were then subjected to fractionation, and the extractive yield of various obtained fractions ranged from 2.86% to 59.85% (Fig. 1). Among fractions, the order of percentage extraction yield was AF(A) (59.85%) > BF(A) (27.75%) > HF(A) & EF(A) (3.71%) > CF(A) (2.86%) for Soxhlet fractions, whereas, in case of maceration fractions, it was AF(B) (59.51%) > BF(B) (10.78%) > HF(B) (7.83%) > CF(B) (3.76%) > EF(B) (3.15%), and for ultrasonication fractions, it was AF(C) (58.05%) > HF(C) (17.45%) > BF(C) (17.11%) > CF(C) (7.05%) > EF(C) (3.69%). The study results indicate that the maximum yield was obtained in aqueous fractions, followed by butanol and hexane fractions, while the lowest yield was obtained in ethyl acetate and chloroform fractions. Notably, the extractive yield of fractions was

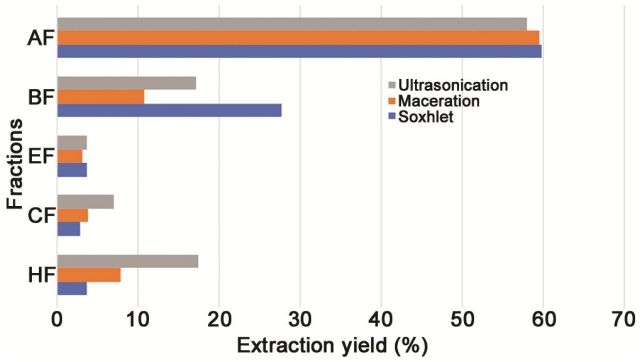

Fig. 1 — Extraction yield (%) of different fractions.

greatly affected by the varying polarity of solvents. The hike in yield with increased polarity can be explained by the plausible fact that polar solvents likely have a potent ability to dissolve polar compounds and sequester the active ingredients into the extracts[35]. The dielectric constant of solvents may also affect the percentage yield by abating the intensity of electric fields in the solvent medium[23]. Moreover, regarding extraction techniques, the maximum yield was obtained in ultrasonication, then in maceration, and the nominal yield was obtained in Soxhlet for HFs and CFs. Likewise, the order of percentage yield for EFs and BFs was Soxhlet>ultrasonication>maceration, but for AFs, the yield was approximately consistent in all extraction procedures. Differences observed in percentage yield with respect to extraction methods can be ascribed to various key factors governing the processes. The prolonged dwelling of hot solvent flowing through the powdered sample increases the solubility and diffusion coefficient of active metabolites, which may account for the higher yield in Soxhlet extraction. However, the underlying reason for the high yield in ultrasonication within a short period might be the acoustic cavitation induced by the ultrasonic field in a liquid medium, which breaches the cell wall readily to release the content in the solvent.

Total phenol content

Plant polyphenolic groups are important plant-derived metabolites that possess medicinal and therapeutic properties[36]. These phenolic components display significant antioxidant potency due to the highly reactive nature of phenolic moiety, which scavenges free radicals by donating electrons or hydrogen atoms and also neutralises the free radical chain of lipids[37]. Diverse responses have been obtained using the Folin-Ciocalteu method for different fractions based on the number of phenolic groups present in the test samples.

The phenolic content in the different fractions of *V. radiate* seeds varied from 16.32±1.67 to 56.82±0.88 mg of GAE/g of the sample with a significant difference (p<0.05) in-between (Fig. 2). Among fractions, the highest phenolic concentration was shown by BF(A), followed by CF(A), then HF(A), and EF(A), and at last AF(A) for fraction obtained from Soxhlet extract, while in case of fraction obtained from maceration extract the order was AF(B) > BF(B) ≈ HF(B) > EF(B) > CF(B), and for fractions obtained from ultrasonication extract the order was CF(C) ≈ BF(C) > AF(C) ≈ HF(C) > and EF(C). In 2011, Chung *et al*. also observed varied polarity order for total phenol content in different fractions of mungbean seed extract[38]. The chemical nature and polarity of solvents utilised for extraction have a considerable impact on the solubility of phenolic groups in fractions of different polarities. The observed variability can be explained by the fact that these polyphenols are usually coupled with other elements, including proteins, carbohydrates, lipids, terpenes, etc., which may affect their solvability in different solvents[39]. On the other hand, among extraction techniques, the maximum phenol concentration was found in HFs obtained from maceration, followed by Soxhlet extraction and ultrasonication, while an exact inverse order was observed in CFs, i.e., ultrasonication > Soxhlet > maceration. In the case of EFs, almost similar phenol concentrations were observed for Soxhlet and maceration (p>0.05), and lower phenol concentrations were observed in ultrasonication. For AFs, the highest phenolic value was shown by maceration, then by ultrasonication and Soxhlet extraction methods. On the contrary, no significant difference was observed for BFs obtained from all three extraction procedures (p>0.05). All in all, the results showed higher phenolic concentration in fractions derived from maceration and Soxhlet than sonication. The key factor behind improved phenol concentration in Soxhlet fractions over other fractions was the extensive extraction by repeatedly washing the plant material in a hot solvent at a constant temperature.

Total flavonoid content

Flavonoids, which also include condensed tannins, flavones and flavanols, are an important metabolic group of plants, which contain a free OH group that aids in their antioxidant properties[40]. Plant-derived flavonoids have proven benefits against various

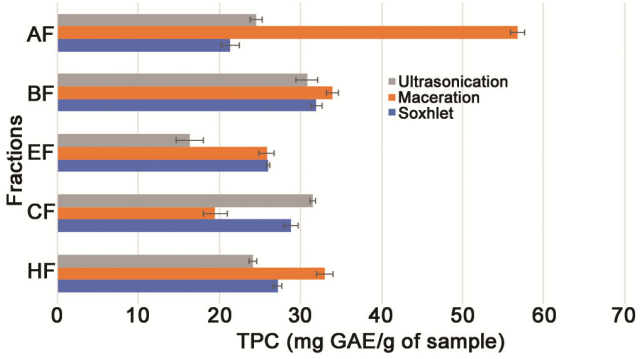

Fig. 2 — Total phenol content.

degenerative diseases along with *in-vitro* and *in-vivo* antioxidant, antimicrobial and anticancer activities[41].

As presented in Fig. 3, the total flavonoid concentration of all the tested fractions ranged from 38.21±0.07 to 149.95±0.53 mg QE/g of the sample with a statistically significant difference (p <0.05). With respect to solvent polarity in fractions obtained from all three extraction techniques, the higher flavonoid concentration was observed in EF and BF, followed by AF, while the lowest flavonoid concentration was observed in HF and CF. The findings of the study revealed higher flavonoid content in polar fractions compared to non-polar fractions. This may be due to the high solvability of flavonoids in polar solvents. Considering the extraction techniques, the concentration of flavonoids was reduced as follows: ultrasonication > Soxhlet > maceration for HF, maceration > Soxhlet > ultrasonication for CF and EF, and ultrasonication > maceration > Soxhlet for BF. However, the inverse order for AF is Soxhlet > maceration > ultrasonication. The observed disparities suggested that changes in flavonoid solubility could be influenced by specified extraction parameters.

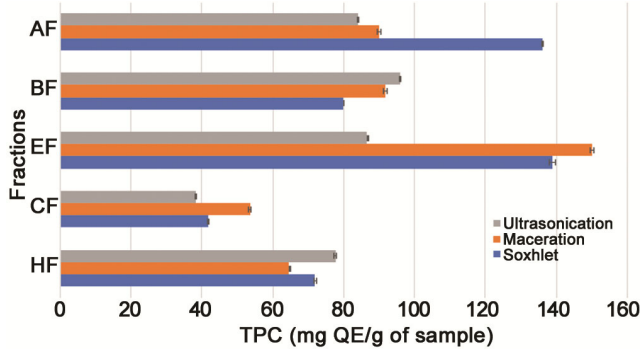

Fig. 3 — Total flavonoid content.

FTIR

The FTIR (Fourier Transform Infrared) spectra were used to determine the characteristic functional groups concordant to the bioactive compounds found in plant samples based on the values of absorption peaks obtained in the infrared region[42]. In Fig. 4a-c, the profiles of FTIR spectra were depicted for different fractions. The assignment of functional groups to the respective peaks was done in accordance with Coates[43]. The broad bands between the wavelength of 3570-3200 cm^{-1} indicated the OH stretching of the phenol group. However, the stretching peaks at 2996 and 2914 cm^{-1} might be assigned to asymmetric C-H (alkene) stretch, the latter probably assigned to methylene; another asymmetric/symmetric C-H stretch at 2851.4 cm^{-1} also representing methylene was observed in CF(B) and

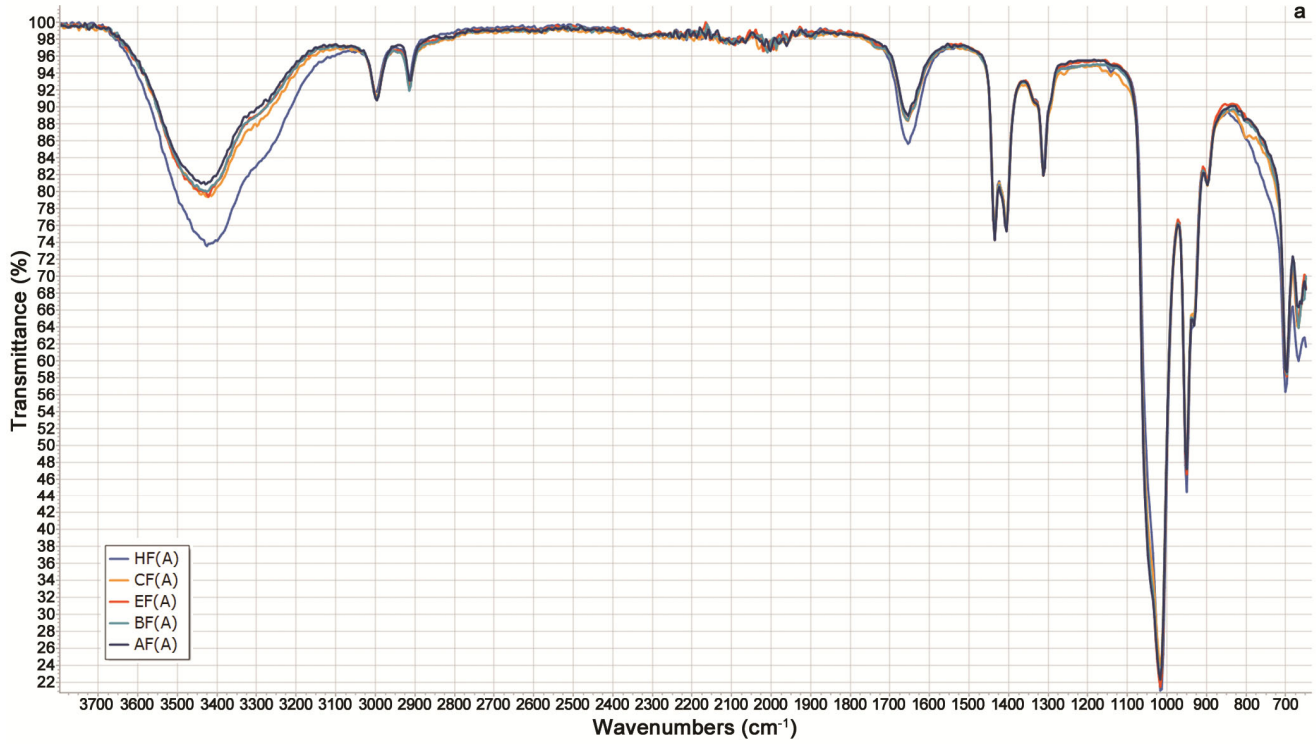

Fig. 4 — FTIR comparison spectra a) Soxhlet extraction fractions (*Contd.*)

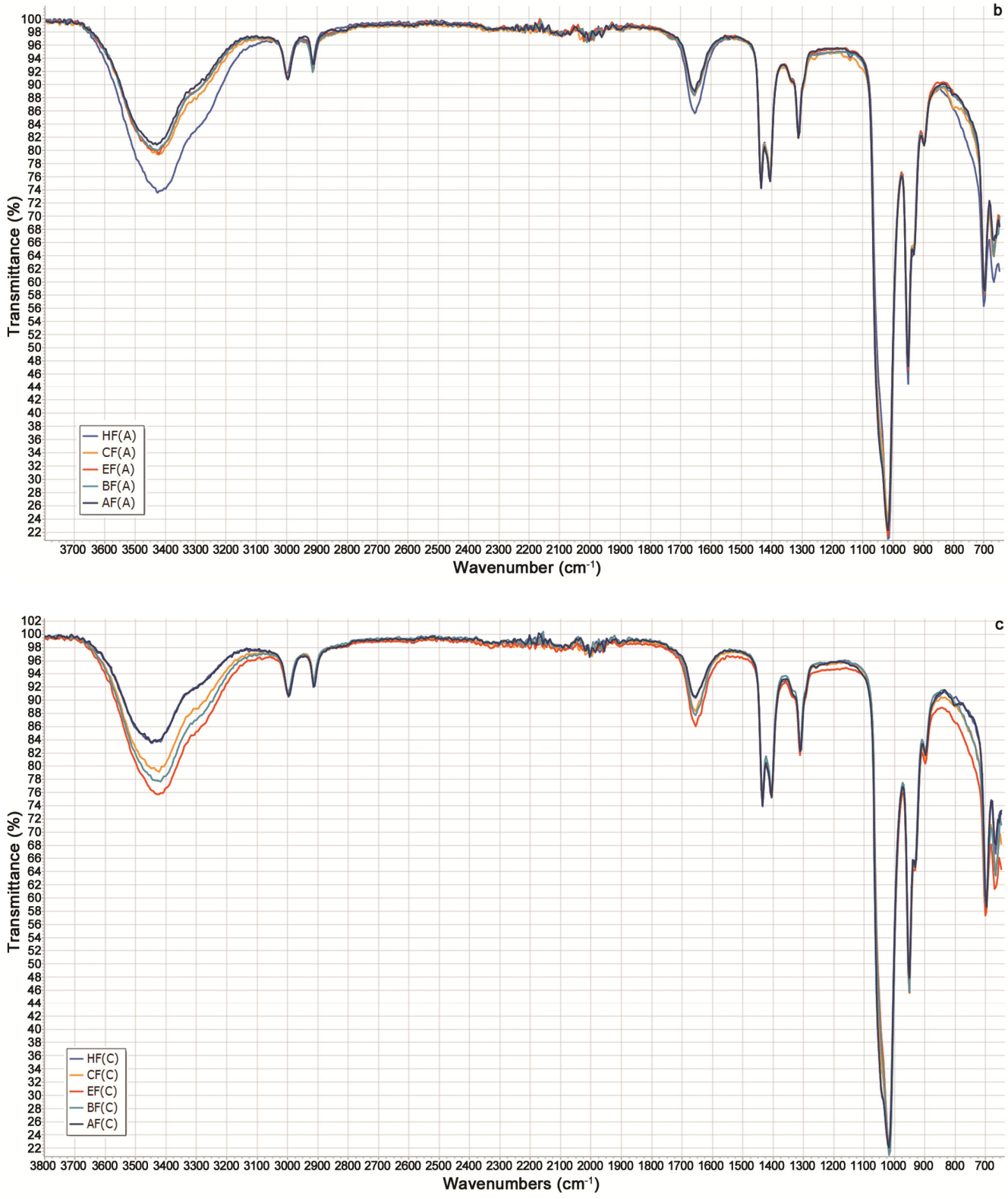

Fig. 4 — b) FTIR comparison spectra of maceration fractions; and c) FTIR comparison spectra of ultrasonication extraction fractions.

EF(B). The absorption peaks between 2000-1660 cm^{-1} may show the presence of C-H bending of aromatic compounds. The distinct peaks at 1740.7 and 1654.9 cm^{-1} may represent C=O stretching of carbonyl groups, although the former was only present in CF(B). Furthermore, the band at 1435 cm^{-1} might have revealed the presence of O-H bending of carboxylic acid salts and the band at 1408.9 cm^{-1} revealed to be the O-H bend of phenol or tertiary alcohol. The peak appearing at 1312.0 cm^{-1} is due to the C-O stretching of aromatic esters. In addition, the minor peaks at 1226.3 cm^{-1} and 1237.5 cm^{-1} might be assigned to C-O stretching of aromatic ether, probably vinyl ether, which is only found present in HF(C), CF(B) and EF(B). The strong peak near 1021.3 cm^{-1} could be assigned to silicon-oxy compounds. Also, the distinguished band at 954.2 cm^{-1} represents the trans-C-H out-of-plane bending. The peaks at 700.7 might be assigned to either cis-C-H out-of-plane bend or di or tri-substituted aromatic C-H bends. Lastly, the peak at 667.2 might represent the aromatic C-H out-of-plane bending. A higher number of peaks were observed in CF(B) and EF(B), both fractions obtained from the maceration technique. The results indicated the presence of several functional groups associated with the active phytocomponents, but no distinct variations were observed between the different fractions.

Antioxidant activity – FRAP assay

FRAP assay was employed to estimate the antioxidant activity, which quantifies the reducing capability of an antioxidant that reduces Fe(III)-TPTZ to Fe(II)-TPTZ to give a coloured complex at low pH[44]. The intensity of the complex formed reflects the antioxidant potential of studied samples.

The antioxidant activity of the tested fractions was represented by FRAP values that ranged between 41.54±0.56 to 104.81±0.48 mg Fe (II) E/g of the sample with significant difference (p<0.05) (Fig. 5. The results revealed that the sequence of FRAP values was almost identical in fractions obtained from Soxhlet and maceration i.e., EF(A) > AF(A) > BF(A) > HF(A) > CF(A) and EF(B) > AF(B) ≈ BF(B) > CF(B) ≈ HF(B) respectively, while the order for fraction obtained from ultrasonication extract was BF(C) ≈ EF(C) > AF(C) > HF(C) > CF(C). In the same way, when we talk about extraction procedures, the highest reducing power was observed in sonication, while the lowest was in maceration and Soxhlet for HF and BF. However, the exact inverse order was observed for AF, in which the Soxhlet and maceration gave statistically similar FRAP values (p>0.05), followed by ultrasonication. However, in the case of CF and EF, the best FRAP value was achieved in maceration, followed by Soxhlet and the lowest in ultrasonication, with nearly equal values in Soxhlet and maceration for EF (p>0.05).

The dependency of antioxidant activity (FRAP assay) in relation to TFC and TPC was also evaluated using Pearson's correlation analysis. The findings revealed a remarkable positive correlation between FRAP assay and TFC (r^2 = 0.905; p<0.05), indicating that flavonoids were highly implied in the antioxidant activity of the plant. Fig. 6 displayed the Heat map, which presented the quantitative analysis of Yield, TPC, TFC and FRAP.

Antimicrobial activity

Significant antimicrobial activity was exhibited by various fractions of *Vigna radiate* (L.) R. Wilczek

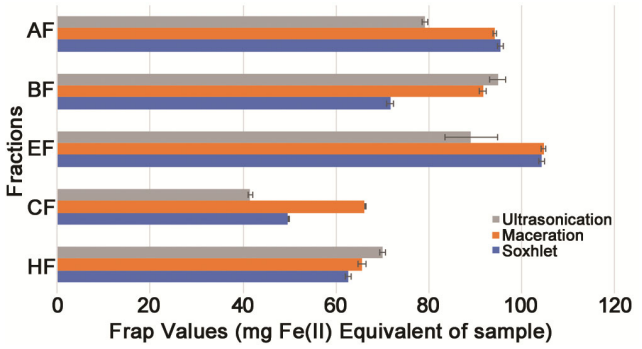

Fig. 5 — Antioxidant activity by FRAP assay.

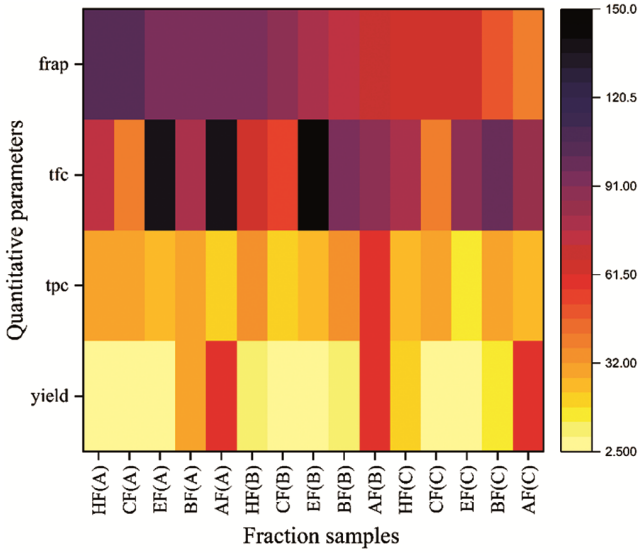

Fig. 6 — Heatmap presenting the quantitative analysis of Yield, TPC, TFC and FRAP.

seeds against tested microorganisms. According to the results presented in Fig. 7 the most susceptible bacterial strain proved to be *S. aureus* (gram-positive), followed by *B. subtilis* (gram-positive). All the same, *Pseudomonas sp.,* a gram-negative bacterium, was shown to be the most resistant as no fractions developed a zone of inhibition against it. The higher susceptibility in gram-positive bacteria can be explained by the different cell wall topology because gram-positive bacteria have a cell wall with only a peptidoglycan layer, which is an ineffective permeability barrier, while gram-negative bacteria have another outer phospholipid layer, which is a more significant barrier[35]. In the case of fractions, the utmost activity was exhibited by fractions obtained from the ultrasonication extract, with a 20 mm zone of inhibition for EF(C) and AF(C), which is nearly equivalent to standard antibiotic gentamycin (25 mm), followed by BF(C) and HF(C) (inhibition zone; 15 and 14 mm respectively) against the gram-positive bacterium, *S. aureus.* In addition, fractions obtained from maceration extract were equally potent against *S. aureus* except for AF(B), which had no zone of inhibition, albeit from the Soxhlet fraction, only CF(A) showed activity. Kanatt and co-workers corroborated significant inhibition of *S. Aureus* by an aqueous extract of mungbean hull[45]. With respect to *B. subtilis*, only EF(A) and HF(C) from Soxhlet and ultrasonication, respectively, showed effectual inhibition. However, in the case of activity against fungal strain, the fractions EF(A) and BF(A) (inhibition zone; 10 mm) obtained from Soxhlet extract displayed accentuated inhibition to *S. cerevisiae*, with results comparable to the standard antifungal drug Fluconazole (inhibition zone; 20 mm). Overall, ethyl acetate fractions from all the extraction techniques presented the best results against tested microbial samples. It was reported that mungbean seeds create an acidic environment, which may be a potential mechanism for antimicrobial activity by disrupting bacterial cell membranes[46].

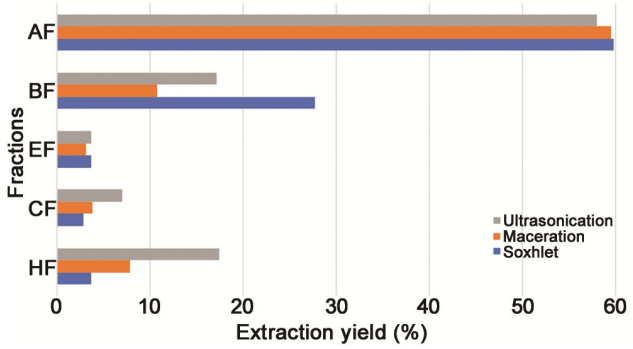

Fig. 7 — Antimicrobial activity.

Principal component analysis

Principal Component Analysis (PCA), an important multivariate technique, was employed for quantitative phytochemical data sets to discriminate the different extraction methods along with different solvents used in the study to identify the possible interrelationships and clusters among them. The first three PCA components explained 97.93% of total cumulative variance, of which 51.09% of variance accounted by PC1, 32.27% of variance accounted by PC2, and 14.57% of variance accounted by PC3. The highest contribution to PC1 was attributed to FRAP (0.664)and TFC (0.648), followed by yield (0.359) and TPC (0.096); all parameters were found to be positively correlated to PC1. However, PC2 was highly contributed by TPC (0.762), followed by yield (0.568), while TFC (-0.270) and FRAP (-0.154) contributed the least, in which TPC and yield were found to be positively correlated, and TFC and FRAP were found to be negatively correlated to PC2. In the case of PC3, the highest contribution was attributed to yield (-0.740), followed by TPC (0.629), FRAP (0.219) and TFC (0.093); all parameters were found to be positively correlated to PC3 except yield. According to the PCA biplot (Fig. 8), TFC and FRAP show a strong correlation with each other, and both presented a strong correlation with AF(A), BF(B), EF(A), BF(C) and EF(B), which was also consistent with quantitative data, suggesting that polar solvents should be preferred for flavonoid and FRAP

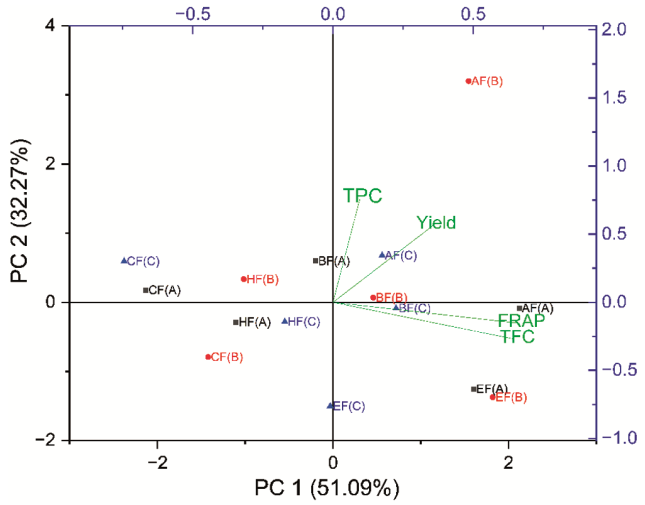

Fig. 8 — PCA biplot.

quantification. AF(B), BF(A), HF(B), HF(A), CF(C), and CF(A) present a strong correlation with TPC, indicating that the Soxhlet extraction method with chloroform and hexane solvent might be optimal for the TPC. Moreover, AF(B) and AF(C) were found to be strongly correlated to yield, which was congruent with higher yield in aqueous fractions.

Conclusion

The current study offered a thorough investigation of the effect of different extraction techniques (Soxhlet, maceration, and ultrasonication) and solvents on yield, quantitative phytochemical profile, and biological activities. The various fractions of mung bean (*V. Radiate* (L.) R. Wilczek) seeds prepared from the extract obtained from different extraction techniques showed significant variation in biochemical profiling, including antibacterial activity. No discernible pattern was observed, although maceration techniques gave comparatively best results, and Soxhlet also presented well among extraction techniques. In the case of fractions, the ethyl acetate fraction outperformed others in terms of quantitative estimation and antioxidant and antimicrobial activity. FTIR also revealed the presence of the maximum components in the ethyl acetate fraction obtained from the maceration extract. PCA analysis corroborated the above quantitative estimations and showed significant discrimination among extraction techniques and solvents. The study also highlighted the need for future studies to focus on comprehending the mechanism of action for such activities to validate the effectiveness of mung bean and its derivatives as therapeutic agents.

Conflict of interest

The authors declare no conflict of interest.

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