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Solvent pH extraction effect on phytochemical composition and antioxidant properties of Algerian *Matricaria Pubescens*

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ABSTRACT

This study investigated the phenolic content, flavonoid content, flavonols and antioxidant and anti-inflammatory activities of different pH of solvent aqueous (water) extraction of aerial part from *Matricaria Pubescens*. Three phenolic acids (gallic acid, chlorogenic, caffeic acid) and two flavonoids (rutin and vanillin) were identified and quantified using HPLC. The total phenolic content, flavonoids content and flavonols were determined by a spectrophotometric method. The assessment of the antioxidant potential of different pH extract (3, 4, 5, 6 and 7) using the ferric reducing antioxidant power (FRAP) assays, diammonium salt (ABTS). Total phenolic contents flavanoïds and flavonols from leaves were the highest at pH 5, but gradually decreased with elevated pH. The FRAP scavenging effects of extraction pH value 5 and 4 were superior to those at other pH, and paralleled the trend of total phenolic content. The ABTS scavenging capacity was found to be the highest for extract at pH between 5 and 4. HPLC analysis showed rutin to be the most abundant phytochemical, followed by gallic acid. The relative phenolic compounds surface hydrophobicity of all extracts was influenced by pH. The highest anti-radical activity is that in the presence of ascorbic acid. Extraction of phenolic compounds and there antioxidant activity can be completely inhibited by some chemicals and extreme pH.

KEYWORDS: *Matricaria Pubescens*, FRAP, ABTS, hydroxyl, phenolic content, HPLC

INTRODUCTION

The increasing demand for herbal medicines encourages collectors and traders to decimate natural populations of important medicinal plants. About one out of eight of flowering plant species are used for medicinal purposes[1], Plants have been an integral part of pharmacotherapy throughout the history of humanity, and they have served as an invaluable source for the discovery of bioactive molecules since the very beginning of rational drug discovery in the 19th century[2]. Since that time, natural products have played a crucial role for the discovery of new drug leads. Medicinal plants are the source of primary health care throughout the world for thousands of years. However, in the middle of 20th century, the use of medicinal plants was reduced one fourth because researchers favor the use of synthetic chemicals for curing diseases[3].

The World Medicines Situation has reported unequivocally that between 70% and 95% of the population in developing countries consume traditional medicine[4]. In Africa, various studies found that between 80% and 85% in Algeria, up to 90%, 85% South Africans[5], 75% Malians[6]. Tradional medicine use remains universal in countries where conventional medicine is predominant in the national health care system. In the Republic of Korea estimates that about 76% and 86% of the respective populations still commonly use traditional medicine[7]. Other national reports posit that almost every Chinese has ever used at least one form of traditional medicine with more than 90% prevalence[8].

Matricaria Pubescens is a well known medicinal plant used in the south east of Algeria against several diseases[9], is frequently used in the traditional medicine against rheumatic aches and muscular pains, pains in bones and joints, coughs, allergies, ocular affections, dysmenorrhoea, scorpion stings, dehydration, and toothache[10]. It is used for gastro-intestinal troubles and calculus, and is a much-appreciated medicinal herb. The crushed stems and leaves are used as a filter for goat's butter, giving a nice aroma to the butter and helping to conserve it. The whole plant is collected fresh in spring in the south and south east of Algeria (El Oued, Béchar, Djanet, El Golea). It is prepared as an infusion or powder and used internally. The antioxidant activity of plant extracts has been mostly accredited to the presence of phe-

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nolic compounds[11]. The phenolic compounds and antioxidant activity of those by-products from chestnut have been examined in recent years[12].The phenolic compounds are large and heterogeneous groups of these secondary metabolites, that are distributed throughout the plant kingdom. Phenolic compounds are amongst the most desirable phytochemicals due to antimicrobial, antiviral, anti-inflammatory properties, and high antioxidant capacities. Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals[13]. Phenolics have been considered powerful antioxidants in vitro. Regular intake of plant products rich in phenolics have been reported to reduce risks of chronic diseases, such as cancer, heart diseases and diabetes.

The objective of this study was to examine the effects of various extraction pH values on the extraction of phenolics content, flavonoid, flavonols from *Matricaria Pubescens* and then evaluate scavenging activities towards FRAP, ABTS, hydroxyl radicals and antibacterial activity.

Figure 1. ***Matricaria Pubescent***

MATERIALS AND METHODS

Plant material:

The *Matricaria Pubescent* were collected from southeast of Algeria, state of El Oued on October 2014. The leaves then separated from each other, washed and dried at room temperature. All these organs were ground to a powder with a basic electric grinder, and stored in the dark at room temperature before use [14]. Then the powder was put in a hot air oven at 60 °C until complete drying. Depending on the physical characteristics of the samples, the time ranged from 24 at 30 h.

Soxhlet process extraction:

Thirty grams of powdered (30 g) were mixed with 160 ml of water in various extraction pH value (pH ranged from 3 at 7). The above solution extracted in a Soxhlet apparatus for 6 h. The extracts concentrated under vacuum at 40 °C by using a rotary evaporator. To obtain aqueous extracts, air dried powdered plants were boiled with 200 ml of aqueous for 30 min. The extract aqueous were filtered and concentration using rotary-evaporated under vacuum at 45 ?C to dryness. Extracts were stored at +4 °C in dark until use.

Total phenolic concentration:

The total phenolic contents in all organs were determined by the folin-Ciocalteu method[15]. Briefly, 100 µl of both the sample and the standard (gallic acid) of known concentrations were made up to 2.5 ml with water, and mixed with 0.25 mL of 1N Folin-ciocalteu reagent. After 5 min, 2.5 ml of sodium carbonate aqueous solution (2%, w/v) was added to the mixture and was completed the reaction for 30 minutes in darkness at room temperature. The absorbance was read at 765 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). For the blank the same protocol was used but the extract was replaced by solvent. The concentration of total polyphenols in the extracts was expressed as mg gallic acid equivalent (GAE) per g of dry weight using UV-Visible (Shimadzu UV-1800, Japan) and the equation of calibration curve: $Y = 0.00778x$, $R^2 = 0.991$, x was the absorbance and Y was the gallic acid equivalent. All results presented are means (±SEM) and were analyzed in three replications.

Total flavonoids and flavonols concentration:

The determination of flavonoids was performed according to the colorimetric assay[16]. Distilled water (4 ml) was added to 1 ml of leaf extract. Then, 5% sodium nitrite solution (0.3 ml) was added, followed by 10% aluminum chloride solution (0.3 ml). Test tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1 M NaOH were added to the mixture. Immediately, the volume of reaction mixture was made to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink colour developed was determined at 510 nm. A calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents (CE)/g of dry weight. For the flavonols[17,18], 25 µL of the crude extracts was added to 25 µL HCl (0.1 %) in 95 % ethanol, all were mixed with 500 µl HCl (2 %) and incubated for 30 min at room temperature and then the absorbance was measured at 360 nm using UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). The is prepared with the same procedure described above but we replace the simple extract by the quercetin. Total flavonol content was expressed as quercetin equivalent (QE)/ g of dry weight.

HPLC analysis

The composition of the extracts was analyzed by high performance

liquid chromatography (HPLC)[16]. We used Shimadzu (LC 20, Japan), system comprised of a LC-10AD pump, a CTO-10A column oven, a SPD-10A UV-DAD detector, a CBM-10A interface and a LC-10 Workstation was utilized. A LC-18 column (250 mm x 4 mm i.d. x 5 mm) was employed. Samples were injected. The components of the samples were separated by gradient elution at at 30 °C. The mobile phases were: A, 98:2 (v/v) acetic acid, and B, acetonitrile and the elution gradient was: 0–5 min, 5% B; 10 min, 10% B; 11 min, 20% B; 20 min 20% B; 30 min 40% B; 40 min 50% B; 50 min 80% B. The flow rate was 0.8 ml/min and the detection wavelength was 285 nm. Phenolic compound standards gallic acid, Adcorbic acid, Querecetin, Chloregenic acid, Vanillin, Caffeic acid and Rutin were dissolved in solvents extraction and used for identification of the phenolic acids present in different extracts of *Matricaria Pubescent*. Peak identification in HPLC analysis was achieved by comparison of retention time and UV spectra of reference standards. Quantification of individual phenolic compounds in the extracts was done using the peak area of reference compounds, and reported as mg/g of extract.

Measurement of ferric reducing power (FRAP assay):

Briefly, the FRAP reagent contained 2.5 mL of 10 mM tripyridyltriazine (TPTZ) in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3M acetate buffer (pH 3.6), was freshly prepared. A volume 0.2 mL, of ethanolic extract) various concentrations) or standard was mixed with 1.8 mL of freshly prepared FRAP reagent. The absorbance of each sample solution was measured at 595 nm[19]. For the calibration curve, $FeSO_4$ was prepared in same solvent extraction in the range of 100–700 µM and querecetin was used as positive controls. The results were expressed as mg/ml of Fe(II), using the equation obtained from the calibration curve of $FeSO_4$: $Y = 6.908x$, $R^2 = 0.998$.

ABTS assay (2, 20-azinobis [3-ethylbenzothiazoline-6-sulfonate]):

The ABTS scavenging assay was carried out in triplicate, ABTS reagent was prepared by 10 mL of 7 mM ABTS solution and 178 µL of 140 mM potassium persulfate aqueous, the mixture was incubated at room temperature in darkness for for 13 h before use. 2 µL ethyl acetate extracts or standard was added to 1.588 µL diluted ABTS solution to react in the dark at room temperature for 10 min[20], and the absorbance was measured at 732 nm. The percentage inhibition of ABTS radical by the extract and BHT as calculated and compared following the equation:

ABTS radical scavenging activity=[($Abs_{control}$-Abs_{sample})/ $Abs_{control}$] x100

Where,

$Abs_{control}$:Is the absorbance of ABTS radical + ethyl acetate

Abs_{sample} : Is the absorbance of ABTS radical + ethyl acetate extract or standard. Test on

Statistical analyses

The data obtained in this study were expressed as the mean of three replicate determinations plus or minus the standard deviation (SD). Statistical comparisons were made with Student's test. *P* values <0.05 were considered to be significant.

RESULTS

Total phenolic contents: Aqueous extracts of *Matricaria Pubescent* obtained by various pH value, extract of pH 5 was found to be rich in total phenolics contents. The total phenolic content is given in figure 2. Extract of pH 5 was found to have the highest value 9.76 ± 0.32 mg GAE/g DW, following by pH 7: 6.94 ± 0.29 mg GAE/g DW, pH 6 : 6.75± 0.32 mg GAE/g DW, pH 4: 5.5± 0.22 mg GAE/g DW and the lowest value in extraction pH value 3: 3.81 ± 0.11 mg GAE/g DW. Similar results were observed in quantification of total flavonoids, the content of total flavonoids was also found to vary significantly ($p < 0.05$) and content ranged from 6.36±0.2 mg CE/g DW to 2.16± 0.13 mg CE/g DW. The Total flavonoids in increasing order was: pH 5 (6.36±0.2> pH 7 (4.88±0.12)> pH 6 (3.94±0.17)> pH 4 (2.16 ±0.17)> pH 3 (2.14±0.19). The flavonols content is given in figure 2. Extract of pH 5 was found to have the highest value 1.55 ± 0.02 mg GAE/g DW, pH 6: 0.81± 0.04 mg GAE/g DW, pH 4: 0.7 ± 0.05 mg GAE/g DW, pH 7: 0.65 ± 0.03 mg GAE/g DW and the lowest value in pH 3: 0.64 ± 0.03 mg GAE/g DW).

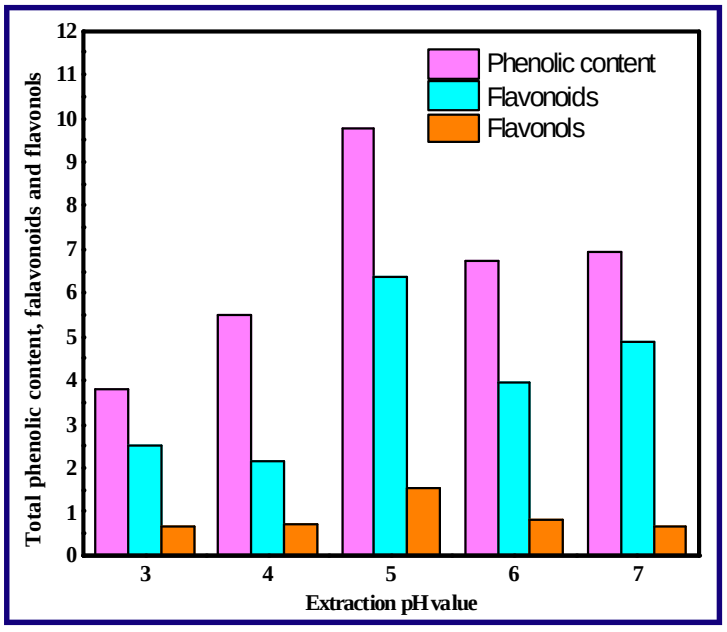


Figure 2. Total phenolic content (mg GAE/g DW) and total flavanoïds (mg CE/g DW) and flavonols (QE/g DW) of *Matricaria Pubescent* extracts obtained by different extraction pH value.

HPLC analysis:

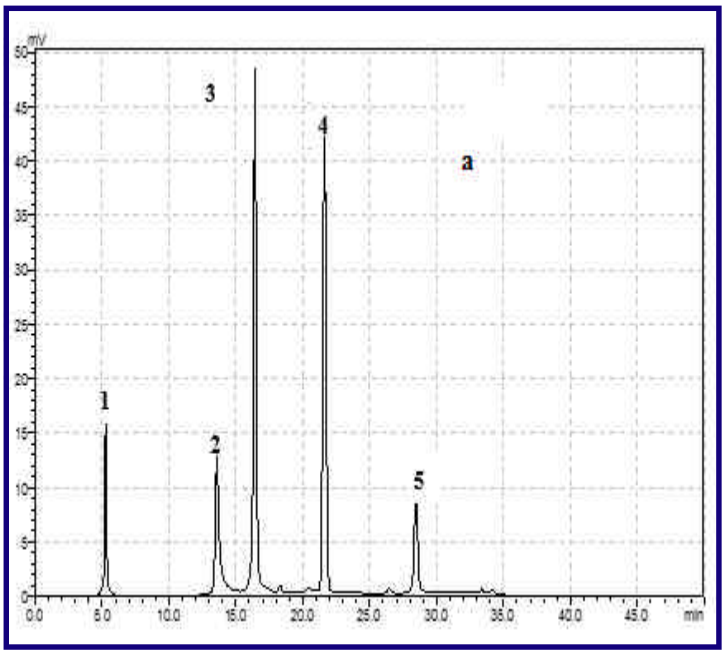
The constituents in the different extracts were analysed by HPLC.

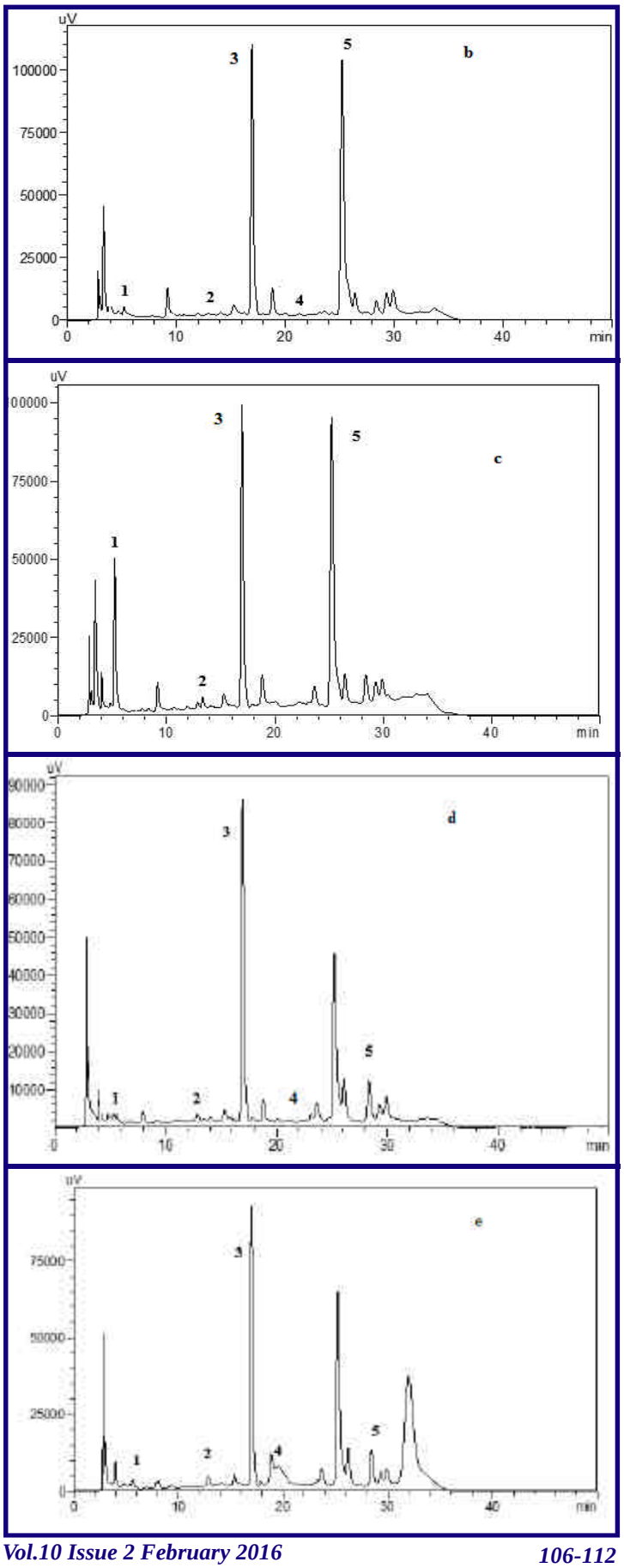
Figure 3 showed the chromatograms of three extracts sample and standard markers mixture. Peaks 1, 2, 3, 4 and 5 were ascorbic acid, chlorogenic acid, caffeic acid, vanillin and rutin respectively. The contents of these components in different extracts were determined according to the calibration curves of ascorbic acid $y = 2260.81x$ ($r^2 = 0.998$), chlorogenic acid $y = 37492.06 x$ ($r^2 = 0.999$), caffeic acid $y = 70429.77x$ ($r^2 = 0.999$), vanillin $y = 80555.42x$ ($r^2 = 0.989$) and rutin $y = 3118.94x$ ($r^2 = 0.988$). Where y was the peak area and x was the concentration of compound (0–80 µg/ml). The quantitative results are summarized in Table 2. As shown, Vanillin was the most dominant constituent and similar in ethanolic, methanolic extracts and no detected in petroleum ether extract. The extract of pH 5 and 4 contains five compounds with considered quantity. While extract of pH 3 and 7 were considered poor from compounds. However, because of its strong polarity, some compounds not concentrated by aqueous extraction.

Table 1. Identification and quantification of phenolic compounds of *Matricaria Pubescent* extracts obtained by different extraction pH value analysed by HPLC

Compounds	Concentration (µg/mg)				
	pH 7	pH 6	pH 5	pH 4	pH 3
Gallic acid	0.3163	0.3247	0.3750	0.2927	-
Chlorogenic Acid	0.1701	0.3977	0.0789	0.0763	4.1790
Caffeic Acid	0.1182	0.1319	0.0362	0.0202	0.0157
Vanillin	0.1118	-	0.0111	0.0193	-
Rutin	8.3369	4.4397	16.1787	8.8324	6.5949

- : not detected





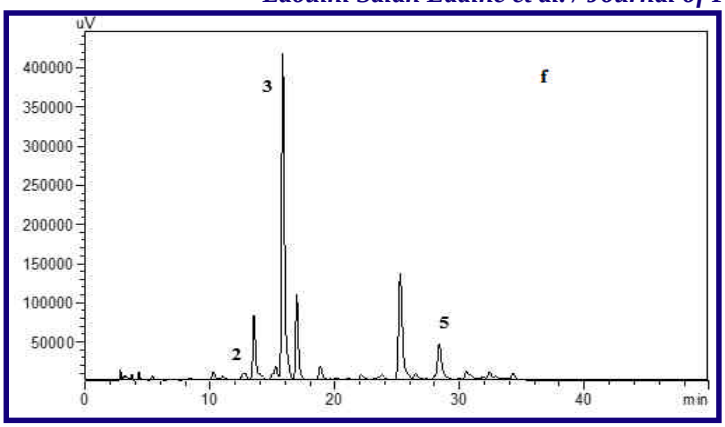


Figure 3. Chromatograms of standard mixture (a), extraction pH 7 (b), extraction pH 6 (c), extraction pH 5(d), extraction pH 4 (e) and extraction pH 3 (f). Peak 1: Gallic acid; Peak 2: Chlorogenic acid; Peak 3: Caffeic acid; Peak 4: Vanillin; Peak 5: Rutin

FRAP assay: The antioxidant properties of a given compound depends not only on its chemical structure but also on the type of the generated radical, it can neutralize. For this reason, we tested the antioxidant potential of *Matricaria Pubescent* extracts obtained by different extraction pH value (3, 4, 5, 6 and 7) against more than one radical type. The percentage inhibition of scavenging activities of the ethyl acetate extracts for ferric reducing were shown in Figure 4. The IC_{50} values of extract pH value 3, 4, 5, 6 and 7 were found to be 19.08 ± 0.31 µg/ml, 11.63 ± 0.31 µg/ml, 9.63 ± 0.28 µg/ml, 15.47 ± 0.33 µg/ml and 18.39±0.28 µg/ml respectively.

Table 2. Scavenging activity of FRAP and ABTS of *Matricaria Pubescent* extracts obtained by different extraction pH value. Antioxidant activity was expressed as % inhibition IC_{50} values (µg/ml).

Extraction pH value	FRAP (IC_{50}= µg/ml)	ABTS (IC_{50}= µg/ml)
3	19.08 ± 0.31	20.08 ±0.82
4	11.63 ± 0.31	17.97 ± 0.84
5	9.63 ± 0.28	16.76± 0.65
6	15.47 ± 0.33	18.75 ± 0.70
7	18.39±0.28	19.35 ± 0.70

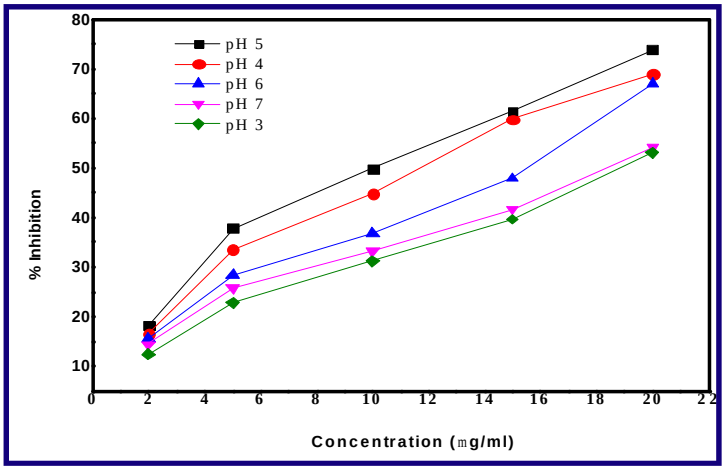


Figure 4. The ferric reducing power (FRAP assay) of *Matricaria Pubescent* extracts obtained by different extraction pH value.

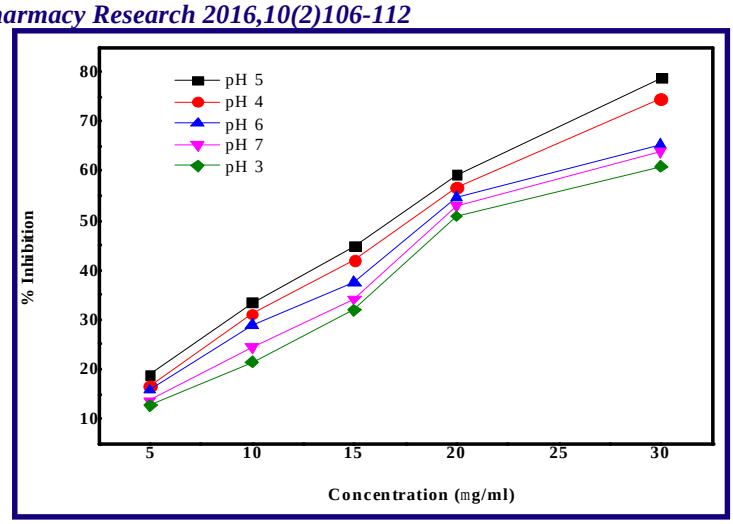


Figure 5. The ABTS radical scavenging activity of *Matricaria pubescent* extracts obtained by different extraction pH value.

ABTS scavenging activity

The extracts from five extraction pH value of leaves from *Matricaria Pubescent* were also measured and compared for their free radical scavenging activity against the ABTS radical. Figure 4 shows that all extracts used in this study had significant ABTS radical scavenging activities. The IC_{50} values ABTS radical scavenging activities of extraction pH value 3, 4, 5, 6 and 7 were in the range of 16.76± 0.65± µg/ml at 20.08 ±0.82 µg/ml. The highest ABTS radical scavenging activities was found in extract pH value 5 (IC_{50}=16.76 ± 0.65 ± µg/ml, the medium in pH 4 (17.97 ± 0.84 µg/ml), pH 6 (18.75 ± 0.70 µg/ml), pH 7 (19.35 ± 0.70 µg/ml) and the lowest value in pH extract 3 (20.08 ± 0.82 ± µg/ml).

DISCUSSION

In the present study, various extraction pH value used to evaluate the total phenolic contents, total flavonoids, flavonols and antioxidant activity of *Matricaria Pubescent*. Among the different solvent extraction pH value. Extraction pH value 5 of medium could provide comparable or even better results than the pH 4, 6, 7 and 3 for extracting phenolic compounds and showed a significant antioxidant activity over the other extraction pH value.

The extraction amount of phenolic compounds from *Matricaria pubescent* increased as the pH values increased from 3 to 5, but it decreased as the pH values higher than 5 were used, which indicated that the extraction pH value significantly affected the extraction of phenolic compounds. The increased extraction of phenolic content under the low pH conditions could be due to the inhibition of the enzymatic oxidation of phenolics and/or the maintenance of the extracted[21].

Activity of different extraction pH value significantly increases with

increasing pH of the medium ($P < 0.05$). It also appears that there is a slight decrease at pH 6 at 7. In general, most plants including lettuce show maximum polyphenoloxidase (PPO) activity at near pH of weak acid values. pH is a determining factor in the expression of enzymatic activity. It alters the ionization states of amino acid side chains or the ionization of the substrate. One reason for the loss of antioxidant activity at pH 4, 5 and 6 could be higher activity of extracts at this pH resulting in higher phenol degradation. The extracts catalyzes the oxidation of phenolic such as chlorogenic acid and rutin to quinones to form dark condensation products. Moreover, the different actions measured by each extraction pH medium value could produce some discrepancies between the results obtained. As a simple assay that provides all the antioxidant information does not exist, it is necessary to apply different extraction pH value of medium to obtain the more complete antioxidant profile of the extracts. Free radicals are produced in higher amounts in many pathological conditions, and involved in the development of the most common chronic degenerative diseases. Degenerative diseases and lysis of the cells and tissues. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplement as traditional medicines.

Phenolic compounds, flavonol and flavonoids have been reported to have multiple biological effects, including antioxidant and anti-inflammatory properties. Recent evidences suggest that diets rich in polyphenolic compounds play a significant role against oxidative stress related disorders because of their antioxidant activities.

CONCLUSION

In this study, phytochemical investigation, in vitro antioxidant activities of extraction pH value (3, 4, 5, 6 and 7) of leaves from *Matricaria Pubescent* obtained by Soxhlet method have been evaluated. The results indicated that the extract obtained by pH value 5 exhibited highest phytochemical compounds and strongest antioxidant activities. HPLC analysis showed rutin to be the most abundant phytochemical, followed by gallic acid. The contents of polyphenols and flavonoids extract were significantly higher than other extraction pH value, which were possibly responsible for higher antioxidant activities. From the results, we can draw the conclusion that not only the more bioactive components obtained but also the extract has better free radical and reactive oxygen species scavenging activities through extract pH value 5. These findings further illustrate that extraction of pH 5 has a bright prospect for extracting active ingredients from plant materials than the other extraction pH value 3, 4, 6 and 7.

Conflict of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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