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Comparative extraction methods, phytochemical constituents, fluorescence analysis and HPLC validation of rosmarinic acid content in *Mentha piperita, Mentha longifolia* and *Osimum basilicum*

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Abstract
The present investigation is aimed to compare the percentage value of *Mentha Piperita, Mentha longifolia and Osimum basilicum* extracts by using different solvent and extraction methods, phytochemical screening of constituents present in dry leaves, also to study fluorescent characteristics of leaves powder which are responsible for the medicinal properties of the plant using various standard tests and HPLC analysis of rosmarinic acid content in three labiatae species. The highest percentage extract was yielded from *Mentha longifolia* (18.5%) by using maceration methods and ethanol 80% solvent. Phytochemical analysis revealed the presence of alkaloids, saponins, phenols, flavonoids, terpenoids, cardioactive glycosides, tannins, carbohydrates in dry leaf extracts. Presence of alkaloids and phenolic acid was determined in high quantity in *Mentha longifolia*, while saponins present in high quantity in *Mentha Piperita* and flavonoids in *Osimum basilicum*. The fluorescent characteristics of leaves powder with various chemical reagents were noted under visible and UV light. HPLC analysis of dry leaf extracts revealed presence of rosmarinic acid among the investigated plants and in higher percentage in *Osimum basilicum* (0.306%).

Keywords: Labiatae species; phytochemical constituents; Fluorescence analysis; HPLC validation; Rosmarinic acid.

1. Introduction
The Labiatae family is one of the most employed medicinal plants as a worldwide source of spices and also as a consolidated source of extracts with strong antibacterial and antioxidant properties. Within this family, the genus *Ocimum* and *Mentha* provides various species, and *Ocimum basilicum* is one of the more than 60 *Ocimum species* [1, 2]. The *Ocimum basilicum* is an annual herb which grows in several regions around the world. The plant probably originated in India, Afghanistan, Pakistan, Northern India and Iran, and now is cultivated worldwide. Traditionally has been extensively utilized in food as a flavoring agent, and in perfumery and medical industries [3]. The leaves and flowering tops of the plant are perceived as carminative, galactogogue, stomachic and antispasmodic in folk medicine [4]. However, recently the potential uses of *O. basilicum* essential oil, particularly as antimicrobial and antioxidant agents have also been investigated [5, 6]. Externally, they have been applied for the treatment of acne, loss of smell, insect stings, snake bites, and skin infections [7]. The activity is chiefly attributed to a variety of phenolic compounds and composition of essential oil. The main compounds responsible for typical aroma are chavicol methyl ether (estragol), linalool, eugenol, 1, 8-cineole and methyl cinnamate [8]. The non-volatile compounds were found to be rich in phenolic acids with major part of caffeic and rosmarinic acid [9]. The genus Mentha belonging to the family Labiatae, and consists of about 25-30 species, are herbaceous, perennial plants, widespread distribution in Iraq [10] Europe, Australia and South Africa [11, 12]. *Mentha longifolia* which can reach up to 1.5 m high in favourable conditions [10]. *Mentha piperita* which can reach 100 cm in height (40 inches) has four-sided stem [13]. Various biological activities have been reported for species of *Mentha*, such as antibacterial [14, 15], antifungal [16], and insecticidal properties [17, 18]. *Mentha spp* possesses antioxidant properties due to the presence of active constituents like menthone, menthol, flavonoids, rosmarinic acid and carvone [19]. The aim of this research is the evaluate the influence of using different extraction methods on the yield of extracted substances, also phytochemical study, fluorescence analysis of active constituents and HPLC for rosmarinic acid content in *Mentha Piperita, Mentha longifolia* and *Osimum basilicum* and compare the results among these three labiatae species.

2. Materials and Methods

2.1. Collection of plant materials

Fresh leaves of *Mentha Piperita, Mentha longifolia* and *Osimum basilicum* were collected from Kurdistan regions, Iraq. The plant materials were taxonomically identified the department of Pharmacognosy, Hawler Medical University. The plant materials were shade dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were ground well using mechanical blender into fine powder and transferred into airtight containers with proper labeling for future use.

2.2. Preparation of plant extracts

2.2.1. Maceration

Thirty gm of each plant leaves were extracted first with n-hexane by maceration method stand for 6 day [20] and then the residues were further extracted with ethanol 80% stand for 6 day separately. The solvents were used based upon their increasing polarity index. The solvents were evaporated in a vacuum evaporator model then crude extracts were stored at -4c°and yielded quantities of leaves extracts of different plants in different solvents were obtained recorded and were further taken to evaluate the phytochemical studies.

2.2.2. Ultrasonic bath

Thirty gm of each plant leaves were extracted first with n-hexane using ultrasonic assisted extractor for 1 hr at 40 ^{0}C [21], then the residues were further extracted with ethanol 80% using ultrasonic assisted extractor for 1 hr at 40 ^{0}c. The solvents were used based upon their increasing polarity index. The solvents were evaporated in a vacuum evaporator model then crude extracts were stored at -4 ^{0}C and yielded quantities of leaves extracts of different plants in different solvents were obtained recorded and were further taken to evaluate the phytochemical studies.

2.3. Phytochemical screening

2.3.1. Qualitative analysis on phytochemical constituents of *Mentha Piperita, Mentha longifolia* and *Osimum basilicum*.

The n-hexane and ethanol 80% extracts of leaves were analyzed for the presence of various phytoconstituents by standard phytochemical tests [22, 23, 24].

2.3.2. Quantitative analysis on phytochemical constituents of *Mentha Piperita, Mentha longifolia* and *Osimum basilicum*.

Total alkaloids, flavonoids, phenolic acids and saponins were determined using the method described by Krishnaiah *et al* [25].

2.3.2.1. Determination of alkaloids

Ten grams of each plants were placed in a 250 ml beaker and 200 ml of 10% CH_3CO_2H in C_2H_5OH was added. The mixture was covered and allowed to stand for 4 hours. It was then filtered and the filtrate was concentrated on a water bath until it reaches a quarter of its original volume. Concentrated NH_4OH was added until precipitation was complete. The mixture was allowed to settle and the precipitate collected on a weighed filter paper and washed with dilute NH_4OH. The precipitate of alkaloid was dried and weighed. The percentage alkaloid was calculated as:

$$\text{Percentage of total alkaloid} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

2.3.2.2. Determination of flavonoids

Ten grams of each plants were repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250 ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was calculated as:

$$\text{Percentage of total flavonoids} \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

2.3.2.3. Determination of saponins

Ten grams of each plants were weighed into a 250 ml conical flask. 100 ml of 20% C_2H_5OH was added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at about 55 °C. It was then filtered with a Whatman No.42 paper. The residue was re-extracted with another 200 ml of 20% C_2H_5OH. The combined extract was reduced to 40 ml over a water bath at about 90 °C. The concentrated extract was then transferred into a 250 ml separator funnel and 20 ml of $(CH_3CH_2)_2O$ was added to the extract and shaken vigorously. The aqueous layer was recovered while the $(CH_3CH_2)_2O$ layer was discarded. This purification process was repeated. 60 ml of n-butanol was added and the combined n-butanol extract was washed twice with 10ml of 5% NaCl. The remaining solution was then heated on a water-bath in a pre-weighed 250 ml beaker. After evaporation the residue was dried in a Gallenkamp moisture extraction oven (Size 1) to a constant weight. The % saponin was calculated as:

$$\text{Percentage of total saponin} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

2.3.2.4. Determination of simple phenolic compounds

Each plant separately were grinded to powder and 10g was defatted by petroleum ether, the residue was extracted with 80% methanol using sonication for 2h., the crude extract was separated by simple filtration, the filtrate was evaporated, and the residue was partitioned between chloroform and water (using separation funnel). The dark brown residue left after evaporation of chloroform was used as crude phenolic compounds. The % simple phenolic was calculated as:

$$\text{Percentage of simple phenolic} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

2.4. Fluorescence Analysis

A small quantity of dried and finely powdered leave of each plant separately were taken into clean and dried test tubes. To each tube 5 ml of different organic solvents like methanol, 1% glacial acetic acid, 10% NaOH, dilute ammonia solution, 1M H_2SO_4, 1M HCl, 10% $Fecl_3$, acetone + methanol, and 10% iodine were added separately. Then, all the tubes were shaken and they were allowed to stand for about 20-25 min. The solutions obtained were observed under the visible and UV light for their characteristic colour reaction was recorded [26, 27].

2.5. HPLC analysis

2.5.1. Sample preparation

The accurately weighed dried *Mentha Piperita, Mentha longifolia and Ocimum basilicum* powder 1 gm was extracted

with ethanol 80% using ultrasonic assisted extractor for 1hr at 40 ^{0}C [21] then filtered and which was after drying dissolved in 10 ml (5%) HCl and refluxed for 1hr, liquid-liquid fractionation using chloroform (10 x 3 ml) resulted an organic fraction on drying in vacuum that used for evaluation of phenolic acid constituents. The organic extract was filtered through a 0.45-µm membrane prior to injection into the HPLC system.

2.5.2. Preparation of Stock and Standard Solutions

Rosmarinic acid (1 mg), was accurately weighed into a 5 ml volumetric flask, dissolved in methanol: water (1:1) filled up to volume for preparing stock solutions. Standard solutions were prepared for each compound at five different concentration (200, 100, 50, 10 and 5 µg) levels in 5 ml volumetric flasks for the establishment of calibration curves.

2.5.3. Chromatographic condition

2.5.3.1. Method Validation and quantification [28, 29]

The qualitative and quantitative analysis of rosmarinic acid was performed on Knauer HPLC instrument equipped with ChromGate HPLC software provided by Knauer was use with Eurospher 100, C18 column (4.6 mm i.d. x 250 mm, 5 mm) and UV/Visible detector. The flow rate of the mobile phase for rosmarinic acid was kept at 1 ml/min. Mobile phase A was Methanol and B water containing 0.1% acetic acid the isocratic conditions were as 80% A and 20% B. The temperature of column was controlled at 30 °C. Injection volume was 20 µl. The detection wavelength 280 nm. The retention time (RT) values for standard compounds were determined 10 min. prior to each run, the HPLC-UV/Visible system was allowed to warm, and the baseline was monitored until it was stable before sample analysis.

2.5.3.2. Linearity

One mg of rosmarinic acid was weighed into a 5 ml volumetric flask, dissolved in methanol: water (1:1) filled up to volume for preparing stock solutions. Standard solutions were prepared for each compound at five different concentration (5, 10, 50, 100, and 200 µg/ml) levels in 5 ml volumetric flasks for the establishment of calibration curves. The prepared dilutions were injected in series, peak area was calculated for each dilution, and concentration was plotted against peak area.

2.5.3.3. Accuracy

Accuracy was determined by the standard addition method for the three concentrations (50, 100, and 200 µg/ml) and the recovery was calculated by comparison of the found amounts with the added ones. The experiment was performed in triplicate. Recovery (%) was calculated for each concentration.

2.5.3.4. Precision

Precision was determined as both repeatability and intermediate precision, in accordance with ICH recommendations. Repeatability of sample injection was determined as intra-day variation and intermediate precision was determined by measurement of inter-day variation. For both intra-day and inter-day variation, standard solutions at three different concentration (50, 100, and 200 µg/ml) were determined in triplicate.

2.5.3.5. Detection (LOD) and Quantification (LOQ) Limits

LOD and LOQ were determined by the standard deviation (Sy/x) method. Blank samples were injected in triplicate and the peak area of the blanks were calculated. LOD and LOQ were determined from the slope (S) of the calibration plot and the standard deviation of the response for the blank sample, Sy/x, by use of the formulae LOD = 3.3 × Sy/x/S and LOQ = 10 × Sy/x/S.

3. Results and Discussion

The results showed that percentage yield of different extracts of the given powdered leaves of three labiatae species influenced by use different extraction method and solvent. (Table 1) revealed that amount yielded by maceration method higher than ultrasonic method, which is modified maceration method, maceration is that the process can be quite time-consuming [30, 31] but yield higher quantity of extract. During use different solvent, amount of ethanol 80% extracts more than n-hexane extracts, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material [32], ethanol was the most effective one producing the highest extraction yield and n-hexane gave the lowest yield in extracting bioactive compounds by these methods [33]. Among three labiatae species higher amount extract yielded by *Mentha longifolia* (18.5%) by maceration process and minimum amount by *Mentha piperita* (0.81%) by Ultrasonic bath extraction process.

Table 1: The percentage yield of leaf extracts of three labiatae species by using different solvent and extraction methods.

Plant sample	Extraction methods	n-Hexane	Ethanol 80%
Mentha piperita	Maceration	1.4%	10.4%
	Ultrasonic bath	0.81%	2.74%
Mentha longifolia	Maceration	3.4%	18.5%
	Ultrasonic bath	0.92%	5.46%
Osimum basilicum	Maceration	1.4%	11%
	Ultrasonic bath	1.33%	6.05%

The phytochemical active compounds of *Mentha Piperita, Mentha longifolia* and *Osimum basilicum* were qualitatively analyzed for leaves separately and the results are presented in (Table 2 and 3). In these screening process alkaloids, saponins, phenols, flavonoids, terpenoids, cardioactive glycosides, tannins, and carbohydrates were present in ethanolic 80% extracts of three labiatae species, flavonoids and cardioactive glycosides present in *Osimum basilicum* in higher concentration followed by *Mentha longifolia* then *Mentha Piperita*, while alkaloids present in higher concentration in *Mentha longifolia* followed by *Mentha Piperita* and *Osimum basilicum*. Steroids and anthraquinone glycosides were found to be present in *Osimum basilicum* only and were absent in other plants. In n-hexane extracts alkaloids, saponins, terpenoids, carbohydrates, cardioactive glycosides were present in three labiatae species, and phenol, flavonoids, anthraquinone glycosides and tannins found to be absent in all plants. Steroids was found to be present only in *Osimum*

basilicum. Presence of alkaloids, saponins, flavonoids, terpenoids, phenols in *Mentha longifolia* and absence of steroids and anthraquinone glycosides which were supported by previously recorded data [34], while presence of cardioactive glycosides and tannins was not in agreement with the opinion of same study. Presence of a tannins, flavanoids, phenols and absence of anthraquinone in *Mentha Piperita* supported the data recorded previously [35,36], while presence of alkaloids, saponins, carbohydrates, cardioactive glycosides in Iraqi species are in contrast to this study. Also presence of tannins, saponins, flavonoids, terpenoids, carbohydrates, phenols, cardioactive and anthraquinone glycosides, were present in *Osimum basilicum* are in agreement with previously recorded data [37, 38, 39]. While reports from workers in other areas indicated the absence of alkaloids and steroids, this is contrary to what was obtained in this work [40]. On quantitative estimation in (Table 4) alkaloids and phenolic acids present in high percentage in *Mentha longifolia* (0.81%) and (1.6%), while saponins present in high percentage in *Mentha Piperita* (6%) and flavonoids in high percentage in *Osimum basilicum* (11.4%).

Table 2: Qualitative analysis of ethanol 80% extracts of *Mentha Piperita, Mentha longifolia* and *Osimum basilicum* leaves.

Plant sample	Alkaloids	Saponins	Phenols	Flavonoids	Steroids	Terpenoids	Cardioactive glycosides	Anthraquinone glycosides	Tannins	Carbohydrates
Mentha piperita	++ve	+ve	+ve	+ve	Ve-	+ve	+ve	-ve	+ve	+ve
Mentha longifolia	+++ve	+ve	+ve	+ve	-ve	+ve	++ve	-ve	+ve	+ve
Osimum basilicum	++ve	+ve	+ve	++ve	+ve	+ve	+++ve	+ve	+ve	+ve

(+++ve) Highly present, (++ve) moderately, (+ve) low, (-ve) absent

Table 3: Qualitative analysis of n-Hexane extracts of *Mentha Piperita, Mentha longifolia* and *Osimum basilicum* leaves

Plant sample	Alkaloids	Saponins	Phenols	Flavonoids	Steroids	Terpenoids	Cardioactive glycosides	Anthraquinone glycosides	Tannins	Carbohydrates
Mentha piperita	+ve	+ve	-ve	-ve	Ve-	+ve	+ve	-ve	-ve	+ve
Mentha longifolia	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve
Osimum basilicum	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve

(+++ve) Highly present, (++ve) moderately, (+ve) low, (-ve) absent

Table 4: Quantitative estimation of *Mentha Piperita, Mentha longifolia* and *Osimum basilicum*

Plant sample	Alkaloids	Saponins	Simple phenolic acid	Flavonoids
Mentha piperita	0.012 gm (0.12%)	0.60 gm (6%)	0.092 gm (0.92%)	0.22 gm (2.2%)
Mentha longifolia	0.081 gm (0.81%)	0.57 gm (5.7%)	0.16 gm (1.6%)	0.40 gm (4%)
Osimum basilicum	0.010 gm (0.1%)	0.59 gm (5.9%)	0.046 gm (0.46%)	1.14 gm (11.4%)

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. In these study leaf powder of *Mentha Piperita, Mentha longifolia* and *Osimum basilicum* showed different colouration under visible and UV light using different chemical reagents such as methanol 1% glacial acetic acid, 10% NaOH, dilute NH3, 1M H_2SO_4, 1M HCl, 10% Fecl3, Acetone + Methanol, 10% Iodine (Table 5-7) and (Figure 1-9). One of the important features of fluorescence is that UV light induces a fluorescent nature in many natural products where fluorescence is not seen in natural day light.

Table 5: It shows fluorescence analysis of powdered leaves of *Mentha Piperita*.

Powdered drug	Visible/day light	UV 254 nm	UV 366 nm
Powder + Methanol	Yellow green	Green	Light green
Powder + 1% glacial acetic acid	Darkkhaki	Medium purple	Dark purple
Powder +10% NaOH	Dark brown	Reddish brown	Dark reddish brown
Powder + dil. NH3	Saddle brown	Brown	Light brown
Powder +1M H2SO4	Pale golden rod	Thistle	Rose brown
Powder +1M HCl	Pale golden rod	Thistle	Rose brown
Powder + 10% FeCl3	Dark brown	Black	Dark blue
Powder +Acetone + Methanol	Olive	Green	Light green
Powder +10% Iodine	Dark golden rod	Brown	Reddish brown

Table 6: It shows fluorescence analysis of powdered leaves of *Mentha longifolia*

Powdered drug	Visible/day light	UV 254 nm	UV 366 nm
Powder + Methanol	Green	Light green	Dark green
Powder + 1% glacial acetic acid	Darkkhaki	Yellowish grey	Thistle
Powder +10% NaOH	Golden rod	light golden rod	Dark golden rod
Powder + dil. NH3	Brown	Dark brown	Dark reddish brown
Powder +1M H2SO4	Light golden rod yellow	Rosy brown	Thistle
Powder +1M HCl	Light golden rod yellow	Rosy brown	Thistle
Powder + 10% FeCl3	Reddish brown	Dark green	Black
Powder +Acetone + Methanol	Yellowish green	Light green	Green
Powder +10% Iodine	Dark golden rod	Light brown	Reddish brown

Table 7: It shows fluorescence analysis of powdered leaves of *Osimum basilicum*.

Powdered drug	Visible/day light	UV 254 nm	UV 366 nm
Powder + Methanol	Olive	Yellow green	Olive drab
Powder + 1% glacial acetic acid	Light yellow	Lavender bluish	Light gray
Powder +10% NaOH	Dark golden rod	Golden rod	Gold
Powder + dil. NH3	Dark brown	Brown	Light brown
Powder +1M H2SO4	Light golden rod yellow	Lavender bluish	Thistle
Powder +1M HCl	Light golden rod yellow	Lavender bluish	Thistle
Powder + 10% FeCl3	Dark brown	Reddish brown	Dark reddish brown
Powder +Acetone + Methanol	Olive drab	Light green	Green
Powder +10% Iodine	Peru	Brown	Medium purple

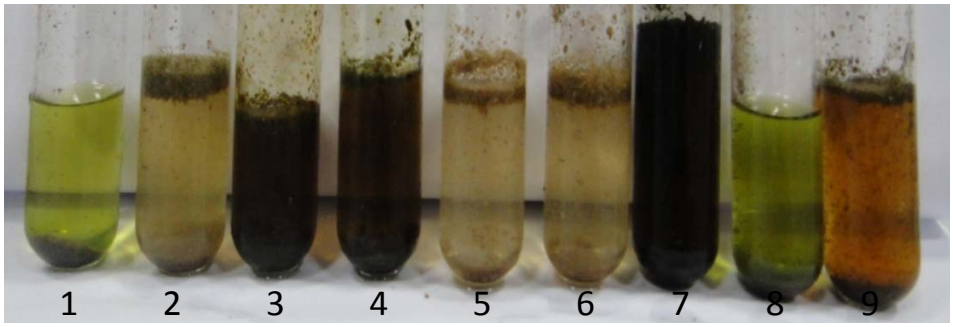

Fig 1: Fluorescent analysis of leaf powder of *Mentha Piperita* (Day light) (1. Methanol; 2. 1% glacial acetic acid; 3. 10% NaOH; 4. dil. NH3; 5. 1M H2SO4; 6. 1M HCl; 7. 10% FeCl3; 8. Acetone + Methanol; 9. 10% Iodine

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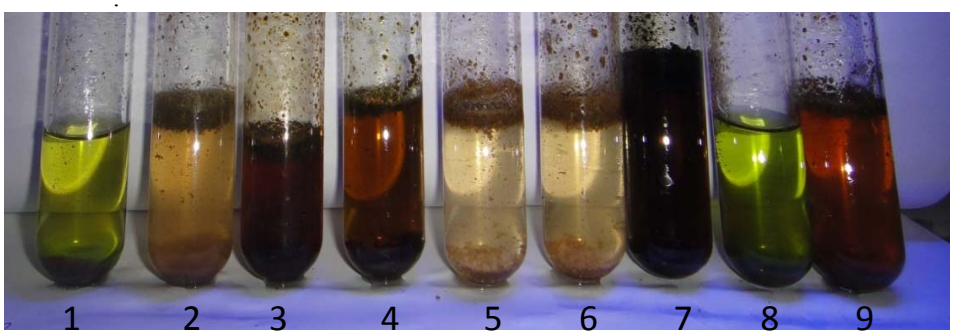

Fig 2: Fluorescent analysis of leaf powder of *Mentha Piperita* (254 nm) (1. Methanol; 2. 1% glacial acetic acid; 3. 10% NaOH; 4. dil. NH3; 5. 1M H2SO4; 6. 1M HCl; 7.10% FeCl3; 8.Acetone+Methanol; 9.10%Iodine.

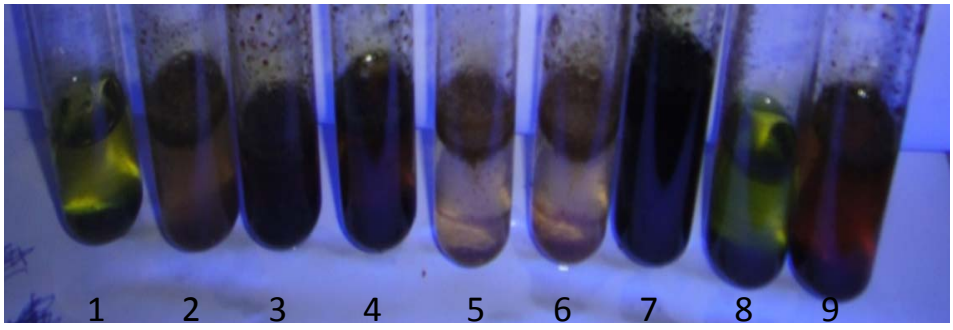

Fig 3: Fluorescent analysis of leaf powder of *Mentha Piperita* (366 nm) (1. Methanol; 2. 1% glacial acetic acid; 3. 10% NaOH; 4. dil. NH3; 5. 1M H2SO4; 6. 1M HCl; 7. 10% FeCl3; 8. Acetone + Methanol; 9. 10% Iodine.

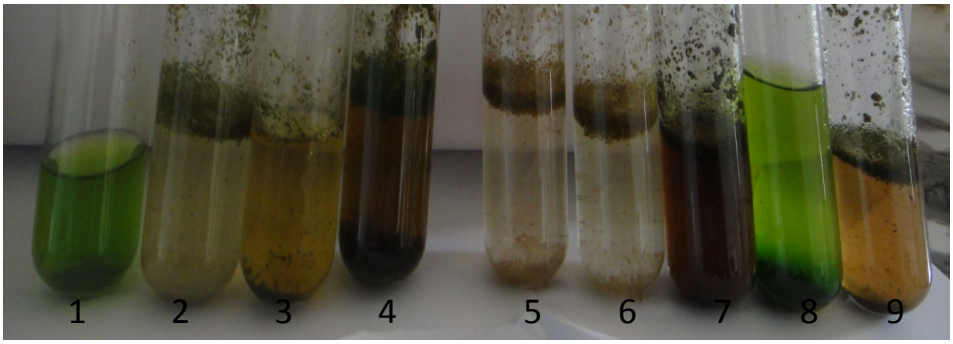

Fig 4: Fluorescent analysis of leaf powder of *Mentha longifolia* (Day light) (1. Methanol; 2. 1% glacial acetic acid; 3. 10% NaOH; 4. dil. NH3; 5. 1M H2SO4; 6. 1M HCl; 7. 10% FeCl3; 8. Acetone + Methanol; 9. 10% Iodine.

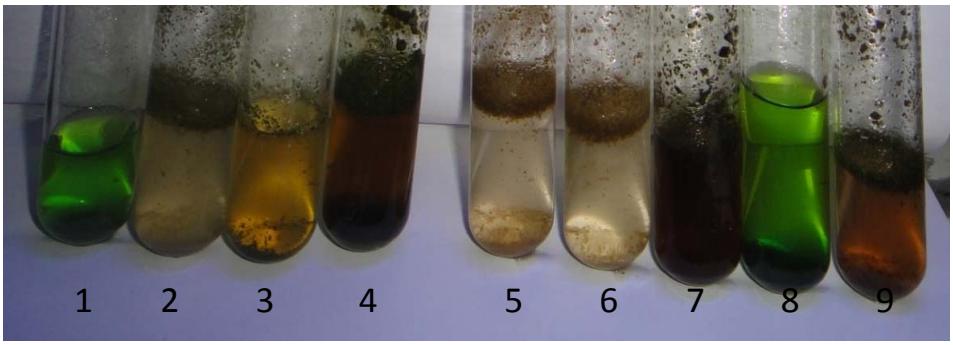

Fig 5: Fluorescent analysis of leaf powder of *Mentha longifolia* (254nm) (1. Methanol; 2. 1% glacial acetic acid; 3. 10% NaOH; 4. dil. NH3; 5. 1M H2SO4; 6. 1M HCl; 7. 10% FeCl3; 8. Acetone + Methanol; 9. 10% Iodine.

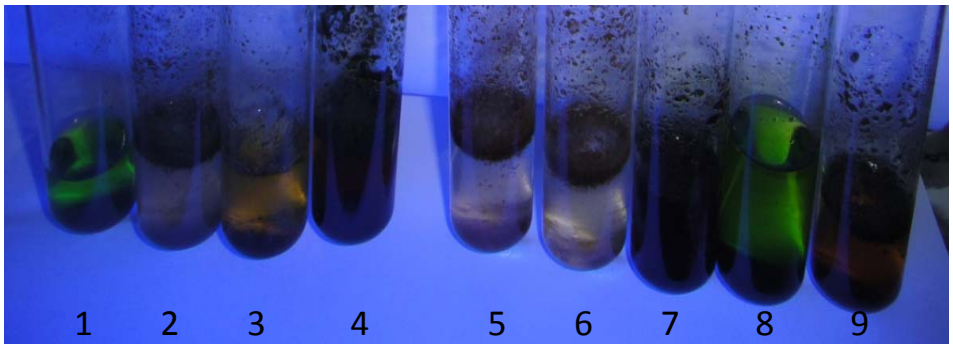

Fig 6: Fluorescent analysis of leaf powder of *Mentha longifolia* (366 nm) (1. Methanol; 2. 1% glacial acetic acid; 3. 10% NaOH; 4. dil. NH3; 5. 1M H2SO4; 6. 1M HCl; 7. 10% FeCl3; 8. Acetone + Methanol; 9. 10% Iodine.

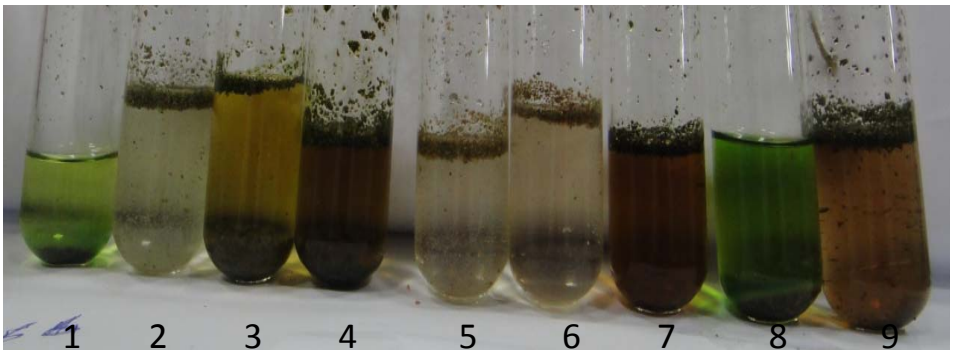

Fig 7: Fluorescent analysis of leaf powder of *Osimum basilicum* (Day light) (1. Methanol; 2. 1% glacial acetic acid; 3. 10% NaOH; 4. dil. NH3; 5. 1M H2SO4; 6. 1M HCl; 7. 10% FeCl3; 8. Acetone + Methanol; 9. 10% Iodine.

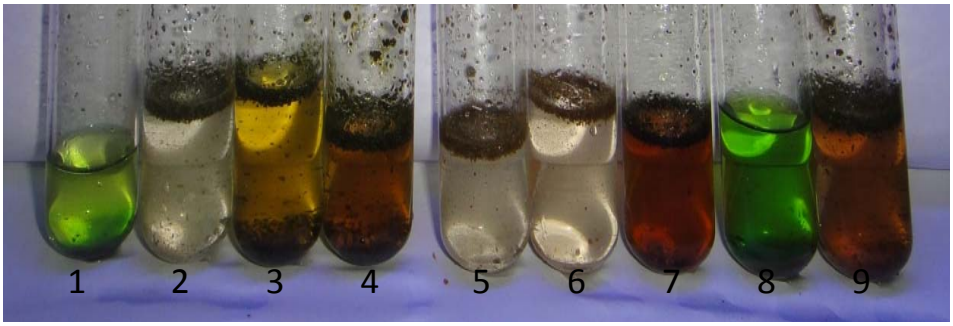

Fig 8: Fluorescent analysis of leaf powder of *Osimum basilicum* (254 nm) (1. Methanol; 2. 1% glacial acetic acid; 3. 10% NaOH; 4. dil. NH3; 5. 1M H2SO4; 6. 1M HCl; 7. 10% FeCl3; 8. Acetone + Methanol; 9. 10% Iodine.

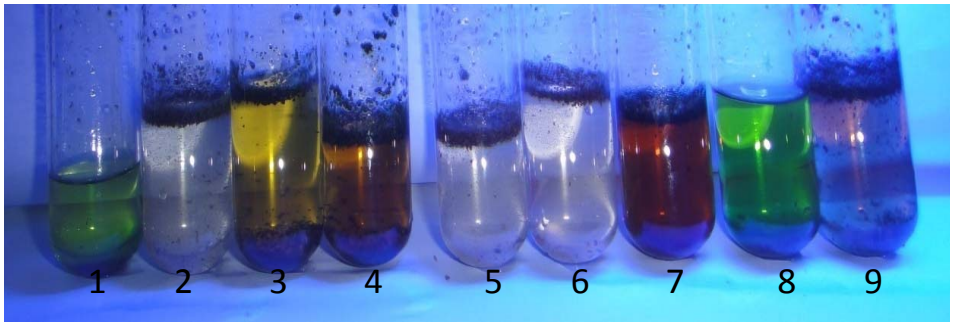

Fig 9: Fluorescent analysis of leaf powder of *Osimum basilicum* (366 nm) (1. Methanol; 2. 1% glacial acetic acid; 3. 10% NaOH; 4. dil. NH3; 5. 1M H2SO4; 6. 1M HCl; 7. 10% FeCl3; 8. Acetone + Methanol; 9. 10% Iodine.

The qualitative and quantitative study for rosmarinic acid was performed on Knauer HPLC instrument, C18 column and UV/Visible detector recording at 280 nm from leaves extract. An isocratic system was chosen to minimize the variation of the baseline and ghost peaks. Representative chromatograms are shown in (Figure 10) which indicated the presence of rosmarinic acid in *Mentha Piperita, Mentha longifolia and Osimum basilicum* which were supported by previously recorded data [19, 41]. The calibration curve was linear over the concentration range 5 to 200 µg/ml for standard solutions (Table 8). Correlation coefficients (r) was 0.998 showing a good linearity response for the method. LOD and LOQ were calculated 14.26 and 43.23 µg/ml and the results indicated that the proposed method exhibits a good sensitivity (Table 9). A repeatability test was performed in order to estimate intra-day variation in the peak areas and retention times. Highest value for RSD was 2.05 % (n = 3) proving that repeatability reproducibility of the method is satisfactory. Recovery ranged between 100.01–97.91 % showing that the presented methods had good accuracy (TABLE 10). A validated simple HPLC method for the qualitative determination of rosmarinic acid has been developed. Quantity of rosmarinic acid in *Mentha Piperita, Mentha longifolia and Osimum basilicum* are given in the (Table 11). On comparison of quantity of rosmarinic acid in three Labiatae species, results of study demonstrated that high quantities of rosmarinic acid present in *Osimum basilicum* (0.306%) followed by *Mentha longifolia* (0.208%) then *Mentha Piperita* (0.143).

Table 8: Calibration data for the proposed HPLC methods

Rosmarinic acid	
Concentration (µg/ml)	**Peak area 10^5 (mAU)**
5	2.1
10	4.06
50	17.83
100	39.16
200	80.66

Table 9: System suitability and validation parameters

Parameters	**Rosmarinic acid**
Linearity range (µg/ml)	5-200
Regression equation	Y=aX-b
Correlation coefficient(r)	0.998
Slope	0.4039
Intercept	0.7171
SE of intercept	0.7809
SD of intercept	1.7461
LOD (µg/ml)	14.26
LOQ (µg/ml)	43.23

Table 10: Precision and recovery data of HPLC methods

Compound	Amount added (µg/ml)	Amount recovered (µg/ml)[a]	Recovery (%)[a]	RSD (%)	
				Intra day[b]	Inter day[c]
Rosmarinic acid	50	49.60± 0.69	100.01 ± 0.69	1.39	1.77
	100	100.60 ± 1.82	99.01 ± 1.82	1.81	2.05
	200	199.81 ± 3.65	97.91 ± 3.65	1.82	1.86

[a]Mean ± SD (n=3)
[b]Samples were analyzed three times a day
[c]Sample were analyzed three times a day over two consecutive days

Table 11: Quantitative study of rosmarinic acid in three Labiatae species extract by HPLC

Compound name	RT	Concentration % *Mentha Piperita*	Concentration % *Mentha longifolia*	Concentration % *Osimum basilicum*
Rosmarinic acid	2.2	0.143%	0.208%	0.306%

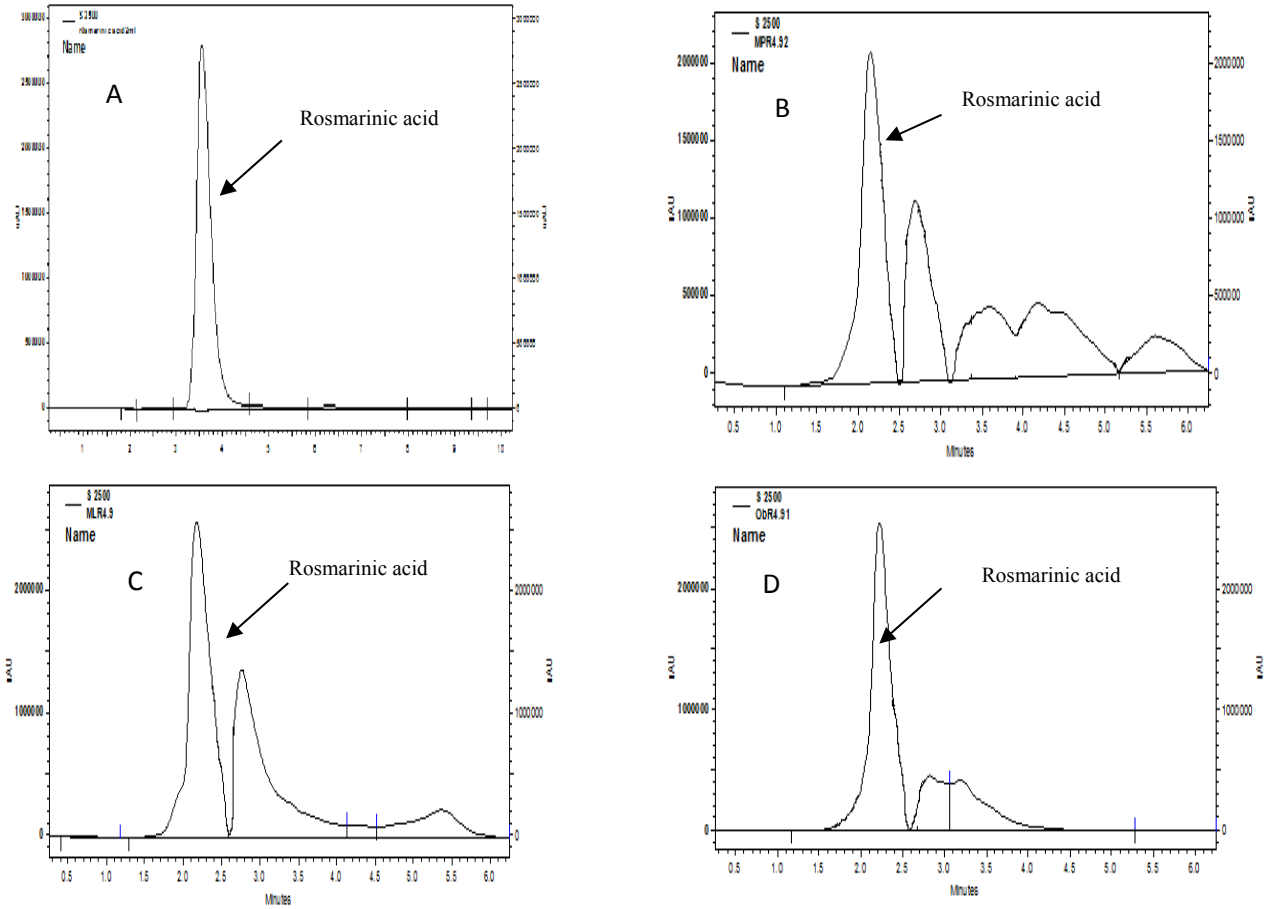

Fig 10: HPLC chromatogram of A-Standard rosmarinic acid; B- *Mentha Piperita*; C- *Mentha longifolia*; D- *Ocimum basilicum*

4. Conclusion

From the comparison study on *Mentha Piperita, Mentha longifolia and Ocimum basilicum* it was concluded that the highest percentage extract was yielded from *Mentha longifolia*, a number of important phytoconstituent natural product groups were detected on phytochemical screening in three labiatae species, rosmarinic acid was qualitatively and quantitatively determined by HPLC and demonstrated that maximum percentage of rosmarinic acid present in *Osimum basilicum.*

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