Archives of Pharmaceutical Sciences Ain Shams University 2019; Vol. 3(1):78-89

OPEN ACCESS

Medicinal Chemistry

Research Article

Microwave-assisted extraction of the gallic acid biomarker from *Acacia arabica* bark followed by HPLC analysis

Hend Z. Yamani*, Lobna A. Hussein, Maha F. Abdel Ghany

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo 11566, Egypt

ABSTRACT

An efficient and fast microwave-assisted extraction (MAE) technique was developed for extracting gallic acid as an indicative biomarker for the quality control of *Acacia arabica* bark. The MAE technique was optimized and compared with other conventional extraction techniques. The optimal conditions of MAE were 20% methanol as solvent, solid/liquid ratio 1:40 (g/mL), irradiation power 20% and two extraction cycles, 5 min each. The proposed extraction technique produced a maximum yield of 10.59 (mg/g) gallic acid in 10 min, which was 1.03 and 1.15 times more efficient than 6 h of heat reflux and 24 h of maceration extraction, respectively. This high yield, along with saving of time, energy, and solvent would position MAE as a valuable and cost-effective technology suitable for today's highly competitive industries, with growing demand for increased productivity, improved efficiency, and reduced cycle time. Moreover, a new high-performance liquid chromatography method was developed and validated for the determination of gallic acid in *Acacia arabica* bark extract. The method was found to be rapid, sensitive, accurate, precise, and robust. The method showed good linearity over concentration range 1-100 (µg/mL) with LOD 16.08 (ng/mL) and LOQ 48.73 (ng/mL). The average recovery obtained using standard addition technique was 100.36% with a low value of RSD% (1.19%) indicating the accuracy of the proposed method for determination of gallic acid in *Acacia arabica* bark extract.

Keywords: *Microwave-assisted extraction; Gallic acid; Acacia arabica; HPLC.*

***Correspondence** | Hend Z. Yamani, Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo 11566, Egypt.
Email: hend.z.yamani@pharma.asu.edu.eg
Citation | Yamani H Z, Hussein LA, Abdel Ghany MF. 2019. Microwave-assisted extraction of the gallic acid biomarker from *Acacia arabica* bark followed by HPLC analysis. Arch Pharm Sci ASU 3(1): 78-89
DOI: 10.21608/aps.2019.20240
Online ISSN: 2356-8380. **Print ISSN:** 2356-8399.
Received 15 April 2019. **Accepted** 24 May 2019.

Published by: Ain Shams University, Faculty of Pharmacy

1. INTRODUCTION

Acacia nilotica (L.) Delile syn. *Acacia arabica* (Lam.) Willd is a tree from family *Fabaceae*. It is commonly known as Egyptian acacia, gum Arabic tree, thorny acacia, and babul. This multipurpose tree which is native to Africa, the Middle East and India have been widely used for the treatment of various diseases. Different parts of this plant including bark, gum, roots, leaves, flowers, fruits and pods were reported to have anti-diabetic, anti-pyretic, anti-asthmatic, anti-carcinogenic, anti-bacterial, anti-fungal, antiplasmodial, antihypertensive, anti-spasmodic, antioxidant, anti-Alzheimer's and gastroprotective activities. It is also reported to have inhibitory activity against Hepatitis C virus (HCV), and human immunodeficiency virus (HIV) **[1]**.

Several reports focused on therapeutic activities of gallic acid as natural polyphenol having hypoglycemic, hypocholesterolemic **[2]**,

anti-oxidant **[3]**, anti-tumor **[4]**, anti-inflammatory **[5]**, anti melanogenic **[6]**, anti-bacterial **[7]**, anti-viral **[8]**, neuroprotective **[9]** and cardioprotective **[10]** activities.

For both the qualitative and quantitative analysis of medicinal plants, extraction is considered a vital step to obtain the desired chemical constituents that are subjected to further separation and characterization **[11]**.

Medical plants are complex matrices. Each plant may contain up to several thousand secondary metabolites. Consequently, the development of modern extraction techniques with high and rapid performance has become a vital necessity **[12]**. The conventional extraction techniques, such as Soxhlet extraction (SE), heat reflux extraction (HRE) and maceration extraction (ME) need long extraction time. This would decrease the number of processed samples and increase the risk of thermal degradation of plant components **[13]**. Moreover, the large volumes of extraction solvents used in these techniques require successive evaporation in order to concentrate the extract **[14, 15]**. Hence, these conventional techniques are considered the time and solvent consuming. Therefore, modern extraction techniques that provide various advantages over conventional ones ensure high-quality herbal products.

Microwave-assisted extraction (MAE) is considered a potential alternative to conventional extraction techniques as it provides many advantages such as higher extraction yield, shorter extraction time, lower solvent consumption and cost **[16]**. Several applications of MAE have appeared in the literature in different fields, including the extraction of phytoconstituents from herbal extracts **[17-19]**, drugs from biological fluids **[20]** and pollutants from environmental and food samples **[21]**.

MAE of gallic acid from *Acacia arabica* bark has not been reported in the literature before. The objective of the present study was the optimization of the parameters affecting the MAE efficiency of gallic acid from *Acacia arabica* bark. The extraction efficiency of MAE was compared with conventional extraction techniques. The other objective was to develop a simple, rapid, and selective HPLC method for determination of gallic acid in *Acacia arabica* bark extract.

2. MATERIALS AND METHODS

2.1 Instruments

MAE was performed in a household microwave oven (Campomatic, KOR22A1, China) that was modified in our laboratory with the addition of a water condenser **[22]**. The microwave equipped with a magnetron of 2450 MHz with a nominal maximum power of 700 W, a reflux unit, 5 power levels, and a timing controller.

HPLC analysis was conducted on an Agilent 1200 series HPLC system (Agilent, San Jose, CA, USA) equipped with a quaternary pump, an on-line solvent vacuum degasser, an autosampler with a 20 μL injection loop and a diode array detector. An Agilent Zorbax Eclipse XBD RP C18 column (150 mm × 4.6 mm I.D., 5 μm) fitted with an Alltech C18 guard column (8 mm × 4.6 mm I.D., 5 μm) was used. Instrumental control and data acquisition were operated by ChemStation software (Rev. B. 04. 01, Agilent Technologies).

2.2. Chemicals and plant material

Gallic Acid (purity 99.50%) was purchased from Merck [Hohenbrunn, Germany). Methanol, isopropanol, and water were purchased from Scharlu (Barcelona, Spain). O-phosphoric acid was purchased from (Ridel-deHaën, Sigma Aldrich, Germany. All solvents and additives

used were of HPLC grade. *Acacia arabica* bark was collected from (New Valley, Egypt) and authenticated by Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University (Cairo, Egypt).

2.3. Preparation of standard solutions

2.3.1. Gallic acid standard stock solution (1 mg/mL)

Gallic acid standard stock solution was prepared by dissolving accurately weighed 50 mg gallic acid in the least volume of mobile phase (methanol: 0.025% o-phosphoric acid, 20:80, v/v), transferred to the volumetric flask 50 mL then completed to the mark with mobile phase

2.3.2. Gallic acid standard working solutions (0.1 mg/ml and 0.01 mg/ml)

Gallic acid standard working solutions (0.1 mg/mL and 0.01 mg/mL) were prepared by transferring 5 mL and 0.5 mL, respectively, from the previously prepared standard stock solution to volumetric flasks 50 mL and completed to the mark with mobile phase (methanol: 0.025% O-phosphoric acid, 20:80, v/v).

2.4. Extraction techniques

Acacia arabica bark samples were cleaned manually to remove all foreign materials then milled, passed through a stainless steel sieve (20-40 mesh) and stored at 4 °C until use. Two conventional extraction techniques as given below were used for comparison with MAE.

2.4.1. Microwave-Assisted Extraction (MAE)

An accurately weighed sample (1 g) of the bark powder was mixed with the solvent in a round-bottom flask. The extraction process was performed under different experimental conditions for the optimization of the extraction parameters. The effects of methanol concentration, solid/liquid ratio, irradiation time, irradiation power and extraction cycles on the extraction yield of gallic acid were investigated. After the extraction, the suspension was centrifuged at relative centrifugation force 3743 g for 10 min. The supernatant was subjected to HPLC analysis.

2.4.2. Heat Reflux Extraction (HRE)

An accurately weighed sample (1 g) of the powdered bark was extracted at 88 °C for (1, 2, 3, 4, 5, 6, and 7 h) under reflux with 100 mL of 20% methanol in a round-bottom flask heated in a water bath. After the extraction, the suspension was centrifuged at 3743 g for 10 min. The supernatant was subjected to HPLC analysis.

2.4.3. Maceration Extraction (ME)

An accurately weighed sample (1 g) of the powdered bark was macerated in 100 mL of 20% methanol for (12, 15, 18, 21, 24, 27, and 30 h) in a closed conical flask at room temperature. After the extraction, the suspension was centrifuged at 3743 g for 10 min. The supernatant was subjected to HPLC analysis.

2.5. Thermal Stability of Gallic Acid

Two concentration of standard gallic acid (100 and 10 µg/mL in 20% methanol) were subjected to MAE under the optimum extraction conditions obtained from the systematic study to determine its thermal stability. The mean percentage recoveries and RSD% was calculated.

2.6. HPLC analysis

The mobile phase consisted of 0.025% O-phosphoric acid in water (solution A) and methanol (solution B). The elution scheme was 0-5 min, 20% B; 5.1-15 min, increasing gradually from 50% to 80% B; 15.1-18 min, 20% B. All analyses were carried at flow rate 1 mL/min and at room temperature using diode array detector at 272 nm. All solvents were filtered through 0.45 µm membrane filter immediately before use then degassed in an ultrasonic bath for 15 min.

Samples were filtered through 0.45 µm syringe filters before injection into the HPLC.

2.7. HPLC method validation

The proposed HPLC method was validated according to the International Conference of Harmonization (ICH) guidelines **[23]**.

2.7.1. Linearity

Accurately measured volumes (0.25-25 mL) of gallic acid standard working solution (0.1 mg/mL) were transferred into a series of 25 mL volumetric flasks separately and diluted with the mobile phase to obtain concentrations of (1, 5, 10, 30, 50, 70 and 100 µg/mL), respectively. The specified chromatographic conditions were set. A 20 µL of each concentration was injected in triplicates. The average peak areas were calculated. The calibration curves relating the integrated peak area and its corresponding concentration were constructed and the regression equations were computed.

2.7.2. Limit of Detection and Limit of Quantification (LOD and LOQ)

Accurately measured volumes (0.25-2.5 mL) of gallic acid standard working solution (0.01 mg/mL) were transferred into a series of 25 mL volumetric flasks separately and diluted with the mobile phase to obtain concentrations of (0.1, 0.5, 0.7 and 1 µg/mL), respectively. The specified chromatographic conditions were set. A 20 µL of each concentration was injected in triplicates. The average peak areas were calculated. The calibration curve for LOD and LOQ relating the integrated peak area and its corresponding concentration was constructed and the regression equation was computed. LOD was calculated as 3.3 σ/S while LOQ as 10 σ/S, where σ is the standard deviation of intercepts and S is the slope of the calibration curve.

2.7.3. Accuracy

The pre-analyzed samples of *Acacia arabica* bark extract were spiked with extra 50, 100, and 150% of pure gallic acid. The mixtures were reanalyzed in triplicates by the proposed method. The mean percentage recoveries and RSD% was then calculated.

2.7.4. Precision

Three concentrations of pure gallic acid samples (1, 10, and 100 µg/mL) were analyzed in triplicates within the same day and for three successive days in order to determine intra- and inter-day variation. The mean percentage recoveries and RSD% was then calculated.

2.7.5. Specificity

Specificity of the method was confirmed by testing the peak purity of gallic acid peak in *Acacia arabica* bark extract.

2.7.6. Robustness

Robustness of the method was done by introducing small changes in the methanol ratio (± 2 %), pH (± 0.2), flow rate (± 0.2 min) and wavelength (± 2 nm). Robustness was studied at a concentration level of (50 µg/mL). The mean percentage recoveries and RSD% was then calculated.

2.7.7. System Suitability Tests (SST)

System suitability test parameters of the proposed method were calculated on *Acacia arabica* bark extract by half height method used by British pharmacopeia **[24]**.

2.8. Application to Acacia arabica bark extract analysis

2 mL of the extract supernatant was transferred to a volumetric flask 25 mL and diluted with the mobile phase. The specified chromatographic conditions were set.

3. RESULTS

In this study, the effects of several extraction parameters (methanol concentration, solid/liquid

ratio, irradiation time, irradiation power and extraction cycles) were systematically investigated to obtain the maximum yield of gallic acid. The influence of each parameter was studied by single-factor experiments. The optimal conditions of MAE were 20% methanol as solvent, solid/liquid ratio 1:40 (g/mL), irradiation power 20% and two extraction cycles, 5 min each. The results of gallic acid thermal stability under optimum MAE conditions are summarized **(Table 1)**.

MAE, HRE and ME techniques were compared for their extraction efficiency of gallic acid from *Acacia arabica*. The extraction yields of gallic acid obtained by the three extraction techniques under their optimum conditions are summarized **(Table 2)**. The proposed extraction technique produced a maximum yield of 10.59 (mg/g) gallic acid in 10 min, which was 1.03 and 1.15 times more efficient than 6 h of heat reflux and 24 h of maceration extraction, respectively.

The validation parameters of the proposed HPLC method and the system suitability testing data are summarized **(Tables 3 & 4)**.

Table 1. Thermal stability of standard gallic acid under optimum MAE conditions

Initial Concentration (µg/mL)	Found Concentration [a] (mg/mL)	Recovery%
100.00	99.96	99.96
10.00	9.98	99.80
Mean ± RSD%		99.88 ± 0.11

[an] Average of three determinations

Table 2. Comparison of MAE of gallic acid from *Acacia arabica* bark with other conventional methods under optimized conditions

Extraction method	Extraction time	Solvent volume (mL)	Yield (mg/g)	RSD%[a]
MAE[b]	10 min	80	10.58	0.28
HRE[c]	6 h	100	10.26	3.41
ME[d]	24 h	100	9.21	17.48

[an] (n=2), [b] MAE; Microwave-assisted extraction, [c] HRE; Heat reflux extraction, [d] ME; Maceration extraction

Table 3. Validation parameters of the proposed HPLC method for analysis of gallic acid

Parameter	Value
Range	1 – 100 µg/mL
Regression equation	y = 62.08x + 2.5178
The correlation coefficient (*r*)	0.9999
Limit of detection (LOD)	16.08 ng/mL
Limit of quantification (LOQ)	48.73 ng/mL
Accuracy (Mean ± RSD%)	100.36 ± 1.19
Repeatability (RSD%)	0.73
Intermediate precision (RSD%)	1.06
Robustness	100.17 ± 1.27

Table 4. System suitability testing (SST) data of the proposed HPLC method for analysis of gallic acid in *Acacia arabica* bark extract

SST	Value	Reference Value **[24]**
Number of theoretical plates (N)	2616.75	Increase with the efficiency of separation
Resolution (R)	3.67	≥ 1.5
Asymmetry Factor (A_s)	1.03	$A_s = 1$ corresponds for ideal symmetry

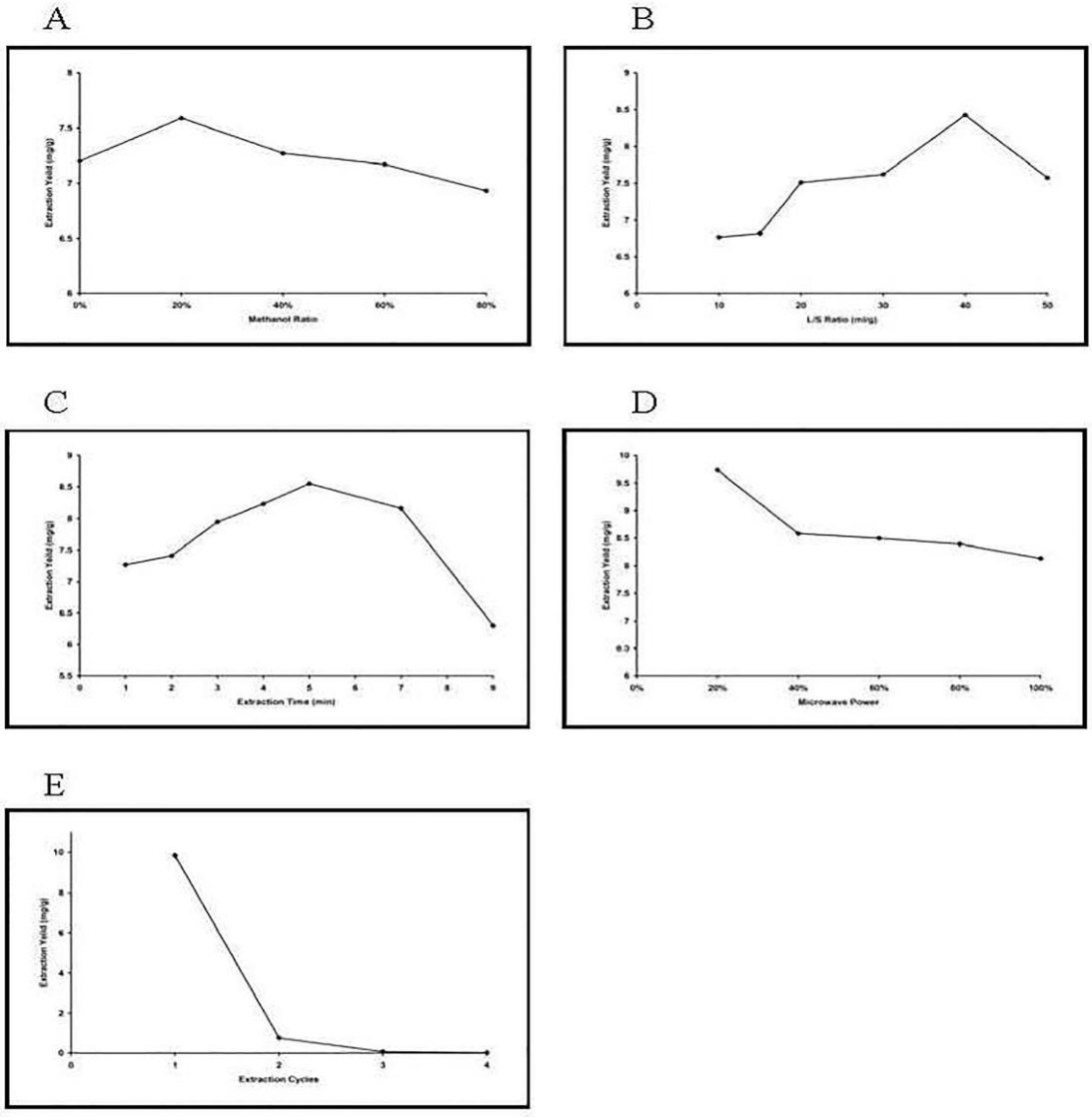

Fig. 1. Effect of different extraction parameters on gallic acid extraction yield from *Acacia arabica* bark in single-factor experiments: (A) methanol concentration; (B) L/S ratio; (C) Irradiation time; (D) Irradiation power; (E) Extraction cycles.

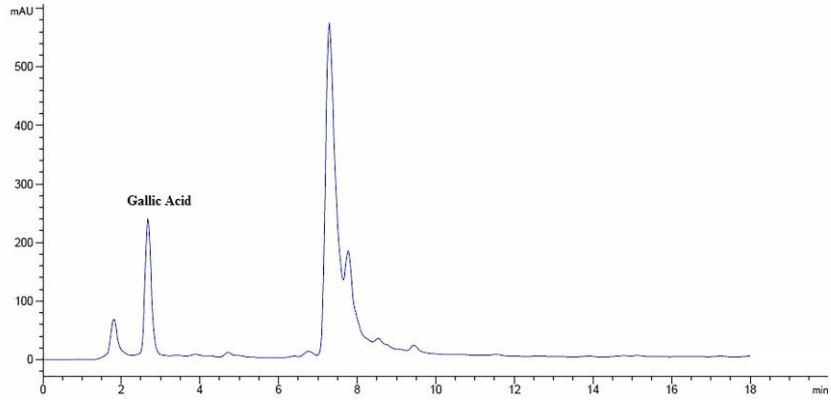

Fig. 2. HPLC chromatogram of *Acacia arabica* bark extract.

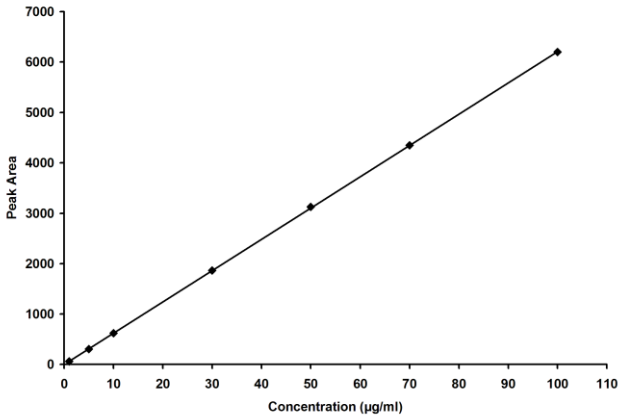

Fig. 3. Calibration curve of gallic acid correlating the pak area to the corresponding concentration of gallic acid (1-100 µg/mL) using the proposed HPLC method

4. DISCUSSION

4.1. Optimization of Microwave-Assisted Extraction (MAE) parameters

4.1.1. Effect of methanol concentration

In developing any extraction method for extracting the desired analytes from the sample matrix, the choice of the most appropriate solvent is a vital step. The solvent choice for MAE depends on several factors including the solubility of the desired analyte, the microwave absorbing properties of the solvent and the interaction between the plant matrix and solvent **[25]**. The solvents used in MAE should have (high tan δ value) which means good heating efficiency under the microwave. Generally, aqueous methanol and ethanol are good candidates **[26-28]**. Methanol has a relatively higher dissipation factor, which means that it has a better ability than other solvents to absorb microwave energy and transform it into heat **[29]**. Different ratios of methanol-water were tested in order to determine the effect of methanol concentration on the extraction yield. The other extraction parameters were set as follows: solid/liquid ratio 1:30 (g/mL), irradiation time 5 min, irradiation power 60% and one extraction cycle.

The yield of gallic acid was greatly influenced by the aqueous methanol concentration as shown in **(Fig. 1A)**. 20% of methanol showed higher yield than absolute methanol. The presence of some water increases the solubilizing capacity of the solvent by increasing the relative polarity of

the solvent and the mass transfer process **[14]**. Moreover, water may enhance the swelling of plant materials, resulting in increasing the contact surface area between the solvent and plant matrix **[30]**. Further increase in methanol content resulted in significant decreases in extraction yield. Therefore, 20% of methanol was chosen to be the optimum extraction solvent.

4.1.2. Effect of solid/liquid ratio

To ensure that the whole sample is immersed, the solvent volume must be sufficient. Generally, a higher volume of solvent will increase the extraction yield in conventional extraction techniques. In contrast to that in MAE, a higher solvent volume may give a lower yield **[31]**. To investigate the influence of solid/liquid ratio on the yield of gallic acid, different ratios of solid/liquid were tested. The rest of the extraction parameters employed were 20% methanol as the extraction solvent, irradiation time 5 min, irradiation power 60% and one extraction cycle. It can be seen in **(Fig. 1B)** that the yield of gallic acid increased with increasing the ratios of solid/liquid until 1:40 (g/mL) at which the yield reached its highest value. Further increase in solid/liquid ratio caused a decrease in yield. This was probably due to an inadequate stirring of the solvent when microwaves were applied at larger volumes. Moreover, a larger volume of solvent will cause more absorption of microwave energy and thus sufficient microwave energy may not be available for facilitating the cell breakage for effective leaching out of the target analyte **[32]**. From the above observations, a ratio of 1:40 (g/mL) was considered the optimum.

4.1.3. Effect of irradiation time

It is necessary to select a proper irradiation time to guarantee completion of the extraction. Generally, by increasing the extraction time, the quantity of analytes extracted is increased, but overexposure may lead to thermal degradation of effective constituents [14]. To investigate the effect of irradiation time on the extraction yield, different irradiation times were tested. The rest of the extraction parameters were as follows: 20% methanol as the extraction solvent, solid/liquid ratio 1:40 (g/mL), irradiation power 60% and one extraction cycle.

As confirmed in **(Fig. 1C)**, by increasing irradiation time, the extraction yield of gallic acid increased and reached its maximum at 5 min. Then, the extraction yield decreased with the extension of the irradiation time. This may be due to thermal decomposition of gallic acid upon overexposure to microwaves. Thus, 5 min was considered as the appropriate irradiation time.

4.1.4. Effect of irradiation power

In order to evaluate the effect of microwave irradiation power on gallic acid yield, different microwave irradiation powers were controlled. The rest of the extraction conditions were as follows: 20% methanol as the extraction solvent, solid/liquid ratio 1:40 (g/mL), irradiation time 5 min and one extraction cycle.

As shown in **(Fig. 1D)**, the highest yield was obtained when 20% of microwave irradiation power was used. Further increase in power resulted in a decline in yield. Microwave irradiation energy disrupts hydrogen bonds, because of microwave-induced dipole rotation of molecules and ionic conduction. This enhances the penetration of the solvent into the matrix, allowing the dissolution of components to be extracted **[33]**. However, at higher power levels, solvent temperature increases drastically with a rapid loss in extracting solvent due to evaporation **[16]**. Hence, 20% of irradiation power was chosen as the appropriate microwave irradiation power.

4.1.5. Effect of extraction cycles

The effect of successive extractions of the residue, i.e., extraction cycles, was investigated in this experiment. Extraction conditions were set at the optimum parameters obtained so far in the study: 20% methanol as the extraction solvent, solid/liquid ratio 1:40 (g/mL), irradiation time 5 min and irradiation power 20%. The residue was taken back and re-extracted using fresh solvent each time.

A second successive extraction of the residue yielded a further 0.76 (mg/g) gallic acid, taking the final extraction yield to 10.58 (mg/g). This shows that 92.82% of the extraction was complete in the first step itself **(Fig. 1E)**. A successive third and fourth extraction cycles yielded 0.07 and 0.02 (mg/g) gallic acid, respectively. To save solvent, energy, and time, two-cycle extraction was considered enough to release most of the gallic acid into the solvent.

4.2. Thermal stability of gallic acid

The results showed that the average recovery of gallic acid at the optimum extraction conditions was 99.88% with no change in retention time of gallic acid (2.64 ± 0.03). These results confirmed the thermal stability of gallic acid and eliminate the possibility of its thermal degradation under the optimum extraction conditions.

4.3. Comparison of different extraction techniques

In the present study, MAE, HRE, and ME techniques were compared for their extraction efficiency of gallic acid from *Acacia arabica*. In terms of yield of gallic acid, the best results were obtained by MAE, which gave higher values. On extraction time, MAE was significantly the fastest method with only 10 min of extraction time.

If the extraction yield obtained from MAE is to be considered as 100% performance level then, 6 h of heat reflux extraction and 24 h of maceration can attain 96.88% and 87.05% performance efficiency (in terms of extraction yield of gallic acid).

Generally, dried plant samples are used for extraction. However, minute traces of moisture still present inside plant cells. Under microwave effect, this moisture is heated up until evaporation resulting in great pressure on the cell wall **[34]**. The pressure pushes the cell wall from inside and ruptures it resulting in leaching out of the active phytoconstituents to the extracting solvent. Consequently, a higher extraction yield would be obtained. The main constituent of the plant cell wall is cellulose. Ether linkages of cellulose can be hydrolyzed rapidly by higher temperature obtained by microwave radiation. This facilitates easy entry of extracting solvents inside the cellular channels **[35-36]**.

This mechanism of MAE based on exposing the analytes to the solvent through cell rupture is different from that of HRE and ME that depends on a series of permeation and solubilization processes to bring the analytes out of the matrix **[37]**.

4.4. HPLC method development and validation

The mobile phase consisting of methanol: 0.025% o-phosphoric acid in water was tried in varying ratios. 20% methanol was found to give a sharp, symmetric, and well-defined peak at retention time 2.644±0.03 min with good separation from the other phytoconstituents in the **(Fig. 2)**. Methanol ratio was increased from 50% to 80% after separation of gallic acid peak to accelerate elution of other phytoconstituents and hence decreasing run time.

Validation parameters of the proposed method are summarized in **(Table 3)**. The calibration

curve was constructed in the range of (1-100 µg/mL) **(Fig. 3)**. The regression equation was computed and found to be A = 62.08C + 2.5178 (r = 0.9999), where, A is the integrated peak area of gallic acid, C is the concentration in µg/mL and r is the correlation coefficient. The LOD and LOQ indicate the adequate sensitivity of the proposed method. The average recovery obtained using standard addition technique was 100.36% with a low value of RSD% (1.19%) indicating the accuracy of the proposed method for determination of gallic acid in *Acacia arabica* bark extract. There was no interference from other components in the matrix. The low values of %RSD (<1.5%) for inter- and intra-day variation suggest an excellent precision of the method.

The Purity factor of the gallic acid peak in *Acacia arabica* bark extract was found to be 999.935. This confirms the specificity of the proposed method and the absence of interference from other phytoconstituents in the extract. The data presented in **(Table 4)** showed that the measured SST parameters are within the limits of acceptance of British pharmacopeia **[24]**. The results of the robustness study showed the method's capability to remain unaffected by small, but deliberate variations in method parameters demonstrating excellent robustness of the proposed method.

4.5. Study limitations

One of the main limitations of extraction and analysis process is that both steps work independently. The collection and cleanup of the extract from different extraction cycles prior to analysis are time-consuming. Moreover, analyte loss or contamination may occur during the collection and cleanup. These problems can be improved in future research by coupling the extraction and analysis in a single step in a continuous and automatic manner. Besides, in open vessel MAE, an only single sample can be processed in each extraction run, which decreases the sample throughput. Future research may be directed toward modification the design of the microwave extractor so that multiple samples can be extracted simultaneously.

5. CONCLUSION

An efficient and fast MAE method was developed for the extraction of gallic acid from *Acacia arabica* bark. Gallic acid was directly quantified by the HPLC method. The optimum MAE conditions were 20% methanol as the extraction solvent, solid/liquid ratio 1:40 (g/ml), two extraction cycles 5 min each under 20% microwave irradiation power. Compared to conventional extraction techniques, MAE gave the best results due to the highest extraction efficiency within the shortest extraction. Thus, MAE can be accepted as a potential and powerful alternative to conventional extraction techniques.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Contribution of Authors

Hend Z. Yamani designed the study, performed all the practical experiments, collected the analyzed data, interpreted the results, and wrote the manuscript. Lobna A. Hussein and Maha F. Abdel Ghany conceived of the study, helped in designing the study, and revised the results and manuscript. All authors gave final approval for the publication.

6. REFERENCES

1. Ali A, Akhtar N, Khan BA, Khan M S, Rasul A, Shahiq-UZ-Zaman, Khalid N, Waseem K, Mahmood T, Ali L. Acacia nilotica: A plant of multipurpose medicinal uses. J Med Plant Res 2012; 6:1492-6.
2. Punithavathi VR, Prince PS M, Kumar R, Selvakumari J. Antihyperglycaemic, anti-

lipid peroxidative and antioxidant effects of gallic acid on streptozotocin-induced diabetic Wistar rats. Eur. J. Pharmacol 2011; 650:465-471.

3. Yen GC, Duh PD, Tsai H L. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food Chem. 2002; 79: 307-313.

4. You BR, Moon, HJ, Han, YH, Park, WH. Gallic acid inhibits the growth of HeLa cervical cancer cells via apoptosis and/or necrosis. Food Chem.
Toxicol. 2010; 48:1334-1340.

5. Yoon CH, Chung SJ, Lee SW, Park YB, Lee SK, Park, MC. Gallic acid, a natural polyphenolic acid, induces apoptosis and inhibits proinflammatory gene expressions in rheumatoid arthritis fibroblast-like synoviocytes. Joint Bone Spine 2012; 80:274–279.

6. Kim YJ. Antimelanogenic and antioxidant properties of gallic acid. Biol. Pharm. Bull. 2007; 30:1052-1055.

7. Kang MS, Oh JS, Kang IC, Hong SJ, Choi CH. Inhibitory effect of methyl gallate and gallic acid on oral bacteria. J Microbiology. 2008; 46:744-750.

8. Kratz JM, Andrighetti-Fröhner C R, Leal P C, Nunes R J, Yunes R A, Trybala E, Bergström T, Barardi C R, Simões CM O. Evaluation of anti-HSV-2 activity of gallic acid and pentyl gallate. Biol. Pharm. Bull. 2008; 31: 903-907.

9. Mansouri MT, Farbood Y, Sameri MJ, Sarkaki A, Naghizadeh B, Rafeirad M. Neuroprotective effects of oral gallic acid against oxidative stress induced by 6-hydroxydopamine in rats. Food Chem. 2012; 138:1028-1033

10. Priscilla DH, Prince P. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products, and antioxidants in experimentally induced myocardial infarction in Wistar rats. Chem. Biol. Interact. 2009; 179:118-124.

11. Sasidharan S, Chen Y, Saravanan D, Sundaram KM, Latha LY. Extraction, isolation, and characterization of bioactive compounds from plant's extracts. Afr J Tradit Complement Altern Med. 2011; 8:1-10.

12. Nyiredy S. Separation strategies of plant constituents–current status. J. Chromatogr. B Biomed. Sci. Appl. 2004; 812:35-51.

13. Luque de Castro MD, García-Ayuso LE, Soxhlet extraction of solid materials: an outdated technique with a promising innovative future, Anal. Chim. Acta. 1998; 369:1-10.

14. Mandal V, Dewanjee S, Mandal SC. Microwave-assisted extraction of total bioactive saponin fraction from Gymnema sylvestre with reference to gymnemagenin: a potential biomarker. Phytochem Anal. 2009; 20:491-497.

15. Mandal V, Mohan Y, Hemalatha S. Microwave-assisted extraction – an innovative and promising extraction tool for medicinal plant research. Pharmacogn Rev. 2007; 1:7-18.

16. Yan M M, Liu W, Fu YJ, Zu YG, Chen CY, Luo M. Optimisation of the microwave-assisted extraction process for four main astragalosides in Radix Astragali. Food Chem. 2010; 119:1663-1670.

17. Fang X, Wang J, Zhang S, Zhao Q, Zheng Z, Song Z. Simultaneous extraction of hydrosoluble phenolic acids and liposoluble tanshinones from Salviae miltiorrhizae radix by an optimized microwave-assisted extraction method. Sep Purif Technol. 2012; 86:149-156.

18. Wakte PS, Sachin BS, Patil AA, Mohato DM, Band TH, Shinde DB. Optimization of microwave, ultra-sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from Curcuma longa. Sep Purif Technol. 2011; 79: 50-55.

19. Hayat K, Hussain S, Abbas S, Farooq U, Ding B, Xia S, Jia C, Zhang X, Xia W. Optimized microwave-assisted extraction of

phenolic acids from citrus mandarin peels and evaluation of antioxidant activity in vitro. Sep Purif Technol. 2009; 70: 63-70.

20. Madej K. Microwave-assisted and cloud-point extraction in the determination of drugs and other bioactive compounds, Trends Analyt Chem. 2009; 28:436-446.

24. Stationery Office. British Pharmacopoeia 2010. London: UK.

25. Letellier M, Budzinski H, Charrier L, Capes S, Dorthe AM. Optimization by factorial design of focused microwave-assisted extraction of polycyclic aromatic hydrocarbons from marine sediment. Fresenius J Anal Chem 1999; 364: 228-237.

26. Fulzele DP, Satdive RK. Comparison of techniques for the extraction of the anti-cancer drug camptothecin from Nothapodytes foetida. J Chromatogr 2005; 1063:9-13.

27. Waksmundzka-Hajnos M, Petruczynik A, Dragan A, Wianowska D, Dawidowicz AL, Sowa I. Influence of the extraction mode on the yield of some furanocoumarins from Pastinaca sativa fruits. J Chromatogr B Biomed Sci Appl 2004; 800: 181-187.

28. Zhang B, Yang R, Liu C Z. Microwave-assisted extraction of chlorogenic acid from flower buds of Lonicera japonica Thunb. Sep Purif Technol 2008; 62:480-483.

29. Wang Y L, Xi G, Zheng Y, Miao F. Microwave-assisted extraction of flavonoids from Chinese herb Radix puerariae (Ge Gen). J Med Plant Res 2010; 4:304-308.

30. Chen L, Jin H, Ding L, Zhang H, Li J, Qu C, Zhang H. Dynamic microwave-assisted extraction of flavonoids from Herba Epimedii. Sep Purif Technol 2008; 59:50-57.

31. Sparr Eskilsson C, Björklund E. Analytical-scale microwave-assisted extraction, J Chromatogr 2000; 902:227-250.

32. Dhobi M, Mandal V, Hemalatha S, Optimization of microwave-assisted extraction of bioactive flavonolignan – silibinin. J. Chem. Metrol. 2009; 3:13-23.

33. Hudaib M, Gotti R, Pomponio R, Cavrini V. Recovery evaluation of lipophilic markers from Echinacea purpurea roots applying microwave-assisted solvent extraction versus conventional methods. J Sep Sci. 2003; 26: 97-104.

34. Wang L, Weller CL. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci Technol. 2006; 17:300-312.

35. Latha C. Microwave-assisted extraction of embelin from Embelia ribes. Biotechnol. Lett. 2007; 29:319-322.

36. Mandal V, Mohan, Y., Hemalatha, S. Microwave-assisted extraction of curcumin by sample–solvent dual heating mechanism using Taguchi L9 orthogonal design, J Pharm Biomed Anal. 2008; 46:322-327.

37. Materials and MethodsJyothi D, Khanam S, Sultana R. Optimization of microwave-assisted solvent extraction of withanolides from leaves of Ashwagandha, International Journal of Comprehensive Pharmacy. 2010; 1:1-4.