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Sustainable phytochemicals extraction from Conyza Canadensis weed based on green analytical chemistry metrics; optimization of reaction parameters and evaluation of the antioxidant and antimicrobial activities.

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Abstract :

Increasing concern about environmental sustainability and the economic challenges of disposing of wild plants have stimulated researchers to transform waste into valuable resources**.**This paper presents an eco-friendly and cost-effective method for extracting photochemical constituents from Conyza Canadensis (CC) an abundant wild plant in Iraq is considered problematic weed in agricultura sectors, involving the use of green 1-butyl-3-methylimidazolium chloride (BMIMCl) aqueous solution omitting traditional organic solvents. This innovative process employs a 100-ppm solution of each reactant and maintains an optimal heating time of 5 minutes at 50ºC. The biochemical composition of CC reveals the presence of Phenols, Tannins, Terpenoids, Saponins, Glycosides, Steroids and Flavonoids by conducting standard qualitative procedures. Quantitative analysis was studied using a Gas Chromatography-Mass Spectroscopy (GC-MS) indicating the presence of approximately 35 compounds. Ferric reducing antioxidant power (FRAP) procedure measured spectrophotometrically and showed a good antioxidant activity (495 mg mL^{-1}). This result was compared with a green and new µPADs platform (475 mg mL^{-1}) giving a minor difference. The extract was tested for its microbial activity against two Gram-positive bacteria (*Staphylococcus epidermidis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Klebsiella sp* and *Escherichia coli)* as well as one yeast (*Candida albicans*) and revealed antibacterial activity against all isolated except *Klebsiella sp*. Therefore, this research developed a simple, cheap, sustainable, and green platform to extract valuable bioactive compounds from wild plant waste CC to be used as a promising avenue for producing natural antioxidants and antimicrobial agents, in additional to sustainable management of wild plant waste.

Keywords: green phytochemicals extraction, sustainable wild weed recycling, Conyza Canadensis, Ionic liquids, antimicrobial activity, antioxidant activity, lab-on-a-paper.

استخلاص مستدام للمركبات النباتية الفعالة من عشبة حشيشة الجبل استنادًا إلى معايير الكيمياء التحليلية الخضراء؛ تحسين شروط التفاعل وتقييم النشاط المضاد للأكسدة والمضاد للميكروبات.

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مستخلص:

تزايد الاهتمام بالاستدامة البيئية والتحديات الاقتصادية المرتبطة بالتخلص من النباتات البرية دفع الباحثين إلى تحويل النفايات إلى موارد ذات قيمة. يقدم هذا البحث طريقة صديقة للبيئة وفعالة من حيث التكلفة لاستخلاص المركبات النباتية من نبات حشيشة الجبل وهو نبات بري وفير في العراق يُعد من الأعشاب الضارة في القطاع الزراعي. تعتمد الطريقة على استخدام محلول مائي من مركب أيوني أخضر (butyl-3-methylimidazolium chloride - BMIMCl-1) دون الحاجة إلى المذيبات العضوية التقليدية. تتضمن العملية استخدام محلول بتركيز 100 جزء في المليون من كل مادة، مع تسخين لمدة 5 دقائق عند 50 درجة مئوية.

أظهرت التحاليل الكيميائية الحيوية وجود مركبات فعالة مثل الفينولات، التانينات، التربينويدات، الصابونين، الجليكوسيدات، الستيرويدات، والفلافونويدات باستخدام اختبارات نوعية قياسية. كما تم إجراء تحليل كمي باستخدام جهاز كروماتوغرافيا الغاز – مطياف الكتلة (GC-MS)، وكشف عن وجود حوالي 35 مركبًا. تم قياس النشاط المضاد للأكسدة باستخدام طريقة PARF وبلغت القيمة 495 ملغم/ مل، وقورنت بمنصة µPADs الحديثة الخضراء والتي أظهرت نتيجة قريبة (475 ملغم/ مل). تم اختبار المستخلص ضد نوعين من البكتيريا موجبة الغرام (Staphylococcus epidermidis وStaphylococcus aureus) ونوعين من البكتيريا سالبة الغرام (Klebsiella sp وEscherichia coli) بالإضافة إلى فطر Candida albicans، وأظهر فعالية مضادة للميكروبات ضد جميع الأنواع باستثناء Klebsiella. لذلك، طور هذا البحث منصة بسيطة ورخيصة ومستدامة وصديقة للبيئة لاستخلاص المركبات النشطة من نبات بري ضار، مما يشكل مسارًا واعدًا لإنتاج مضادات أكسدة ومضادات ميكروبية طبيعية، إلى جانب إدارة مستدامة للنفايات النباتية البرية.

الكلمات المفتاحية: استخلاص المركبات النباتية الخضراء، إعادة تدوير الأعشاب البرية المستدامة، حشيشة الجبل ، السوائل الأيونية، النشاط المضاد للميكروبات، النشاط المضاد للأكسدة، مختبر على ورقة.

Introduction

A revolutionized development for sustainable analytical practices to extract beneficial bioactive compounds-from agricultural waste/wild plans to produce antioxidant, antimicrobial, anticancer, etc. agents for commercial purposes has extensively increased in recent decades [1]. This can be attributed to the global environmental awareness of agricultural waste / wild plant waste as the second largest contributor to greenhouse gas release due to crop residue burning and indiscriminate dumping arising the global warming and threating the balance of the well-being of society and the ecosystem[2, 3]. In addition to the growth of the world's population, estimated to reach 8.5 billion by 2030, causing bio resource depletion[4-6]. This could be achieved by employing green analytical metrics that contribute to the principles of green chemistry offering an eco-friendly and sustainable analytical procedures with a focus on zero – waste production rather than end-of-pipe treatment [7, 8].

In line with these challenging, the design of biochemical compounds extraction is considered a crucial aspect from green chemistry point of view due to its responsibility for solvent, and energy consumption[9]. To meet these challenges, the choice of suitable combination of green solvents and optimization of extraction steps is one of the strategies to reduce the environmental impact of analytical laboratories activities. Organic solvents including methanol, ethanol, etc and water is widely in extraction procedures. However, water is limited to extract polar compounds. Organic solvent on the other hand is capable for extraction weak polar and polar compounds. Nevertheless, these solvents suffer from their toxicity, environmental hazards high cost and low biodegradability that extremely limit their applications.[10]. A recent green innovation entails the selection of an optimal solvent that must align with a sustainable future, as well as the circular economy. In addition, minimizing human health and environmental impact [11]. Ionic liquids (ILs) have encouraged the elimination of conventional hazardous solvents showing a significant potential for

greener, economic and more environmentally friendly options for diverse applications[12]. Another important factor to be set into consideration is the innovation and use of renewable plant resources that offers unique characteristics, i.e, valuable renewable sources for natural bioactive compounds, minimal environmental impact as it is collected from the environment or originating from the agricultural and food sector not produced, abundantly, easy accessibility, sustainability, biodegradability, and non-toxicity[13-15].

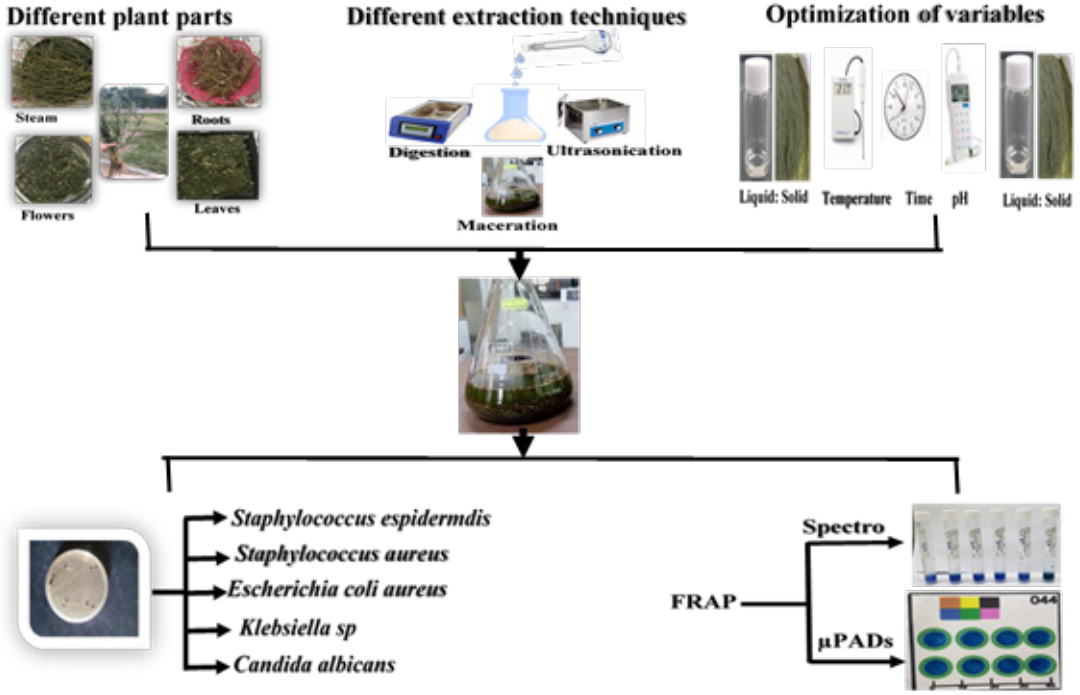
Conyza Canadensis (CC), is a member of Asteraceae family that contain more than 50-80 species originated from North and south America that recognized as an invasive species infesting lawns, roadsides, wastelands, vineyards and field crops such as, cotton and corn [16]. As an invasive species, CC can cause serious problems on the local species diversity and consequently the ecosystem structure[17]. In Iraq, the naturally CC weed is grown in North of Baghdad and is well characterized by hairy long steam (up to 1.5 m tall) with numerous and thin leaves distrusted around the steam and small flower heads. CC is considered as an annual weed (grows in winter and summer) and the extensive light weighted seeds production enables its disperse over long distance by wind[18]. The whole plant is widely used in folk medicine to treat wound, rheumatism, diarrhea, dental pain, headache, microbial infections like urinary infections, swelling, and respiratory tract infections[19, 20]. Several studies have assess the extraction of CC phytochemical but these methods suffer from the use of organic solvent[18, 19, 21] , time consuming [22] , and sophisticate techniques[20]. The aim of this study is to extract beneficial phytochemicals from abundant and abandoned CC weed under the concept of sustainability and green chemistry to be applicable as antioxidant and antimicrobial agents.

Materials and Methods

Conyza Canadensis plant collection and preparation

Abandoned Conyza Canadensis were collected from Al-Rashida, Baghdad, Iraq during early spring (March 2024). The plant was washed under tap water to ensure the removal of any dust

and other contaminants. Each plant was divided into four parts (roots, flowers, leaves, and stems) chopped into small parts, blended using an electrical blender to retain fine powder, dried naturally under sunlight, and stored in a plastic stopper vial. The plant powder was used without any physical or chemical pretreatment other said.



Scheme 1. Represent schematic model of green and sustainable steps involved in phytochemicals extraction from CC weed plant and applications.

Preparation of extract

Green1-butyl-3-methylimidazolium chloride (BMIMCl) (Shanghai Maclean Biochemical Company/ China) solvent (2 % wt/v) was used with two different solvents (deionized water, and ethyl acetate (Sigma - Aldrich / Germany)) to extract CC phytochemicals by mixing one gram of CC with the three solvents, separately. The mixtures were placed into a water bath for 60 minutes at 25° C, and filtered using Whatman Grade No.18 filter paper. In addition, a comparative experiment

was conducted using three different extraction methods. The first method (maceration): included the addition of one gram of CC powdered sample into 10 mL of BMIMCl solvent and leaving the mixture to macerate for one day at room temperature. The second method (sonication): involved mixing 10 mL of BMIMCl with one gram of CC fine powdered. Phytochemical extraction was performed using an ultrasonic bath (Elmasonic S10H, at 25°C, 280 W bath power with 50/60 Hz frequency). The third method (digestion) involved the mixing of one gram of the fine plant powder with 10 mL of BMIMCl and placed in a water bath (Memmert / Germany) at 25° C. The resulting supernatant for each method was filtered using Whatman Grade No.18 filter paper, and dried using an oven set at 50° C, individually. The yield of extraction was calculated using Equation (1.1) [23]

$$Yield\ \% = \frac{Weight\ of\ dried\ crude\ extract\ (g)}{Weight\ of\ dried\ plant\ sample\ taken\ (g)} \times 100 \quad \text{..... Equation 1.1}$$

Operational Conditions Optimization

Batch experiments were conducted including plant type (roots, flower, stem, and leaves), extraction time ranging from (10-90) minutes, solid to liquid ratio (0.5 g: 10 mL, 1 g: 10 mL, 1.5 g: 10 mL, 2 g: 10 mL, 4 g: 10 mL), Liquid to solid ratio (5 mL: 0.5 g, 10 mL:0.5 g, 20 mL:0.5 g, 30 mL:0.5 g), temperature (25, 35, 50, 75, 90) °C, and pH ranging from (3-9) by varying one factor while the other factors are constant to obtain the maximum extraction yield.

Phytochemical Extract Analysis

Qualitative Analysis

Standard procedures were used to investigate the presence of Phenols, Tannins, Terpenoids, Saponins, Glycosides, Steroids and Flavonoids in CC-BMIMCl extract quantitatively [24].

Quantitative Analysis

A gas chromatography-mass spectroscopy instrument was used for the

analysis of the chemical compounds of CC –BMIMCl extract.

Ferric Reducing Antioxidant Power (FRAP)

Spectrophotometric detection: Ferric-reducing antioxidant power (FRAP) described by Javed *et al.* was used to measure of antioxidant activity of CC-BMIMCl with some modification[25]. The mixture of 0.1 mL of CC-BMIMCl, 0.5 mL of potassium ferricyanide (1 %, wt/v) and 0.4 mL of phosphate buffer (0.2 M, pH =6.6) was left for 20 minutes at 50°C. Then, 0.5 mL of trichloroacetic acid (100 mg L^{-1}) was added, and the mixture was vortex and centrifuge centrifuged for 10 minutes at 3000 rpm. Subsequently, the supernatant was mixed with deionized water, and 0.1 % (w/v) ferric chloride (5:5:1 v/v/v) and then left for 10 minutes at room temperature. The absorbance was measured at λ_{max}=700 nm against reagent blank.

Colorimetric detection on la-on-a-chip platform: The paper-based device was fabricated using a method developed by Carrilho *et al* with several variations[26]. Adopting a method from Javed *et al*, the mixture of 0.1 mL of CC-BMIMCl, 0.5 mL of potassium ferricyanide (1 %, wt/v) and 0.4 mL of phosphate buffer (0.2 M, pH =6.6) was mixed and left for 20 minutes at 50°C. Then, 0.5 mL of tri chloro acetic acid (100 mg L^{-1}) was added, and the mixture was vortex and centrifuged for 10 minutes at 3000 rpm. Afterwards, five microliters of deionized water, and five microliters of 0.1% (w/v) ferric chloride, the supernatant were dropped into the detection circular reaction zone, using a micropipette. After pipetting, the detection circular reaction zone was left for 10 minutes at room temperature and photographed using phone camera. Images were analyzed using a freeware Image J (National Institutes of Health, USA).

Antimicrobial activity comparative studies

Agar well diffusion method was used to verify the antibacterial and antifungal activity of CC-BMIMCl extract in vitro on Gram-positive bacteria including *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) and Gram-negative bacteria including *Escherichia coli* (*E. coli*) and *Klebsiella sp.*, in addition

to one *Candida albicans* (*C. albicans*) (yeast) isolate which was kindly provided by the Biology Department, Mustansiriyah University. All strains were grown on Muller Hinton agar for 18 hours at 37˚C. After incubation, a sterile loop was used to pick one colony and then suspended in 5 ml of fresh Muller Hinton broth to reach approximately 0.5 MacFarland (1.5 × 108) by using saline solution. Each of the tested isolates was inoculated and seeded evenly on a fresh Muller Hinton agar plate via a sterile cotton swab and allowed to dry. By using a sterile cork borer, a circular shape (4 mm in diameter) was cut from the agar and 100 µl of the extract was added to determine antimicrobial activity after 24 hours of incubation at 37 ºC by measuring inhibition zone diameter (mm). This test was performed in duplicate[19].

Results and discussion

Optimization of extraction parameters

Batch extraction experiments were conducted to evaluate the influence of different parameters on maximizing the efficiency of the valuable phytochemicals extraction process. A comparative study on the effect of various parts of the CC weed extract including roots, flower, stem, and leaves was studied. The aerial part of the weed gave the highest extraction yield percentage as demonstrate in Figure 1 (A) that agree with previous researches[27]. Therefore, CC leaves extract was chosen for all subsequent experiments. The most important intention of green chemistry is to omit or minimize the utilize of toxic solvents. Ionic liquids (ILs) are characterized with unique properties such as high stability, biodegradability, low vapor pressure and low toxicity etc. Due to these properties ILs are a promising green alternative solvent to organic solvents[28]. Green BMIMCl solvent was used and compared with two different solvents including deionized water, and ethanol. As can observed from Figure 1 (B), BMIMCl gave the maximum extraction yield (20 %) compared with 10 % for deionized water and 7 % for ethanol under the same condition. Thus, BMIMCl was selected for further studies. An essential precursor for preparing the CC extract is the extraction techniques. Mac-

eration (17%), sonication (18%), and digestion extraction technique (20 %) were used and digestion techniques was adopted for phytochemical extraction from CC-BMIMCl extract (Figure 1 C). Another factor that effect the economic cost is the extraction time. Time interval from (10-90) minutes was studied and Figure 1 (D) showed a graduated increase within 30 minutes, which was recommended for optimum extraction yield. Solid to liquid ratio has an important role during the process of extraction. It is correlated to the contact area between liquid (solvent) and solid (plant matrix). Figure 1 (E) stated that the increase of solid(plant matrix) above 0.5 gram caused a decrease in extraction yield percentage which can attributed to the saturation of liquid (solvent) to solid (plant matrix)[29]. In this study, the influence of liquid to solid ratio was found to be ~~in~~ consistent with the principles of mass transfer, the increase in liquid to solid ratio leads to increase in concentration gradient that improves the rate of diffusion and promotes the extraction of greater solutes from the solid (plant matrix) to liquid (solvent)[29]. Although, increasing the amount of solvent enhance the solubility and extraction of more phytochemicals; higher amount of solvent may result in diluted extracts. As a result ,20 mL of BMIMCl solvent was adequate for maximum extraction yield (Figure 1 F). The influence of temperature on CC-BMIMCl extraction efficiency was studied. An increase in extraction yield percentage was detected with the increase in temperature from 25°C to 50°C and a plateau trend was observed above 50°C as cited in Figure 1 (G). Hence, 50°C was selected to avoid hydrolysis and thermolabile compounds degradation[30]. Due to the important impact of pH variation of extraction yield, experiments were conducted using 0.1 M NaOH and HCl solutions to demonstrate the effect of pH in the range of 3-9. Figure1 (H) demonstrate the increase of extraction yield percentage until pH reaches 6, afterwards, no significant increase was observed.

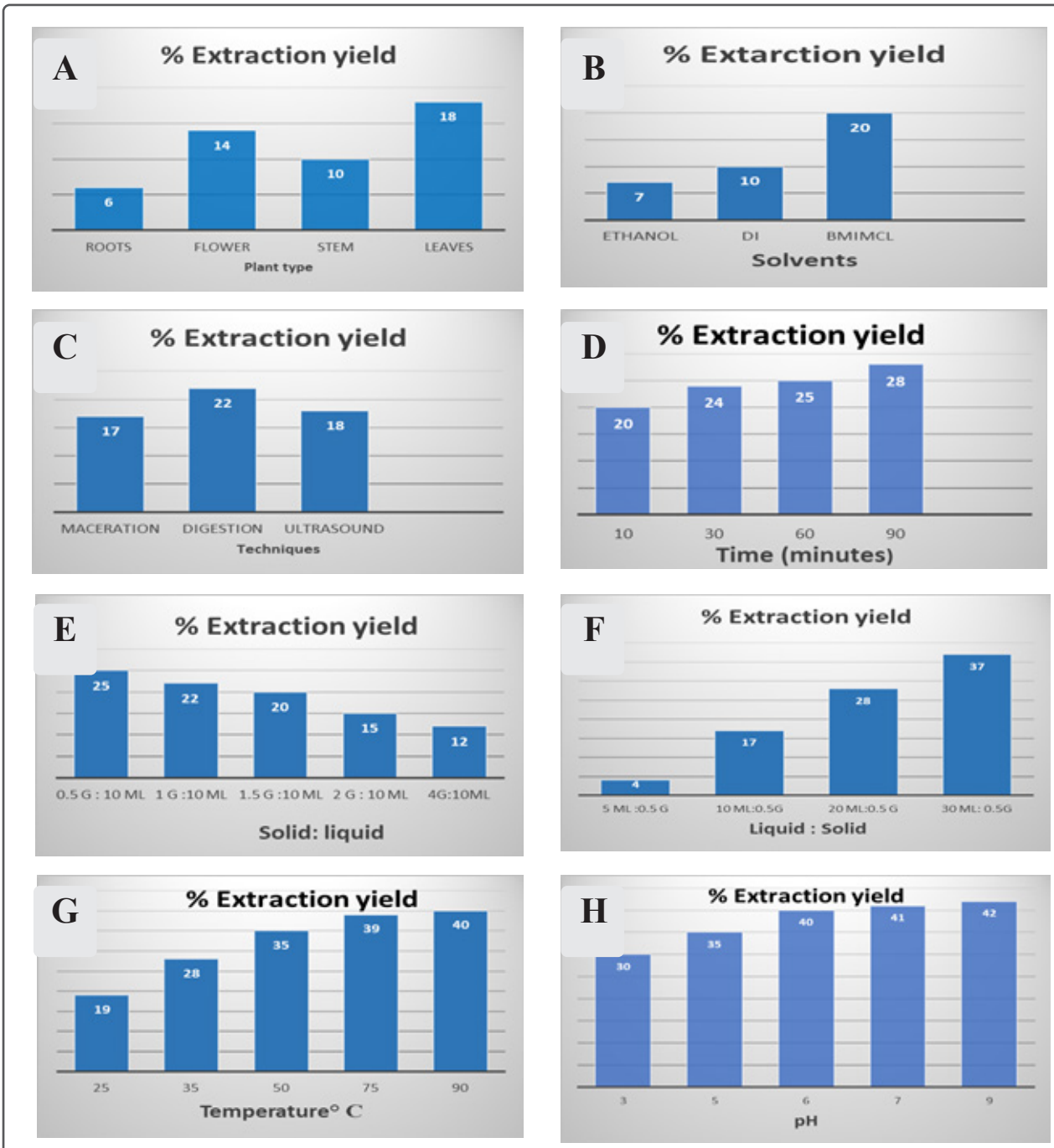


Figure 1. Extraction yield percentage of the optimized extraction system parameters. (A) extraction of different parts of CC weed plant (roots, flower, stem, leaves), (B) different solvents (BMIMCl, ethanol, deionized water), (C) extraction techniques (maceration, ultrasound, Digestion), D) extraction time ranging from (10-90) minutes, E) Solid to liquid ratio (0.5 g: 10 mL, 1 g: 10 mL, 1.5 g: 10 mL, 2 g: 10 mL, 4 g: 10 mL), (F) liquid to solid ratio (5 mL: 0.5 g, 10 mL:0.5 g, 20 mL:0.5 g, 30 mL:0.5 g), (G) temperature (25, 35, 50, 75,90) °C, H) pH ranging from (3-9).

Qualitative phytochemicals analysis

Phytochemical analysis of the CC-BMIMCl extract revealed the presence of Phenols, Tannins, Terpenoids, Saponins, Glycosides, Steroids, and Flavonoids, as tabulated in Table 1 .

These results agreed with previous studies[18, 27]. Among these compounds, phenols play an essential role as antioxidant due to its stable radical intermediates, in addition to the electrons or hydrogen denotation ability[31]. Phenolic-rich byproduct offers a sustainable, green, and economic raw material for free radicals neutralizing that can contribute to the reduction of their effect and preserve human health[32].Aligning with green chemistry principles; the production of antioxidant from byproducts not only reduce environmental impact, but also give a replacement to synthetic pharmaceuticals making them a sustainable and green alternative choice [33].

Table 1. Qualitative phytochemical analysis using standard methods for of CC- BMIMCl extract.

Phytochemicals	Reagents	Remarks	CC- BMIMCl extract.
Phenols	Sodium carbonate and Folin Ciocalteau's Reagent	The formation of blue/green color.	
Tannins	Ferric Chloride	The formation of brownish-green color.	
Terpenoids	Chloroform and H_2SO_4	The formation of reddish-brown color.	
Saponins	boiling water	The presence of foam.	
Glycosides	Benedict reagent	The presence of reddish brown precipitate.	
Steroids	Acetic anhydride and H_2SO_4	The formation of blue to green circle color.	
Flavonoids	lead acetate	The formation of yellow precipitate.	

GC-Mass analysis

The chemical constituents of CC-BMIMCl extract were analyzed using a GC-MS instrument. The GC-MS chromatogram obtained from the extract resulted in the identification of 35 compounds with various retention times (Figure 2). The main constituents that exist in the analyzed CC-BMIMCl are shown in Figure 3 and listed in Table 2. The presence of antioxidants, antibacterial and antifungal compounds has been confirmed. Opening the way for further studies to determine the possible medical applications of these compounds. Several GC-MS peaks were unidentified due to the lack of library data of corresponding compounds.

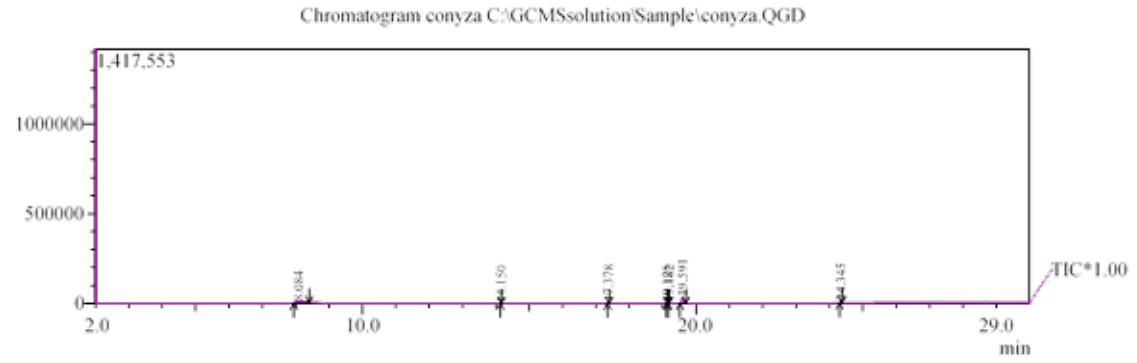


Figure 2. GC-MS chromatogram of CC-BMIMCl extract

Table 2. Retention time, peak area, name of the compound, chemical formula and molecular weight of CC-BMIMCl extract.

Peak	Retention Time	Area%	Name of the compound	Formula	Mol Wt(g/mol)
1	8.083	47.95	2-Amino-3-thiophenecarbonitri l	$C_5H_4N_2S$	124
2	14.150	0.72	4-Hydroxy-6-methyl1-3nitro-2-pyridone	$C_6H_6N_2O_4$	170
3	17.378	3.50	Hexanoic acid,2-methyl	$C_7H_{14}O_2$	130
4	19.126	1.44	2,4-Hexadienal	C_6H_8O	96
5	19.183	1.13	1-Pentyn-ol	$C_6H_{10}O$	98
6	19.591	39.37	5-Eicosyne	$C_{20}H_{38}$	222
7	24.345	5.88	2-Propenoic acid	$C_8H_{12}O_2$	140

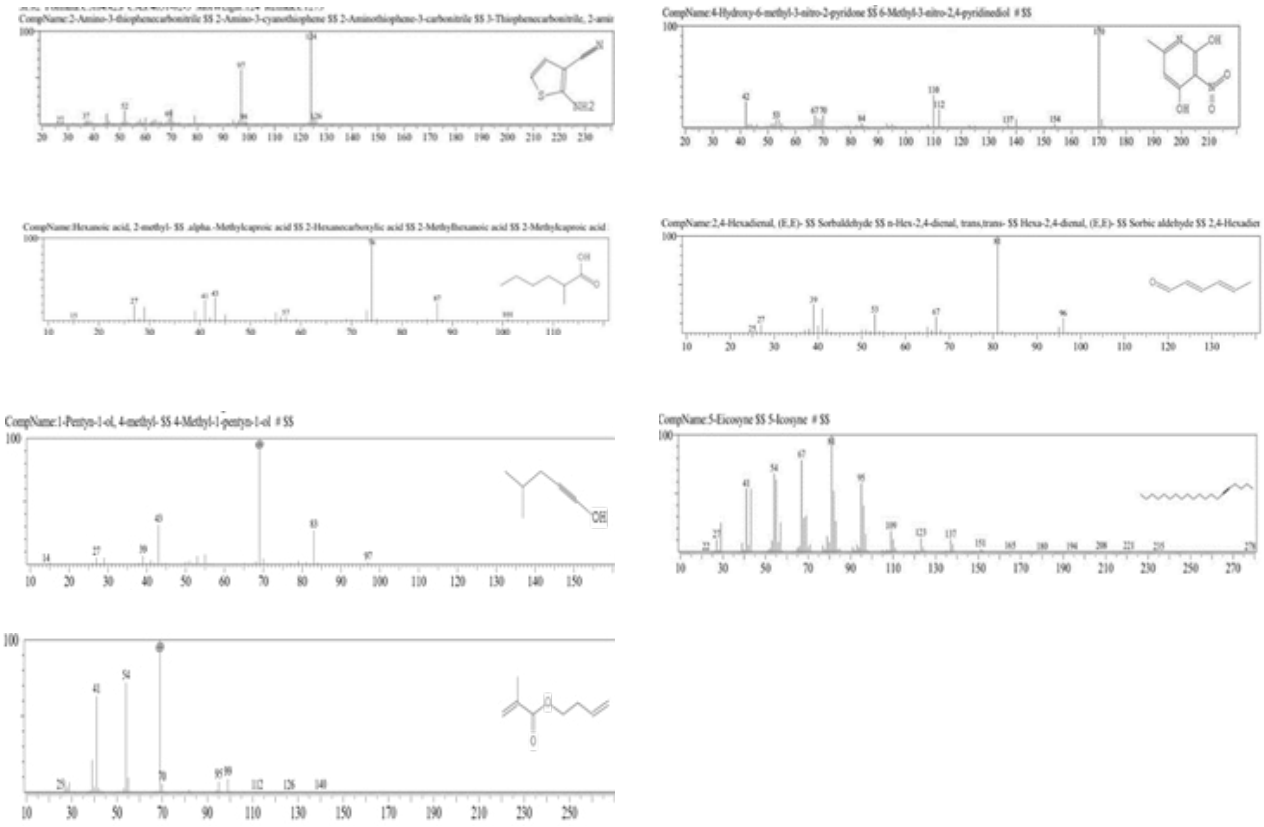


Figure 3. GC-Mass Spectrum of the main compounds detected in CC-BMIMCl extract

Ferric Reducing Antioxidant Power (FRAP)

The reducing power is often used as an indicator of electron donating activity, which is an important approach for testing the anti-oxidative action/anti-oxidant properties/radical scavenging ability of the extracts. previous studies revealed that tannins do not only possess vital anti-inflammatory properties by their ability to inhibit the 5-lipoxygenase enzyme in the arachidonic acid metabolism but are also one of the potent ferric reducing agents[34].As can be seen from Figure 4, the CC-BMIMCl extract exhibited a higher reducing power by spectroscopic method (concentration = 495 mg mL^{-1}) than paper micro fluidic method (concentration= 475 mg mL^{-1}) relative to the ascorbate

standard. Paper-on-lab as a miniaturized form of traditional laboratories have offered a complete diagnostic protocol for antioxidant analysis. Using such systems, improve analytical performance and applying green chemistry principles at the same time by reducing sample reagent volume, portiility, cost-effective, and short time required for analysis[35].

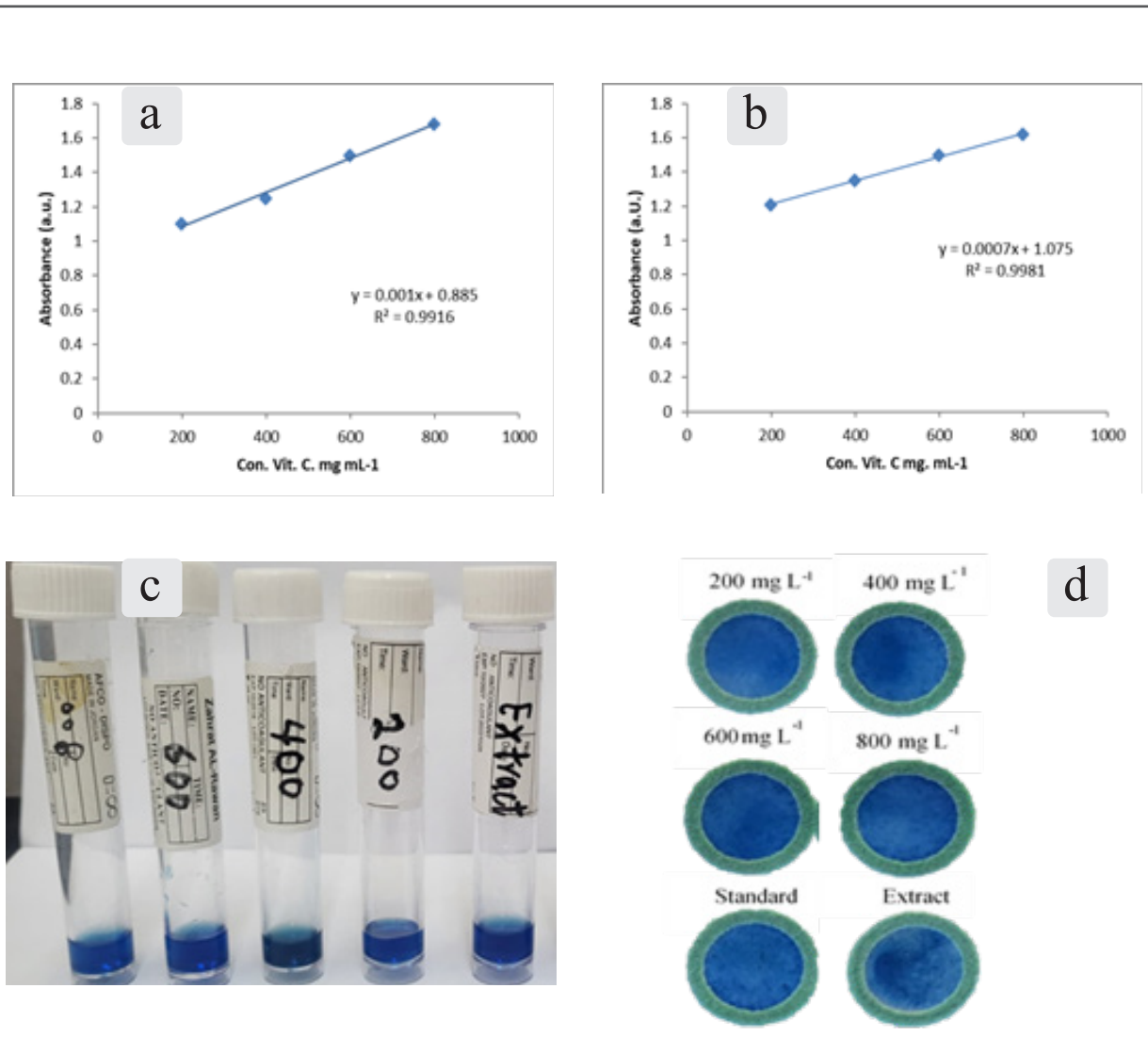


Figure 4. (a) calibration graph for spectrophotometric determination of Vit. C concentration in the range between (200-800) mg L^{-1} , (b) Calibration graph for µPADs determination of Vit. C concentration (200-800) mg L^{-1} , (c) an image of serial concentration of Vit. C in a volumetric flask, (d) an image of the paper microfluidic device with serial standard concentrations of Vit.C spotted inside the sensing zone.

Antimicrobial activity

Finding new alternatives to antibiotics is increasing, as drug-resistant bacteria are becoming a global problem. An excellent source with minimal side effects is plants that are used to treat different bacterial infections[36]. The Conyza species have been known for their biological activity[37]. The BMIMCl extract was tested against different microorganisms including one isolate of *S. aureus*, *S. epidermidis*, *E. coli* and *Klebsiella sp.* and one fungal isolate of *Candida albicans*. As shown in Figure 4; all isolates were inhibited except *Klebsiella sp.* and the extract was most active against *S. epidermidis* (19 mm) while the extract showed same inhibitory effect with 12 mm against *E. coli* and *C. albicans* and 14 mm inhibition zone against *S. aureus* isolate. Therefore, the bactericidal effect of the BMIMCl extract was observed in both gram-positive bacterial isolates tested, more likely resulting from cell wall components. As gram-negative bacteria cell walls are characterized by a more complex structure (outer and plasma membranes), the outer membrane is used for protection by acting as a selective barrier[38]. In addition, the presence of extracellular polymeric substances (EPS) can help bacteria to overcome harsh environmental conditions [39]. While results showed no inhibitory effect against Klebsiella sp., this may be due to the presence of a capsule that protects bacteria from the extract's bactericidal activity [40].

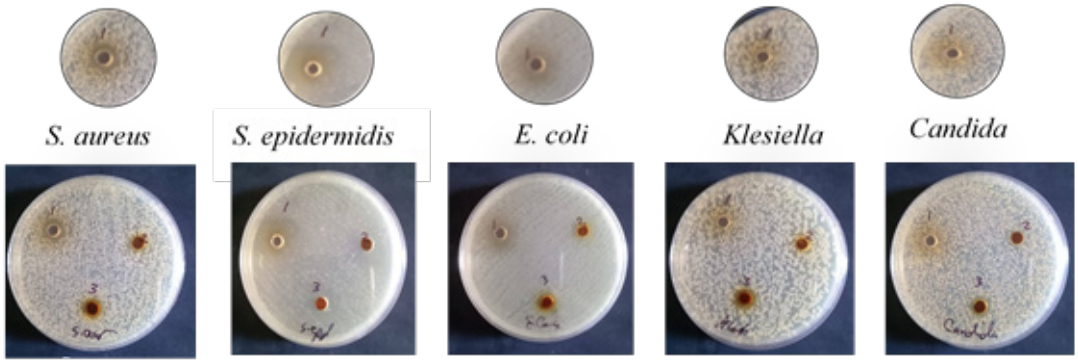


Figure 4. Antimicrobial activity of CC-BMIMCl extract against *S. aureus*, *S. epidermidis*, *E. coli* and *Klebsiella sp.* and one fungal isolate of *Candida albicans*.

Conclusion

To conclude, our study has shown the emergence of the concept of green chemistry and sustainability. This was achieved by extracting valuable biochemical compounds from the invasive, naturally growing Conyza Canadensis weed plant. A green ionic liquid(B-MIMIC) solvent and optimization of various parameters (solid-liquid ratio of 0.5 g: 15 ml, pH = 5, and incubation time of 15 minutes at 75 °C) to order to increase extraction yield was applied to obtain these compounds sustainably and efficiently resulting in designing a greener extraction procedure that can offer a sustainable and environmentally friendly alternative to traditional extraction methods. Qualitative and quantitative analysis evaluated the Conyza Canadensis weed plant richness in antioxidant and antimicrobial agent which can add value to the invasive weed and at the same time reduce or eliminate their environmental impact. Determination of antioxidant concentration and activity was estimated using spectrophotometric method and compared with an *in situ* Paper-on-a-lab platform. The obtained results revealed a minor difference. These results encourage their possible use as antioxidant and antimicrobial agents.

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