IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 5 Ver. III (Sep - Oct. 2015), PP 67-72
www.iosrjournals.org

Physicochemical, phytochemical and biological study of Melissa officinalis growing naturally in Kurdistan Region\Iraq: Comparative study

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Abstract: *Crude drug evaluation method is a systematic process for herbal remedies quality control. The study was aimed to evaluate different parts of Melissa officinalis plant from variant points. Leaf and stem of plant have been assessed from physicochemical, qualitative phytochemical and biological parameters to find out the valuable medicinal plant part. Leaf part of plant expressed it is importance as medicinal part of plant form tested parameters. Leaf part showed higher number of phytochemical constituents, ethanolic extract exhibited antibacterial activity against two strains of evaluated bacterial species Staphylococcus aureus (ATCC 25923) and clinically isolated species Klebsiella spp. while all other bacteria showed resistance for other extracts obtained from both parts. Leaf part of Melissa officinalis was the valuable plant part must taken in consideration in researches. Ethanol solvent of extraction was an efficient solvent of extraction of active phytochemical constituents.*

Keywords: *Fluorescence analysis, Klebsiella spp, Melissa officinalis leaf, Melissa officinalis stem, Physicochemical evaluation*

I. Introduction

Various phytochemical compounds have been endowed from plant source example on these phytochemicals are terpenids, lignins, tannins, stilbenes, betalains, alkaloids, flavonoids, quinones, amines, coumarins and other secondary metabolites which are rich in pharmacological activities [1,2] . Nowadays, crude plant materials have been extensively investigated for phytochemical constituents (secondary metabolites) as a source for medicinal agents [3]. WHO estimated that 70% of the world population relies on the traditional herbal remedies for treatment of various diseases [4].

Crude drug evaluation process over the years undergone systematic changes in both nature and degree view points. In recent years crude drug evaluation include active constituents estimating methods, in addition to the microscopical and morphological analysis, the drugs were evaluated by using different separation technique including the physical assessment of the drugs in both qualitative and quantitative points, in opposite to the initial method which comprise comparing of crude drug with standard ones [5].

Melissa officinalis L, family Lamiaceae is an aromatic perennial herb native to the southern Europe. Medicinally the plant have been used in gastrointestinal disorders because of it is digestive, carminative and antispasmodic properties, other medical activities reported for lemon balm are antioxidant, anti-inflammatory, antibacterial . Traditionally used as aromatic herbal tea and for culinary and cosmetic purposes[6-16]. Main active constituents of Melissa officinalis are essential oils, phenolic compunds, tannins [17, 18]. Melissa leaf and herb were used in different places, while western pharmacopoeias prefer the leaf part over other plant parts for its therapeutic principles [19, 20].

Melissa officinalis herb used by the local community for both medicinal and culinary purposes either the whole aerial parts (leaf and steam) or the leaf alone differs from place to another throughout the Region. The present study was aimed to evaluate various parts of plant alone from different points of evaluation physicochemical, phytochemical and biological and find out the part of plant responsible for the plant value medicinally.

II. Materials and methods

2.1. Plant material collection:

Arial parts of Melissa officinalis were collected in mountain places of Erbil city, Kurdistan Region\Iraq, have been identified by Pharmacognosy Department, Pharmacy College\ Hawler Medical University. Stem and leaf parts of plant were separated and dried in shade. Dried plant material were kept under 21-23 ^{o} C.

2.2. Physicochemical analysis:

Different physico-chemical parameters such as moisture content [loss on drying (LOD)], total ash, acid insoluble ash, water soluble ash were determined. Extractive value, color and consistency of plant material extracts obtained from successive extraction procedure using different polarity solvents for both parts of plant material were determined separately. All the parameters were taken in triplicate and the result which was obtained presented as percentage of mean ± standard deviation (mean ± SD) [21, 22].

2.3. Fluorescence analysis:

Plant part material extracts with different polarity solvents and powdered plant materials have been examined for the presence of fluorescence compounds under UV lamb. Powdered plant materials were placed on a grease free microscopic slide and added 2 drops of freshly prepared reagent solutions (concentrated sulphuric, xylene acid, picric acid, 1M sodium hydroxide, 10% nitric acid, concentrated nitric acid,10% hydrogen peroxide, 10% potassium dichromate, iodine, aqueous ferric chloride, alcoholic ferric chloride, and concentrated hydrochloric acid) mixed thoroughly and waiting for 1-2 minutes, then examine the mixture under visible day light, UV-light (254 nm) and UV light (366 nm), the notified color were recorded [23, 24].

2.4. Qualitative Phytochemical analysis:

Different solvent extracts [petroleum ether and 70% ethanol] of powdered plant part materials were have been applied for different chemical tests for identification of phytochemical active constituents using standard conventional protocols [25, 26].

2.5. Biological analysis:

Different parts plant extract were introduced for biological analysis:

2.5.1. Plant extract preparation:

Powdered plant material (both parts separately) introduced to extraction using petroleum ether (PE) and (70%) ethanol (ETH) as solvent of extraction using ultra sonic extractor method successively [27]. Extracts concentrated and dried under vacuum using rotary vapour machine, 10mg\ml concentration of extracts were analyzed for their biological activity using tween 80 (20%) and dimethyl sulfoxide (DMSO) (10%) as diluent for petroleum ether and ethanolic extract respectively.

2.5.2. Inoculum preparation:

Nine pathogenic bacteria were selected for the antibacterial evaluation:
Standard species: Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 35218), Staphylococcus aureus (ATCC 25923), Streptococcus pyogenes (ATCC 19615), and Streptococcus pneumoniae (ATCC 6303).
Clinically isolated species: Escherichia coli, Staphylococcus aureus, Klepsilla spp. and Bacillus spp.
All strains were maintained on nutrient and blood agar slant, and then stored at 4 ^{0}C until used. McFarland standards 0.5 are used as a reference to adjust the turbidity of bacterial inoculums [28].

2.5.3. Antimicrobial evaluation:

Agar well diffusion method described by Sandhya et al., 2012 [29] used for evaluation of antibacterial activity of plant extracts with slight modification. The bacterial inoculum (0.1 ml) was uniformly spread using sterile cotton swab on a sterile Muller Hinton agar plate. About 0.1 ml of plant extracts was added to each of the wells (6 mm) diameter well cut in the agar using sterile cork borer. Positive control [azithromycine antibiotic (10mcg\ml)] and negative control [DMSO (10%) and tween 80 (20%)] were included in each plate. The plates were incubated under aerobic conditions for 24 h at 37ºC. Following incubation, the zone of inhibition in mm around the wells was measured including the well diameter. Tests were performed in triplicate, the mean ± SD of the results were taken in consideration.

2.6. Statistical analysis:

All data were estimated from triplicate procedure expressed as mean ± standard deviation (SD).

III. Results:

3.1. Physicochemical analysis:

Different physicochemical parameters have been evaluated for both parts of plant material (stem and leaf) shown in Table.1. The tested parameters shows higher moisture content in stem part while greater total ash value in leaf part. Extractive value obtained from different solvents and the corresponding physical characteristics of extracts were expressed in Table.2. and Table.3. A magnificent extractive value were obtained from leaf part using ethanol (70%) as solvent of extraction.

Table 1: Physicochemical parameters for leaf and stem of Melissa officinalis:

Physicochemical Parameters	Melissa officinalis	
	Leaf [1]	Stem[1]
LOD	72.99±0.02%	81.9±0.01%
Total Ash value	17.88±0.15%	13.3±0.21%
Water soluble ash	4.64±0.23%	5.85±0.13%
Acid insoluble ash	5.29±0.41%	3.22±0.51%

1 stands to mean ± SD were expressed as percentage (w\w%), n=3

Table 2: Extractive value and physical characteristics of leaf extracts of Melissa officinalis:

Type of Extract	Extractive value[1] (w\w%)	Physical characteristics	
		Consistency	Color
Petroleum ether	0.5%	Sticky, Pasty Texture	Orange
Ethanol (70%)	13.66%	Non sticky , Hard Texture	Dark green

1 stands to mean ± SD were expressed as percentage (w\w%), n=3

Table 3: Extractive value and physical characteristics of stem extracts of Melissa officinalis:

Type of Extract	Extractive value[1] (w\w%)	Physical characteristics	
		Consistency	Color
Petroleum ether	0.065%	Non sticky, Pasty Texture	Light green
Ethanol (70%)	16.305%	Non sticky, Hard Texture	Brown

1 stands to mean ± SD were expressed as percentage (w\w%), n=3

3.2. Fluorescence analysis:

Both parts of plant material Melissa officinalis have been examined under UV in both forms different polarity solvent extracts and powdered crude drug, variable colours were obtained from dealing the powder drug with different reagent some of the obtained colours with identical reagents gave similar color as in treating powder drugs with potassium dichromate (10%) under UV (254nm) records were shown in Table.4. and Table.5. and dry powdered plant material treating with different reagents in Table.6. and Table .7.

Table 4: Fluorescence analysis of extracts of Melissa officinalis leaf extract:

Plant Extract	UV light (254nm)	UV light (366 nm)
Petroleum ether	Greenish Black	Black
Ethanol (70%)	Dark Green	Black

Table 5: Fluorescence analysis of extracts of Melissa officinalis stem extract:

Plant Extract	UV light (254nm)	UV light (366 nm)
Petroleum ether	Fluorescent light green	Pinkish violet
Ethanol (70%)	Light grey	Grey

Table 6: Fluorescence analysis of extracts of Melissa officinalis leaf powder:

Powder plant + reagent	Visible light	UV (254 nm)	UV (366 nm)
Powder + Xylene	Dark green	Inky blue color	Black
Powder + Concentrated H_2SO_4	Black	Dark brown	Black
Powder + 1 M NaOH	Greenish yellow	Dark green	Greenish black
Powder + 10% HNO_3	Green	Light green	Black
Powder + 10% H_2O_2	Greenish brown	Dark green	Black
Powder + 10% $K_2Cr_2O_7$	Blackish brown	Dark brown	Brown
Powder + I_2	Brown	Black	Black
Powder + Alcoholic $FeCl_3$	Dark green	Dark greyish green	Ink color
Powder + Aqueous $FeCl_3$	Dark green	Greyish green	Blackish green
Powder + Concentrated HCl	Greenish yellow	Dark green	Black
Powder + Picric acid	Greenish brown	Green	Dark green
Powder + Concentrated HNO_3	Caramel	Light green	Greenish black

Table 7: Fluorescence analysis of extracts of Melissa officinalis stem powder:

Powder plant + reagent	Visible light	UV (254 nm)	UV (366 nm)
Powder + Xylene	Light green	Greenish grey	Greenish black
Powder + Concentrated H_2SO_4	Blackish brown	Brown	Black
Powder + 1 M NaOH	Yellow	Yellowish green	Greenish black
Powder + 10% HNO_3	Yellow	Yellow	Black
Powder + 10% H_2O_2	Brown	Yellowish green	Greyish black
Powder + 10% $K_2Cr_2O_7$	Brown	Dark brown	Black
Powder + I_2	Light green	Dark green	Greenish black
Powder + Alcoholic $FeCl_3$	Light green	Dark grey	Ink color
Powder + Aqueous $FeCl_3$	Green	Dark green	Greenish black

Powder plant + reagent	**Visible light**	**UV (254 nm)**	**UV (366 nm)**
Powder + Concentrated HCl	Very light green	Grey	Greyish black
Powder + Picric acid	Yellow	Yellowish green	Green
Powder + Concentrated HNO_3	Orange	Light yellow	Dark green

3.3. Qualitative phytochemical analysis:

The extracts of both parts have been introduced for different phytochemical investigation. Results shows presence of one and six of investigated phytochemicals in both petroleum ether and ethanolic extract respectively. Results were expressed in Table.8. The results reveals the presence of five constituents in leaf and four constituents in stem part in ethanolic extract and only constituents have been detected for the petroleum ether extracts for both parts. Depending on the color intensity the results were expressed as (+) for light colors and (++) for more intense color results.

Table 8: Qualitative phytochemical investigation of Melissa officinalis leaf and stem extracts:

Investigated Phytochemical	**Test**	**Leaf extract**		**Stem extract**	
		PE[1]	**ETH[2]**	**PE**	**ETH**
Alkaloid	Dragendorff test	-	-	-	-
Anthraquinone glycoside	Borntragers test	-	-	-	-
Cardioactive glycoside	Keller- kiliani test	-	-	-	-
Flavonoid	NaOH test	-	++	-	+
Hydrolysable tannin	Ferric chloride test	-	-	-	-
Condensed tannin		-	++	-	+
Terpenoid	Salkowski test	-	+	-	+
Phytosterol	Liebermann Burchard test	-	-	-	-
Phenol	Ferric chloride	-	++	-	+
Quinon	Sulphuric acid test	+	++	+	++
Saponin	Forth test	-	+	-	-

1 PE stands for petroleum ether extract,2 stands for ethanol (70%) extract, (-) stands for absence of phytochemical, (+) stands for presence of phytochemical (trace amounts, faint color), (++) stands for presence of phytochemical (moderate amount, intense color),

3.4. Biological analysis:

Variant parts crude drug extracts [PE and ETH] were assessed for the biological activity by introducing the extracts for antibacterial activity against different bacterial strains, stem extracts did not show activity against tested bacterial strained at investigated concentration while the ETH extract of leaf shows activity against two of the tested strains. Results were expressed in Table.9.

Table 9: Antibacterial activity of Melissa officinalis leaf and stem extracts:

Tested Bacterial strain	**Inhibition zone mm (mean ± SD) (n=3)**				
	Leaf extract		**Stem extract**		**Azithromycine (10mcg\ml)**
	PE[1]	**ETH[2]**	**PE**	**ETH**	
P. aeruginosa ATCC27853	R[3]	R	R	R	28 ±0.013
E. coli ATCC 35218	R	R	R	R	30 ± 0.021
S. aureus ATCC 25923	R	17± 0.052	R	R	26 ±0.014
S. pyogenes ATCC 19615	R	R	R	R	30 ±0.041
S. pneumoniae ATCC6303	R	R	R	R	25 ±0.022
E. coli	R	R	R	R	30 ±0.05
S. aureus	R	R	R	R	17 ±0.91
Klebsiella spp.	R	18 ± 0.142	R	R	34 ±1.02
Bacillus spp.	R	R	R	R	25±0.83

1 PE stands for petroleum ether extract,2 ETH stands for ethanol (70%) extract, 3 R stands for resistance of bacterial strain

IV. Discussion:

Since thousand years phytotherapy have been used by mankind. People used herbal remedies from early history either as religious or magical healing art [28]. Melissa officinalis plant have been used since long history described in pharmacopeias either single part of plant (leaf, stem, flower) or the whole plant incorporated in herbal remedies preparation [19, 20]. Variant physicochemical parameters have been evaluated for both leaf and stem parts of plant. Physicochemical studies were performed to emphasize the quality of crude drug. Relatively high moisture content (81.9±0.01%) have been recorded for the stem part in comparison with leaf part (72.99±0.02%), revealed that the moisture content of leaves were within required range. High percentage of moisture content encourage microorganism growth (bacteria and fungi) [>73% moisture content helps bacterial colonisation] [28, 30, 31]. Results expressed high total ash value in leaf part in correspondence to the stem part which was the indication of physiological matrix of the part, greater ash value reveals to greater

physiological matrix physiological of plant part [32]. Approximately similar values of water soluble ash were obtained for both parts while the acid soluble ash in leaf part was higher than stem part indication for environmental contamination of the part.

Generally low extractive values were obtained for petroleum ether solvent of both parts of plant, high extractive value have been estimated for the hydroalcholic solvent of the leaf part in comparison to the stem part, which reflect on the amount of constituents in leaf part can be extracted by ethanolic solvent (70%).

Various chemical constituents in plant material exhibited fluorescence phenomenon, some were expressed fluorescence in visible light, while some of the phytochemicals can produce fluorescence on UV light application. Fluorescence analysis is considering an important parameter in pharmacognostical evaluation [33-34]. Identical color have been obtained from treating powdered crud drug (stem and leaf separately) as indication of similar compound present in both parts. Further more study on qualitative phytochemical analysis ensured the fluorescence analysis results of both parts. Leaf and stem of Melissa officinalis plant expressed presence of flavonoid, condensed tannin, terpenoid, phenol and quinon in hydroalcholic extract which consistent to the finding of Natália et al, 2011 [35] at different color ranges varied between light to intense color as indication to the concentration of phytochemicals, while the petroleum ether extract were showed the presence of quinon only in trace amounts (light color) in comparison to the ethanolic extract of plant parts. Saponin active constituent have been detected in ethanolic extract of Melissa officinalis leaf extract with absence of latter in stem extract in similar solvent extract, the finding was in contrast to the Natália et al, 2011 [35], which record the absence of the constituent in aerial part extract of plant.

Biologically plant parts have been evaluated by assessing the antibacterial activity of both extracts of both parts at similar concentration. Stem part extracts (PE and ETH) did not exhibit any activity at tested concentration (10mg\ml) against evaluated bacterial strain. Ethannolic leaf extract expressed activity toward S. aureus ATCC 25923 and Klebsiella spp. which a consistent result to the finding of [36, 37], while the PE extract was totally inactive against tested bacterial strain at (10mg\ml) concentration. Generally the leaf part of plant medicinally valuable part of plant from physiological matrix amount (total ash value), qualitative phytochemical constituents and biological point which emphasized by the pharmacopeias reports on preferences of leaf part of plant [20]. Further study on the biological activity of the stem part of plant were recommended from different aspects of clinical evaluations.

V. Conclusion:

In an attempt to find the medicinal plant part of Melissa officinalis plant, we conclude that the leaf part was the valuable plant part from variant evaluated parameters, and ethanol was an efficient solvent of extraction to extract the bioactive phytochemical constituents from Melissa officinalis plant.

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