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Antimycobacterial Activity Some Different Lamiaceae Plant Extracts Containing Flavonoids and Other Phenolic Compounds

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1. Introduction

Mycobacterium tuberculosis is a pathogenic bacteria species of the genus *Mycobacterium*, first discovered in 1882 by Robert Koch, which causes tuberculosis (TB) (Ryan & Ray, 2004). The disease is characterized by symptoms such as sepsis, septic shock, multiple organ failure (Muckart & Bhagwanjee, 1997). It may spread to the central nervous system and cause TB meningitis, intracranial tuberculomas, or abscesses (Harisinghani et al., 2000; Hwang et al., 2010).

After the late 1980s, tuberculosis morbidity and mortality rates became a major health problem for industrialized countries (Raviglione et al., 1995; Heym & Cole, 1997). Multidrug-resistant tuberculosis (MDR TB) and extensively drug resistant tuberculosis (XDR TB) has become a common phenomenon, which cause drugs to be ineffective. MDR-TB results from either primary infection or may develop in the course of a patient's treatment. MDR TB is resistant to at least two first-line anti-TB drugs, isoniazid (INH) and rifampicin (RIF), which are most powerful anti-TB drugs; XDR TB is resistant to INH and RIF, plus fluoroquinolone and at least one of three injectable second-line drugs such as capreomycin, kanamycin, and amikacin. Treatment of XDR-TB is not possible by first-line anti-TB drugs, which are less effective, expensive and toxic; in addition treatment takes two years or more (WHO, 2011a; WHO, 2011b).

Mycobacteria are resistant to most common antibiotics and chemotherapeutic agents due to the mycobacterial cell wall composition of bacterial peptidoglycans (Slayden & Barry, 2000; Lee et al., 1996; Brennan et al., 1995), a lipophilic layer of long-chain fatty acids, and mycolic acids (Barry et al., 1998). The rich lipids of the cell wall has an important role in their virulence (Murray, Rosenthal and Pfaller, 2005). This structure provides a highly hydrophobic and efficient barrier to antibiotics and chemotherapeutic agents (Jarlier & Nikaido 1994). Thus, this cell wall composition restricts the choice of drug treatment. Compounds capable of blocking efflux pumps so that antibiotics can gain access to their targets are of obvious importance (Viveiros et al, 2003). Increased activity of existing efflux

pumps were caused by ineffective therapy of TB patients, which is develops bacterial resistancy to one or more drug. Recent researches showed that mycobacteria have multiple putative efflux pumps which is a key factor for gaining resistance (Braibant, 2000; De Rossi et al., 2002). In addition to, chromosomal gene mutation and then accumulation of these mutations also one of the origine of multidrug-resistant (Ramaswamy & Musser, 1998; Gillespie, 2002; Viveiros et al., 2003).

Some well-known drugs and their mechanism of actions affect bacteria in different ways. Streptomycin (STR) has been used to treat tuberculosis patients since the 1940s; INH was used to treat tuberculosis in the 1960s; RIF was first used at the beginning of the 1970s (Toungoussova et al., 2006); and ethambutol (EMB) was introduced in 1961 as a bacteriostatic first-line drug (Perdigão et al., 2009). RIF inhibits transcription to RNA and translation to proteins by binding its' beta subunit of RNA polymerase in bacteria; however, if bacteria produce a different beta subunit, they are not affected by the drug (O'Sullivan et al., 2005). STR is a protein synthesis inhibitor. STR interacts with a 30S subunit of ribosome and disrupts protein synthesis (Sharma et al., 2007; Springer et al., 2001). Its mechanism of action starts with binding tightly to the phosphate backbone of 16S rRNA in different domains and making contact with the S12 ribosomal protein; finally it causes misreading of the bacterial genetic code during translation (Carter et al., 2000; Hosaka et al., 2006). INH is activated by an enzyme, catalase-peroxidase, called KatG in *M. tuberculosis*. KatG, isonicotinic acyl and NADH form a complex that binds enoyl-acyl carrier protein reductase (InhA) and affects fatty acid synthase. The identification of an enoyl-acyl carrier protein (ACP) reductase plays a role in INH resistance named InhA. In this way, mycolic acid synthesis and cell wall development are inhibited (van Veen & Konings, 1998; Slayden & Barry, 2000; Suarez et al., 2009). As a result, when exposed to INH, Mycobacteria lose their acid-fastness and viability. Changes in the catalase-peroxidase gene (katG) and the inhA genes have been defined as one of the mechanisms of drug resistance in *M. tuberculosis* (Morris et al., 1995; Heym et al., 1995; Mohamad et al., 2004). EMB is a potent synthetic antimycobacterial agent that may cause optic neuropathy in patients (Kozak et al., 1998).

EMB has a bacteriostatic effect and interferes with mycolic acid synthesis, phospholipid metabolism, and arabinogalactan synthesis (Kilburn et al., 1977; Takayama & Kilburn, 1989) and affects nucleic acid metabolism (Forbes et al.,, 1965). EMB has synergistic actions, when combined with other agents, against *Mycobacterium avium* (Inderlied and Salfinger, 1995). TB is currently one of the most serious infectious diseases all over the world. Antimycobacterial drugs cause unpleasant side effects and trigger changes in the antibiotic target, thereby reducing the efficacy of drug therapies. Mycobacteria have recently increased their virulence and tuberculosis (TB) is the most lethal infection in the world. Between 1980 and 2005, 90 million cases of TB worldwide were reported to the WHO (World Health Organization) and over three in every thousand people die of TB, which is the highest rate in the world (Lall and Meyer, 1999). Yang et al. (2010) also reported that the prevalence of MDR-TB among the Chinese people has increased since 1985. The WHO stated, ‘‘The global incidence of TB was estimated to be 136 cases per 100,000 population per year in 2005. In addition, the WHO region of the Americas and the WHO African region represent a total of 8.8 million new cases of TB and 1.6 million deaths from TB every year” (World Health Organization, 2008a). There were 9.5 million TB-related child deaths globally in 2006 (World Health Organization,

2008b). Today, one of the most important global health problems is changes in behavior of TB, such as resistance to anti-TB drugs and the influence of the HIV epidemic (World Health Organization, 2008a). WHO Global TB Control (2009) reported that there were approximately 0.5 million cases of MDR-TB in 2007. The World Health Organization (2010) reported that there were 9.4 million new TB cases globally and approximately 1.7 million people died from TB. The organization also reported that 1.2 million people were living with HIV and 76% of these people were residing in the African region while 14% were living in the South East Asian region in 2009 (World Health Organization, 2010). In South Africa, TB is the most commonly notified disease and the fifth largest cause of death among the black population. The prevalence of TB continues to increase all over the world. Although the main reasons are known to be the human immunodeficiency virus (HIV) and the emergence of drug-resistant strains of TB (WHO, 2009), the other factors include poverty, drug addiction, inadequate health conditions and migration (Antunes et al., 2000; Merza et al., 2011). WHO reports (2011a) estimated that the risk of developing tuberculosis (TB) is between 20 and 37 times greater in people living with HIV than among the general population. In addition, infection with Human immunodeficiency virus type 1 (HIV-1) disrupts immunological control of *Mycobacterium* infections due to the loss of CD4+ T cells. Salte et al. (2011) reported that *Mycobacterium avium* is one of the most common opportunistic infections among AIDS patients. Snider et al. (1985) examined the transmission of MDR-TB strains from adult to child contacts and confirmed the progression of the disease by DNA fingerprint studies. INH-resistant strains caused much infection in children who were in contact with adults.

Mycobacteria are Gram-resistant non-motile pleomorphic rods with a waxy cell wall. These bacteria include high lipid content within the cell wall (Wilbur et al., 2009; Jackson et al., 2007), the complex lipids esterified with long-chain fatty acids. Myobacteria are referred to as acid fast Gram-positive due to their resistance to dilute acid and ethanol-based de-colorization procedures and their lack of an outer cell membrane. When they are stained using concentrated dyes, combined with heat, they do not give up the color by the dilute acid and ethanol-based de-colorization procedures (Ryan & Ray, 2004).

Some medicinal plants have been used to treat the symptoms of TB including *Acacia nilotica*, *Cassine papillosa*, *Chenopodium ambrosioides*, *Combretum molle*, and *Euclea natalensis* from Africa (Watt and Breyer-Brandwijk, 1962; Pujol, 1990; Lall & Meyer, 2001; Bryant, 1966).

Natural products are an important source of new chemical compounds and, hopefully, therapeutic agents for many bacterial diseases. Lall and Meyer (1999) reported antimycobacterial activity of *Euclea natalensis* (Ebenaceae), which is rich in naphthoquinones, against drug-sensitive and drug-resistant strains of *M. tuberculosis.* Gordien et al. (2009) studied two terpenes, sesquiterpene and longifolene; and two diterpenes, totarol and trans-communic acid, obtained from the aerial parts and roots of *Juniperus communis*. They reported that totarol showed the highest activity against *Mycobacterium tuberculosis* H37Rv and that longifolene and totarol exhibited the most activity against rifampicin-resistant variants. Phenolic compounds have some effects on microbial metabolism and growth, depending on their concentration and active compounds (Alberto et al., 2001; Reguant et al., 2000).

Many studies have shown that phenolic compounds inhibit the growth of a wide range of Gram-positive and Gram-negative bacteria (Davidson et al., 2005; Estevinho et al., 2008)

Flavonoids are the most common group of polyphenolic compounds. Flavonoids are plant secondary metabolites with a fused ring system, which are found as glycosides in plants. Of the well-known flavonoids, apigenin has a calming effect, while quercetin and kaempferol have a sedative effect (Jäger & Saaby, 2011).

In previous studies, flavonoids were reported to show antimicrobial (Cushnie & Lamb, 2006, 2011), anti-allergic (Chen et al., 2010), anti-inflammatory (Seo et al., 2000), and anti-carcinogenic (Lee et al., 2008) activities. Until 2004, it was suggested (Cushnie and Lamb, 2005, 2011) that their antibacterial efficacy was dependent upon cytoplasmic membrane damage by perforation (Ikigai et al., 1993), inhibition of nucleic acid synthesis (Mori et al., 1987) and disruption of energy metabolism due to NADH-cytochrome c reductase inhibition (Haraguchi et al., 1998). Currently, some other supporting mechanisms have emerged to indicate the role of flavonoids in antibacterial activity; these mechanisms include damage to the cytoplasmic membrane by generating hydrogen peroxide (Tamba et al., 2007; Kusuda et al., 2006; Sirk et al., 2008), inhibition of nucleic acid synthesis (Gradisar et al., 2007; Wang et al., 2010) and inhibition of ATP synthase (Chinnam et al., 2010). While Puupponen-Pimiä et al. (2001) reported that catechin, rutin and quercetin did not affect the growth of *E. coli*, Vaquero et al., (2007) reported that quercetin was the strongest inhibitor active against bacteria, dependent on concentration.

Lamiaceae, also known as mint, is a family of flowering plants that includes 250 to 258 genera and approximately 6,000 to 6,970 species across the world (Zomlefer, 1994; Mabberley, 1997). The family has a cosmopolitan distribution and contains many plant species with culinary and medicinal purposes; examples of the former are basil, mint, rosemary, sage, savory, marjoram, oregano, thyme, lavender, and perilla (Naghibi et al., 2005). The Lamiaceae family of plants have been used since ancient times as folk remedies for various health problems such as common cold, throat infections, acaricidal, psoriasis, seborrheic eczema, hemorrhage, menstrual disorders, miscarriage, ulcer, spasm and stomach problems (Takayama et al., 2011; Loizzo et al., 2010;. Ribeiro et al., 2010). Their constituents, particularly diterpenoids and triterpenoids, have been found to have antiseptic, antibacterial, anti-inflammatory, cytotoxic, cardio-active and other properties (Ulubelen, 2003).

In our previous studies, we tested more than 100 plant extracts, some of which showed antimycobacterial activity against *Mycobacterium tuberculosis*. In this study, in the light of our past experiences, we present a continuation of the testing of some of the plant extracts and the efficacy of their antimycobacterial properties.

2. Materials & methods

2.1 Plant materials

Aerial parts (herbs in the flowering stage) of plants, *Origanum acutidens* (Hand.-Mazz.) Ietswaart, *Origanum sipyleum* L., *Salvia viridis* L., *Salvia microstegia* Boiss&Bal., *Satureja boissieri* Hausskn. ex Boiss., *Stachys byzantina* C.Koch., *Stachys cretica* L., *Stachys cretica* subsp. *smyrnaea* Rech. fil., *Thymus syriacus* Boiss., and *Thymus cilicicus* Boiss&Bal.*(endemic)* were collected from different parts of Turkey between 2009 and 2010. The plants were identified by Assoc. Prof. Dr. F. Satil at Balıkesir University, Turkey. Voucher specimens were deposited in the herbarium of Balikesir University Department of Biology. Herbarium plant data, such as locality, altitude, and collection time and identification number of species are given in Table 1.

2.2 Preparation of plant extracts

The plants [*O. acutidens* (60 g), *O. sipyleum* (66 g), *Salvia viridis* (12 g), *S. microstegia* (100 g), *Satureja boissieri* (101 g), *Stachys byzantina* (65 g), *S. cretica* (37 g), *S. cretica* subsp *smyrnaea* (71 g), *T. syriacus* (44 g), and *T. cilicicus (*85 g) (endemic)], were air-dried at room temperature. Extracts of dried plants were prepared by the sequential extraction method (Chan et al., 2008) using 1 L of chloroform (CL), ethyl acetate (EA) and methanol (ME) at room temperature over a period of fifteen days. Finally, three extract fractions were obtained from each plants. The extracts were filtered through filter paper concentrated using a rotary evaporator and dried in vacuo at 40 °C. They were stored at −20◦C until use. The total yields from chloroform (CL), ethyl acetate (EA) and methanol (ME) extracts were *O. acutidens* (0.57, 0.74, 4.88g), *O. sipyleum (*2.14, 1.61, 5.50g), *Salvia viridis* (0.22, 0.15, 1.56 g), *S. microstegia* (8.17, 0.77, 6.70g), *Satureja boissieri* (4.05, 1.08, 8.82g), *Stachys byzantina (*7.69, 1.10, 5.15g), *S. cretica (*1.16, 0.59, 3.47g), *S. cretica* subsp. *smyrnaea (*1.92, 1.43, 6.92g), *T. syriacus (*1.80, 1.08, 2.85g), and *T. cilicicus (*2.37, 3.18, 5.35g) respectively. All stocks were stored at -20 °C. To conduct antimicrobial activity tests, samples were dissolved in dimethyl sulfoxide (DMSO) and prepared at a concentration of 100 mg/mL. All the extracts used were sterilized by passing through a syringe filter (Sartorius, Ø 0.22 µm.) before use.

2.3 Chemicals and samples

Gradient grade MeOH and acetonitrile were purchased from MERCK. Gradient grade water (18m) was prepared using a Purelab Option-Q elga dv25 system. All standard stock solutions (1 mg/mL) were prepared by dissolving each compound in MeOH. Standards, rosmarinic acid, trans cinnamic acid, and ferulic acid were purchased from Aldrich, caffeic acid and gallic acid from Sigma-Aldrich and all other chemicals used were obtained from Sigma. All solutions were filtered through a membrane filters (Sartorius, Ø 0.22 µm.) before injection into the capillary.

2.4 LC-MS conditions

Analyses were performed with Agilent LC-MS system (1200 LC with a single quadrupole) with ESI source negative mode. Source parameters were optimized to provide highest sensitivity. The source parameters are: Gas temperature 350 °C, drying gas flow 12 l/min, nebulizer pressure 50 psi, capillary voltage 3500 V., seperation was carried by a C-18 column (EC-C18 4,6x50mm 2.7um). Mobile phases are A: Water (5 mM ammonium formate+ 0.5 % formic acid) and B (acetonitrile). The gradient program is: 5 % B for starting condition and increased up to 45 % B in 1 min, hold 2 min, increase % B to 95 from 3 to 6 min, hold 1 min and decrease % B to 5% at final step. Total run time is 12 min. Injection volume is 5 µl. The detection was accomplished using MS SIM mode. Scan mode is also used. The LC–MS analysis was based in a method described by Pérez-Magariño et al. (1999).

2.5 Preparation of standards

Twenty standards were used for quantitative and qualitative determination: trans-cinnamic acid [(R_t) 4.98 min], ρ-coumeric acid (R_t 3.95 min), vanillic acid (R_t 3.79 min), gallic acid (R_t 1.89 min), caffeic acid (R_t 3.72 min), ferulic acid (R_t 3.99 min),), apigenin (R_t 4.83 min), naringenin (R_t 4.85 min), luteolin (R_t 4.43 min), epicatechin (R_t 3.67 min), quercetin (R_t 4.42

min), carnosic acid (R_t. 8.55 min), chlorogenic acid (R_t 3.59 min), rosmarinic acid (R_t 3.97 min), apigenin 7-glucoside (R_t 3.89 min), oleuropein (R_t 3.969 min), amentoflavone (R_t 5.16 min), naringin (R_t 3.83 min), rutin hydrate (R_t 3.69 min), hesperidin (R_t 3.85 min). Calibration concentrations were 1,4,5 and 20 ppm except one, apigenin 7-glucoside, was 0.9, 1.8, 4.5, 9, and 18 ppm and injection volume was 5 µL for all standards.

2.6 Organisms

The extracts were screened against four strain, *M. tuberculosis* H37Ra (ATCC 25177), *M. tuberculosis* H37Rv (ATCC 25618) and two-positive *M. tuberculosis* isolates obtained from patient from hospital, for antibacterial activity.

2.7 Preparation of *Mycobacterium tuberculosis* inocula

Bacterial suspensions of *M. tuberculosis* were prepared either from Lowenstein–Jensen slants or from complete 7H9 broth cultures. To prepare an inoculum that was less than 15 days old from a culture grown on Lowenstein-Jensen medium, a suspension was prepared in Middlebrook 7H9 broth. The turbidity of the suspension was adjusted to a 1.0 McFarland standard. The suspension was vortexed for several minutes and was allowed to stand for 20 min for the initial settling of larger particles. The supernatant was transferred to an empty sterile tube and was allowed to stand for an additional 15 min. After being transferred to a new sterile tube, the suspension was adjusted to a 0.5 McFarland turbidity standard by visual comparison. One mL of the adjusted suspension was diluted in 4 mL of sterile saline solution.

No	Genus species authority (Lamiaceae)	Locality	Altitude (m)	Collection Time	Herbarium Number
1	*Origanum acutidens* (Hand.-Mazz.) Ietswaart.	Between Elazig-Erzincan	1230	15.Jul.2009	FS 1605
2	*Origanum sipyleum* L.	Between Balıkesir-Savastepe	200	02.Jul.2009	FS1561
3	*Salvia viridis* L.	Balikesir-Cagis	160	02. Jun.2010	FS1560
4	*Salvia microstegia* Boiss&Bal.	Van, Gurpinar	1100	26.Jun.2009	FS 1559
5	*Satureja boissieri* Hausskn. ex Boiss.	Adiyaman-Yazibaşı village	980	20.Sep.2010	FS1562
6	*Stachys byzantine* C.Koch.	Bursa, Mezitler	860	08.Jul.2009	FS1602
7	*Stachys cretica* L.	Balikesir-Edremit, Kazdagi,	350	23.Jun.2009	FS1603
8	*Stachys cretica* subsp. *smyrnaea* Rech.fil.	Balikesir-Edremit, Kazdagi,	1260	17.Jul.2009	FS1604
9	*Thymus syriacus* Boiss.	Gaziantep-Burc forest	850	03.Aug.2009	FS1558
10	*Thymus cilicicus* Boiss&Bal.*(endemic)*	Antalya, Belek	1000	12.Jul.2010	FS1556

Table 1. Herbarium data of plants

To prepare *M. tuberculosis* inoculum using a BACTEC MGIT tube with positive growth, the positive tubes were used beginning from the day after the sample first became positive (day-1 positive), up to and including the fifth day (day-5 positive). The positive tubes that were older than five days were subcultured into fresh growth medium. Tubes that were day-1 and day-2 positive were used in the inoculation procedure for the susceptibility tests. The tubes that were between day-3 and day-5 positive were diluted using 1 mL of the positive broth and 4 mL of sterile saline solution; the 5 mL diluted suspension samples were used for the inoculation procedures.

2.8 Antimycobacterial activity test

Antimycobacterial bioassay was performed using the Microplate Alamar Blue Assay (MABA) method (Collins and Franzblau, 1997). MIC was recorded as the lowest drug concentration that prevented to turn blue to pink colour by adding Alamar blue. MBC was also recorded the minimum extract concentration that do not cause any color changing in cultures reincubated in fresh medium.

2.8.1 Determination of Minimal Inhibitory Concentrations (MICs) for *Mycobacterium tuberculosis*

Microplates were inoculated with the bacterial suspension (20 µL per well except for the negative control wells) and incubated at 37 °C for 6 days. Alamar blue (15 µL, Trek Diagnostic system) was then added to the bacterial growth control wells (without extract) and the microplates were incubated at 37 °C for an additional 24 hours. If the dye turned from blue to pink, (indicating positive bacterial growth) then Alamar blue solution was added to the other wells to determine the MIC values. All tests were performed in triplicate.

2.8.2 Determination of mycobactericidal activity

All the extracts prepared from aerial parts of plants, the herbarium data of these species shown in Table 1, were analyzed by LC-MS. The quantity of chemicals in the methanol extracts are given in Table 2. Chromatograms of phenols in all extracts were compared to chromatograms of standards (Figs. 1–3).

The plant extracts described above were used in mycobactericidal activity tests. Two-fold dilution series in triplicate sets of parallel microplate wells were used for each extract. To determine the minimum bactericide concentrations (MBCs), fresh Middlebrook 7H9 culture broth (185 µL) was transferred to each well. A fifteen microliter of an Mycobacterial suspension, from MIC concentration and higher concentration wells obtained from the MIC test described above was added to each well, in order to determine the minimum bactericide concentration (MBC).

Two microplate wells were used as positive and negative controls, and each test was repeated in triplicate. For the negative controls, 200 mL of fresh broth (Middlebrook 7H9 culture medium and OADC) was used. For positive controls, including 185 µL and inoculums from former positive control wells (15 µL) was used. After 24 hours of incubation and colour development using the Alamar blue solution, MBCs were recorded as the minimum extract concentration that did not cause any colour change in cultures when reincubated in fresh medium.

3. Results

3.1 Phenolics determined by LC-MS analyses

The ten samples and selected standards were analyzed by MS in ESI negative ion mode. Scan mode is also used. In this method, trans-cinnamic acid, p-coumaric acid, vanillic acid, gallic acid, caffeic acid, ferulic acid, apigenin, naringenin, luteolin, epicatechin, quercetin, carnosic acid, chologenic acid, rosmarinic acid, apigenin 7-glucoside, amentoflavone, oleuropein, naringin, rutin hydrate and hesperidin were chosen as standard phenolics to determine the phenolic structures of the samples according to ionization response in ESI mass spectrometry and chromatographic retention time.

Ion profile of negative ion electrospray LC/MS analysis experimental conditions are given above, from plants CL, EA and ME extracts is shown Fig. 1-2 and Table 2. Phenolics of samples were identified by comparing standard phenolic data such as retention times, main ions observed under fragmentation voltage of 80 Volt.

No	Phenolics	R_t min	[M-H]-	Fragment ions
1	*trans*-Cinnamic acid	4,984	147	80
2	ρ-Coumaric acid	3,95	163	80
3	4-Hydroxy-3-metoxybenzoic acid (vanillic acid)	3,747	167	80
4	Gallic acid monohyrate	1,893	169	80
5	Caffeic acid	3,724	179	80
6	Ferulic acid	3,991	193	80
7	Apigenin	4,831	269	80
8	(±)-Naringenin	4,859	271	80
9	Luteolin	4,433	285	80
10	(-)-Epicatechin	3,675	289	80
11	Quercetin	4,427	301	80
12	Carnosic acid	8,555	331	80
13	Chlorogenic acid	3,597	353	80
14	Rosmarinic acid	3,971	359	80
15	Apigenin 7-glucoside	3,896	431	80
16	Amentoflavone	5,169	537	80
17	Oleuropein	3,969	539	80
18	Naringin	3,834	579	80
19	Rutin hydrate	3,699	609	80
20	Hesperidin	3,853	609	80

Table 2. LS-MS characteristics of phenolic compounds

The major phenolic compounds of *T. cilicicus* CL extract were rutin hydrate and naringenin; for EA extract, rosmarinic acid and apigenin; and for ME extract, rosmarinic acid, oleropein, and apigenin.

The highest rosmarinic acid level within all plants were determined in *S. viridis* for CL extracts; in *S. boissieri* and *T. cilicicus* for EA extracts; *O. sipyleum S. byzantine* and *S. boissieri* for ME extracts.

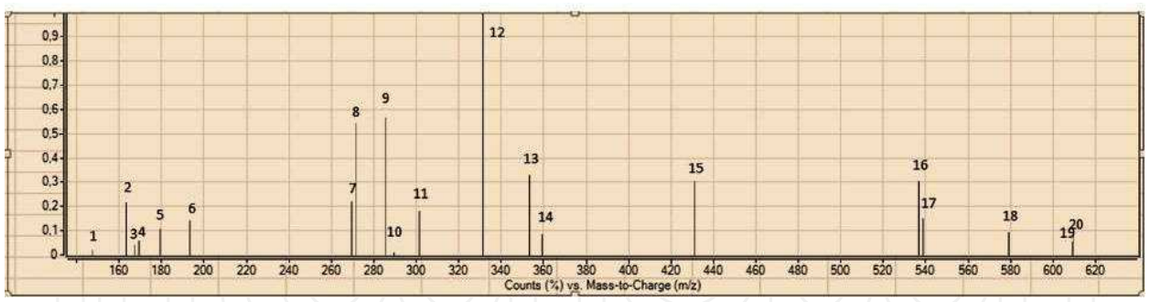


Fig. 1. ESI-MS Spectra of standard phenolics, 1; trans-cinnamic acid 2; p-coumaric acid 3; vanillic acid 4; gallic acid 5; caffeic acid 6; ferulic acid 7; apigenin 8; naringenin 9; luteolin 10; epicatechin 11; quercetin 12; carnosic acid 13; chlorogenic acid 14; rosmarinic acid 15; apigenin 7-glucoside 16; amentoflavone 17; oleuropein 18; naringin 19; rutin hydrate 20; hesperidin

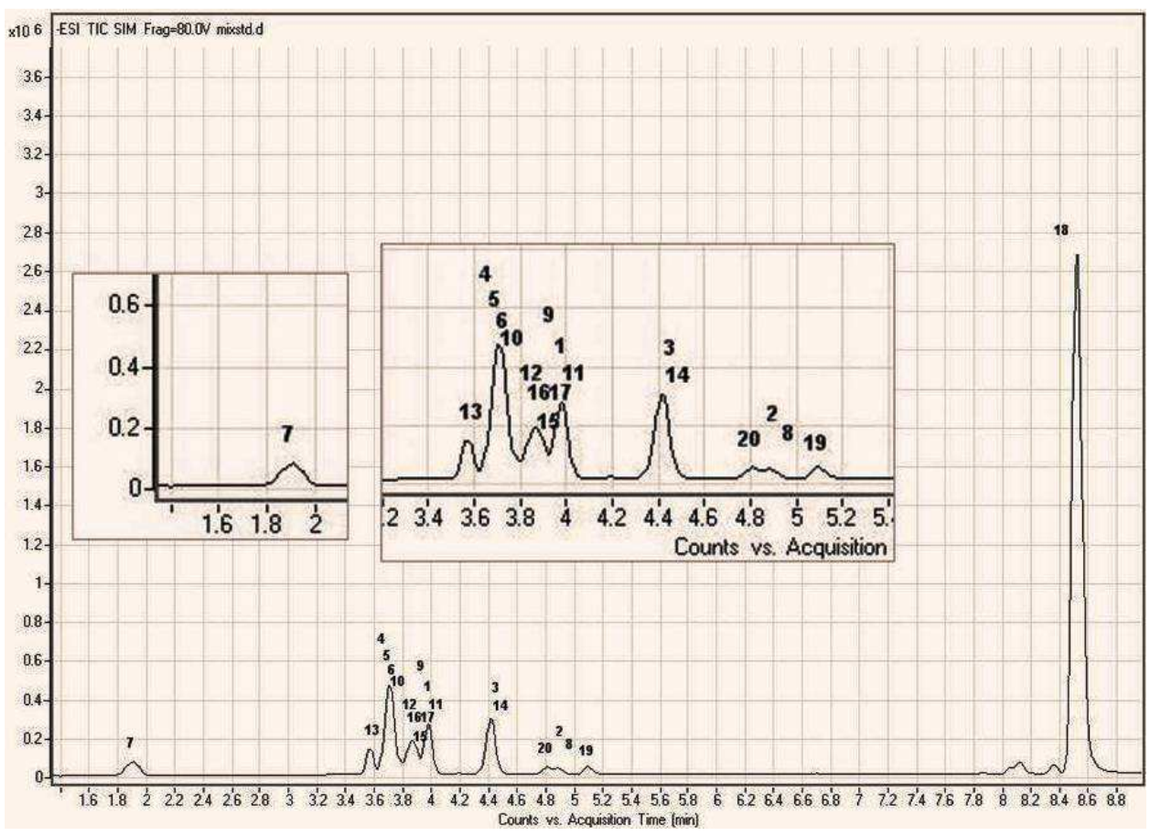
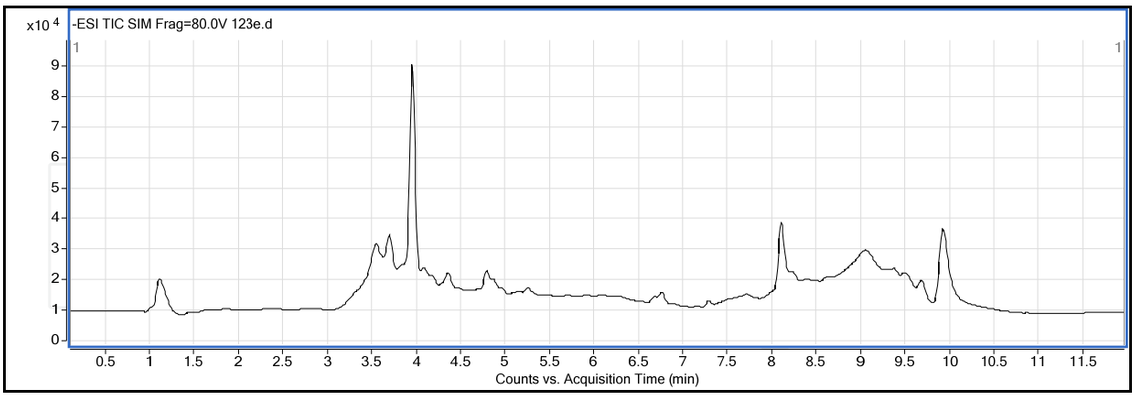


Fig. 2. ESI-TIC SIM chromatogram of standard phenolics, 1; trans-cinnamic acid 2; p-coumaric acid 3; vanillic acid 4; gallic acid 5; caffeic acid 6; ferulic acid 7; apigenin 8; naringenin 9; luteolin 10; epicatechin 11; quercetin 12; carnosic acid 13; chlorogenic acid 14; rosmarinic acid 15; apigenin 7-glucoside 16; amentoflavone 17; oleuropein 18; naringin 19; rutin hydrate 20; hesperidin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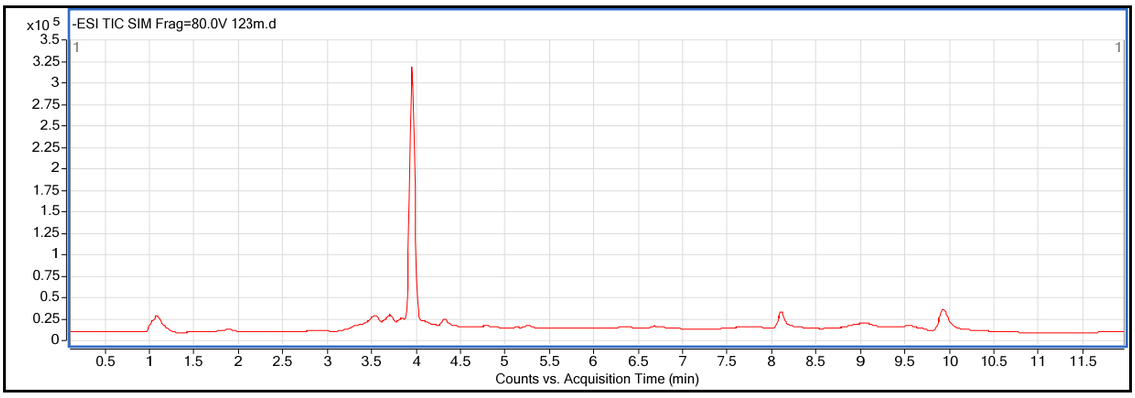
-ESI TIC SIM Frag=80.0V 123e.d
Counts vs. Acquisition Time (min)

A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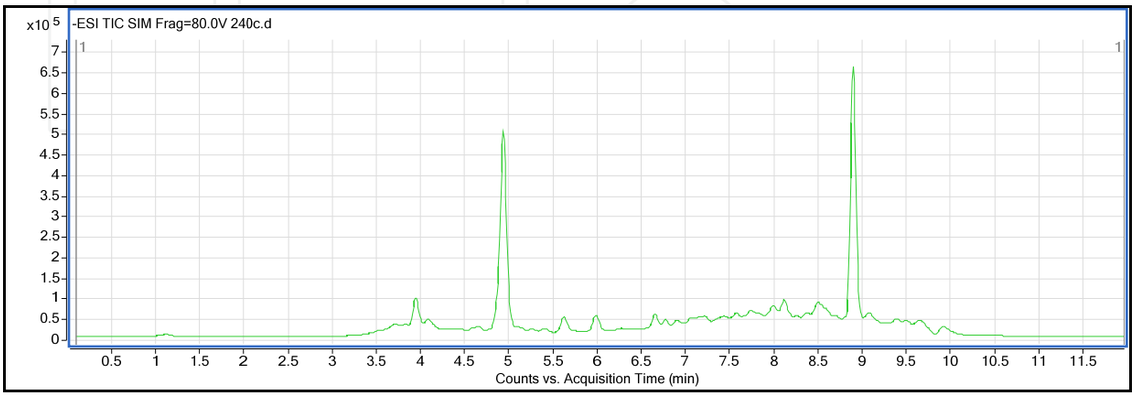
B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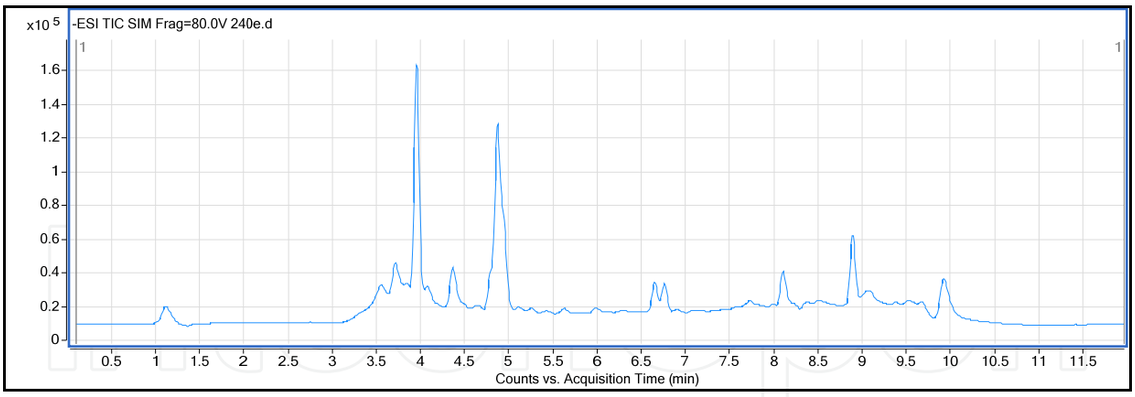
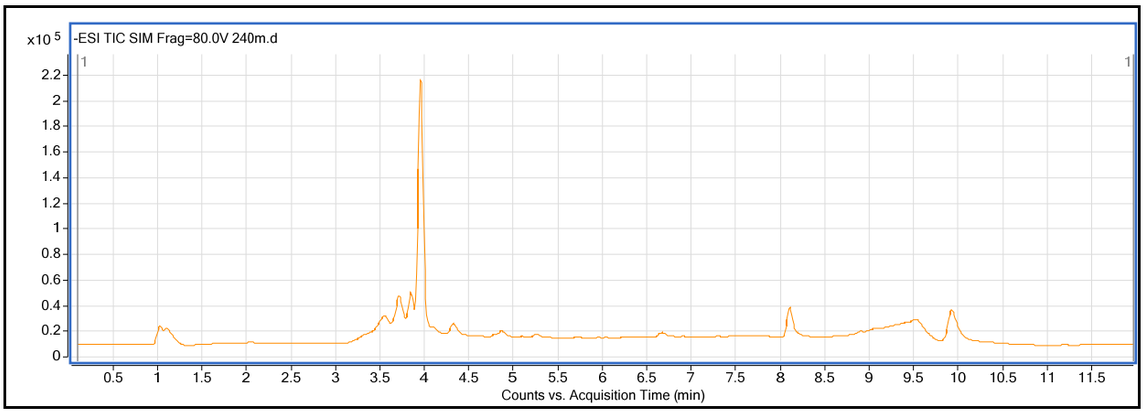
Counts vs. Acquisition Time (min)

C



D



E

Fig. 3. ESI-TIC SIM chromatogram of *O.sipyleum* A) EA extract, B) ME extract; *S. boissieri* C) CL extract D) EA etract and E) ME extract. (Not all chromatograms are included).

The major phenolic compounds for *T. syriacus* were rutin hydrate and naringenin for CL extract; rosmarinic acid, apigenin naringenin, and vanillic acid for EA extract; rosmarinic acid, apigenin, luteolin, and oleropein for ME extract.

The major phenolic compounds of *O. acutidens* determined by LC-MS analyses were rutin hydrate for the CL extracts; rosmarinic acid and oleuropein for the EA extracts, rosmarinic acid; and vanillic acid for the ME extracts. The major phenolics of *O. sipyleum* were rutin hydrate for CL extracts; rosmarinic acid and vanillic acid for EA and ME extracts. The major phenolics of CL extracts of *S. viridis* were rosmarinic acid and rutin hydrate; for EA extracts, oleuropein followed by rosmarinic acid; for ME extracts, rosmarinic acid, chlorogenic acid and hesperidin.

The major phenolic compounds for *S. microstegia* were rutin hydrate for CL extracts; apigenin, luteolin, and rosmarinic acid for EA extract; and rosmarinic acid, apigenin and luteolin for ME extracts. In *S. boissieri*, the major phenolics for CL extracts were apigenin and naringenin; for EA extracts, rosmarinic acid, naringenin and hesperidin; for ME extracts, rosmarinic acid and hesperidin (Fig 3). The major phenolic compounds in the CL extracts of

Pt	Ex	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
O. acutidens	Cl	52,87	*	*	*	104,13	*	*	10,32	*	51,28	*	*	*	*	*	*	*	*	*	*
	Ea	1029,88	*	*	60,54	106,20	34,67	*	47,17	223,42	435,04	61,65	354,02	*	*	*	23,35	723,56	*	*	329,69
	M	27306,44	130,50	*	85,19	208,98	230,09	*	*	345,49	589,89	*	*	280,85	*	150,24	94,88	95,31	*	*	182,66
O. sipyleum	CL	141,64	93,63	*	175,89	606,25	*	*	*	*	77,30	*	*	*	*	*	*	*	*	*	*
	EA	3431,07	131,42	*	293,80	569,54	*	*	*	*	784,83	*	*	*	*	*	*	263,22	*	*	82,75
	ME	58648,70	345,47	*	41,24	25,92	178,81	*	*	13,13	929,00	76,12	*	64,40	66,57	37,05	403,60	352,15	*	*	177,79
S. viridis	CL	409,20	24,37	*	122,01	153,62	*	*	*	*	81,76	5,55	*	*	*	*	*	51,39	*	*	*
	EA	898,43	284,22	*	218,39	607,4	*	*	*	*	76,22	*	*	*	*	*	585,95	1375,02	*	*	*
	ME	7978,30	393,66	*	237,32	246,56	32,53	*6,92	*	15,89	159,48	66,11	*	2316,58	809,02	120,00	1206,49	52,38	*	*	181,48
S. microstegia	CL	50,09	*	*	71,10	121,27	*	*	*	*	15,18	29,92	*	*	*	*	*	50,28	*	*	10,82
	EA	2927,63	111,29	*	194,532	551,48	*	*	*	1019,87	689,43	86,98	376,94	126,95	4883,57	*	96,67	180,79	*	*	5507,09
	ME	20759,50	119,37	*	425,612	167,26	255,86	*	*	318,971	217,88	25,93	*	96,10	1210,50	162,47	775,78	27,62	*	*	1315,47
S. boissieri	CL	52,39	30,28	*	*	122,80	313,40	*	*	*	165,17	364,24	429,47	*	*	*	*	53,72	38,11	*	1423,20
	EA	6380,13	153,53	*	*	575,62	*	*	*	*	465,64	635,25	3778,51	452,64	*	*	1692,63	*	*	*	789,85
	ME	34416,56	239,78	*	225,05	230,25	156,12	*	*	*	247,21	245,99	480,27	411,78	119,31	238,50	15878,94	648,55	*	*	139,60

S. byzantina	CL	75,69	*	*	58,31	165,10	*	*	*	*	*	17,71	*	*	*	*	*
	EA	1675,88	76,60	*	*	475,972	*	*	*	*	318,83	*	*	*	2130,60	107,53	354,41
	ME	42665,98	132,21	*	115,22	309,00	176,08	*	20,66	79,42	127,44	16,16	*	78,23	958,49	1477,40	2187,10
S. cretica	CL	69,61	22,81	*	*	126,01	*	*	249,72	*	209,24	52,39	*	*	*	*	*
	EA	115,74	24,26	*	*	109,84	*	*	*	*	121,07	45,85	*	26,12	*	*	29,81
	ME	598,99	117,17	*	94,41	274,14	*	*	*	*	160,79	20,18	19,44	8090,32	144,33	80,71	232,07
S. cretica subsp. smyrnaea	CL	117,24	*	*	*	111,94	*	*	38,58	*	255,04	14,99	*	*	*	*	*
	EA	144,56	23,26	*	35,22	145,60	*	*	61,36	208,65	509,90	52,02	14,63	481,18	23,51	*	123,65
	ME	114,23	142,70	*	61,72	397,43	*	*	*	25,53	225,07	15,59	*	13986,78	*	285,12	1066,13
T. syriacus	CL	48,34	52,92	*	89,55	112,60	*	*	*	*	210,73	133,71	137,15	*	*	*	17,33
	EA	2783,2	113,76	*	*	228,36	*	*	*	*	891,03	220,39	986,57	*	385,80	*	39,73
	ME	21952,28	333,75	*	40,87	301,74	166,01	*	*	13,56	352,18	109,07	202,50	210,00	1082,13	*	587,01
T. dilicius	CL	60,70	42,24	*	99,72	116,22	*	*	*	*	84,40	34,64	103,27	*	*	*	*
	EA	5120,55	258,34	*	*	1038,34	*	*	*	*	450,38	*	797,61	*	*	*	*
	ME	36529,24	301,38	*	41,87	260,59	853,26	*	*	38,55	334,22	15,11	200,71	15,32	1678,39	*	1025,33
Pt	Ex	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Pt: Plants; Exts: Extracts; CL: Chloroform; EA: Ethyl Acetat; ME: Methanol; 1; Rosmarinic Acid 2; Naringin 3; Querce
Rutin Hydrate 6; Caffeic Acid 7; Gallic Acid 8; Trans-cinnamic Acid 9; ρ-coumaric acid 10; Vanillic Acid 11; Ferul
Naringenin 13; Chlorogenic Acid 14; Luteolin 15; Apigenin 7-glucoside 16; Hesperidin 17; Oleuropein 18; Carnosic
Amentoflavone 20; Apigenin

S. byzantina were rutin hydrate; in the EA extract, apigenin, luteolin and rosmarinic acid; and in the ME extract, rosmarinic acid, hesperidin and apigenin.

In *S. cretica*, the major phenolics in the CL extracts were trans-cinnamic acid and vanillic acid; oleuropein, vanillic acid and rosmarinic acid for EA extract; and chlorogenic acid and rosmarinic acid for ME extract. In *S. smyrnaea*, the major phenolics were rosmarinic acid and rutin hydrate for CL extracts; vanillic acid and chlorogenic acid for EA extract; chlorogenic acid and hesperidin for ME extracts.

The highest rutin hydrate contents were determined in *O. sipyleum* and *S. viridis* for CL extracts; *T. cilicicus, S. viridis, and S. boissieri* for EA extracts*; S. cretica* subsp. *smyrnaea, S. byzantina*, and *T. syriacus* for ME extracts.

Gallic acid was determined only in methanol extracts of *S. viridis*. Carnosic acid was also found in CL extract of *S. boissieri*. Only the EA extracts of *S. microstegia*, S. *byzantina, T. cilicicus* and *T. striacus* included the highest level of apigenin.

Trans-cinnamic acid was found in extracts of four plants (*O. acutidens*, S. *byzantina*, and *S. cretica* subsp. *smyrnaea*). Quercetin and amentoflavone were not found. The highest level of chlorogenic acid was found in ME extracts of *S. cretica* subsp. *smyrnaea*, *S. cretica*, and *S. viridis*. Luteolin occurred mostly in EA and ME extracts in *S. microstegia*, S. *byzantina*, and *T. cilicicus*. The highest hesperidin level was found in *S. boissieri* ME extract and it follows *S. byzantina* ME extracts; In addition, ME extracts of *S. viridis* and *T. cilicicus* also included high levels of hesperidin. The highest oleuropein content was determined in ME extracts of *T. cilicicus*, followed by *T. syriacus* and *S. boissieri*. Within EA extracts*, S. viridis* and *O. acutidens* had the highest level of oleuropein.

3.2 Antimycobacterial activities, MICs & MBCs

The results were evaluated according to the literature. Extracts were tested against four mycobacteria strains (*M. tuberculosis* H37Ra, *M. tuberculosis* H37Rv, and two-positive *M. tuberculosis* isolates) obtained from hospital patients, to determine the MIC and MBC using the micro dilution method (MABA) against reference strains.

All plant extracts showed antimycobacterial activity (Table 4). Within all CL extracts, *O. acutidens, S. microstegia,* and *T. syriacus* exhibited the lowest MIC value of 0.4 mg/mL against *M. tuberculosis* H37 Ra. The lowest MBC value was 6.3 mg/mL for *O. acutidens* and *S. boissieri.* The MBC value for the rest of species was 12.5 mg/mL.

The MIC value of CL extracts against *M. tuberculosis* H37 Rv was 0.4 mg/mL for *S. boissieri*, followed by *S. cretica, T. syriacus,* and *T. cilicus* at MIC 6.3 mg/mL. Although all CL extracts showed bactericidal activity against *M. tuberculosis* H37 Rv, the prominent MBC values are 0.8 mg/mL for *S. boissieri* and 3.1 mg/mL for *T. syriacus*. For TB-positive isolates1, the featured results were 0.8 mg/mL MIC and MBC for *S. boissieri* and 3.1 mg/mL MIC and MBC for *S. cretica* subsp. *smyrnaea. S. boissieri* was also effective at the concentration 0.8 mg/mL as MBC.

In the EA extracts, the most prominent efficacy was observed for *T. syriacus* at MIC 0.8 mg/mL; MBC 1.6 mg/mL for *T. syriacus* against *M. tuberculosis* H37 Ra. *S. boissieri* is also

effective at MIC and MBC 6.3 mg/mL against two TB-positive isolates; *T. cilicus* showed the same effect at MIC and MBC 3.1 on *M. tuberculosis* H37 Rv.

Of the ME extracts, the most effective against *M. tuberculosis* H37 Ra was *T. syriacus* (MIC and MBC 3.1 mg/mL). *Stachys byzantine* also showed considerable efficacy at MIC 3.1 mg/mL against TB-positive isolates1. Among the other extracts, MIC and MBC values ranged between 6.3-12.5 and 6.3-25 mg/mL, respectively (Table 4).

Plants	Extracts	H37Ra		H37*Rv*		Isolate1		Isolate2	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Origanum acutidens	Cl	0.4	6.3	12.5	12.5	12.5	25	6.3	6.3
	Ea	6.3	6.3	6.3	6.3	3.1	6.3	3.1	6.3
	Me	6.3	6.3	25	25	3.1	12.5	6.3	12.5
O. sipyleum	Cl	3.1	12.5	25	25	12.5	25	6.3	12.5
	Ea	1.6	3.1	25	25	6.3	25	6.3	25
	Me	12.5	12.5	25	25	6.3	12.5	6.3	6.3
Salvia viridis	Cl	6.3	12.5	12.5	12.5	n.t	n.t	n.t	n.t
	Ea	1.6	3.1	12.5	12.5	6.3	12.5	6.3	12.5
	Me	12.5	12.5	25	25	6.3	25	6.3	25
S. microstegia	Cl	0.4	12.5	12.5	12.5	12.5	25	6.3	12.5
	Ea	6.3	12.5	25	25	3.1	12.5	3.1	12.5
	Me	12.5	12.5	12.5	25	6.3	25	6.3	25
S. boissieri	Cl	0.8	6.3	0.4	0.8	0.8	0.8	0.8	0.8
	Ea	1.6	3.1	12.5	12.5	3.1	3.1	3.1	3.1
	Me	12.5	12.5	12.5	12.5	6.3	12.5	6.3	12.5
Stachys byzantine	Cl	0.8	12.5	25	25	12.5	25	6.3	6.3
	Ea	12.5	12.5	12.5	12.5	3.1	6.3	6.3	12.5
	Me	12.5	12.5	25	25	3.1	12.5	6.3	25
S. cretica	Cl	0.8	12.5	6.3	12.5	12.5	12.5	6.3	12.5
	Ea	1.6	12.5	6.3	12.5	3.1	12.5	3.1	12.5
	Me	12.5	12.5	12.5	12.5	6.3	25	6.3	25
S. cretica subsp. *smyrnaea*	Cl	6.3	12.5	12.5	12.5	3.1	3.1	6.3	12.5
	Ea	6.3	12.5	12.5	12.5	3.1	12.5	3.1	12.5
	Me	25	25	12.5	12.5	3.1	25	3.1	25
Thymus syriacus	Cl	0.4	12.5	6.3	12.5	6.3	6.3	3.1	6.3
	Ea	0.8	1.6	3.1	3.1	3.1	12.5	3.1	12.5
	Me	3.1	3.1	50	50	6.3	12.5	6.3	25
T. cilicicus	Cl	0.8	12.5	6.3	25	6.3	25	6.3	12.5
	Ea	3.1	6.3	3.1	6.3	3.1	25	3.1	25
	Me	12.5	12.5	12.5	12.5	6.3	25	6.3	25

MIC:(mg/mL); MBC: (mg/mL;). n.t: not tested.

Table 4. Antibacterial activity of extracts of the plants as MIC (mg/mL) and MBC susceptibility test results against M. tuberculosis H37Ra (ATCC 25177) and *M. tuberculosis* H37Rv (ATCC 25618) obtained by MABA (*Microplate Alamar blue* assay) method.

4. Discussion

Lamiaceae plant extracts prepared by using different plant parts such as bark, stem, root, leaves, and fruits used in many biological activity studies. The extracts have been found to have antibacterial activity (Alma et al., 2003; Amanlou et al., 2004; Digrak et al., 2001; Bozin et al., 2006; Karaman et al., 2001), antifungal activity (Bouchra et al., 2003; Askun et al., 2008; Gulluce et al., 2003; Guynot et al., 2003; Souza et al., 2005), antimycobacterial activity (Ulubelen et al., 1997; Askun et al., 2009), antioxidant activity (Alma et al., 2003; Bozin et al., 2006; Mosaffa et al., 2006; Gulluce et al., 2003) and anti-inflammatory activity (Alcar´az et al., 1989; Jim´enez et al., 1986). Inhibitory effects of oregano components on some foodborne fungi were reported (Akgul & Kivanc, 1988). Askun et al. (2009) indicated that *Origanum minutiflorum* and *Thymbra spicata* methanol extracts showed antimycobacterial activity against *M. tuberculosis. T. spicata* var. *spicata* showed greater antimycobacterial efficacy (at MIC 196 µg/ml) than *O. minutiflorum* (MIC 392 µg/ml). They stated that a high quantity of rosmarinic acid might be responsible for antimycobacterial activity.

Recently, investigations of plant extracts are attracting great attentions due to their antibacterial properties (Payne et al., 2007; Rukayadi et al., 2009; Guzman et al., 2010). Previous studies showed that some plant extracts were conciderably effective against *M. tuberculosis*. Lall and Meyer (1999) reported that growth of *M. tuberculosis* is inhibited by acetone and water extracts of *Cryptocarya latifolia*, *Euclea natalensis*, *Helichrysum melanacme*, *Nidorella anomala* and *Thymus vulgaris.* They screened these active acetone extracts against H37Rv and a TB strain that was resistant to the drugs isoniazid and rifampicin. They reported that, while some plants (*Croton pseudopulchellus*, *Ekebergia capensis*, *Euclea natalensis*, *Nidorella anomala* and *Polygala myrtifolia)* exhibited MIC at 0.1 mg/mL against H37Rv, others (*Chenopodium ambrosioides*, *Ekebergia capensis*, *Euclea natalensis*, *Helichrysum melanacme*, *Nidorella anomala* and *Polygala myrtifolia)* inhibited the resistant strain at the same MIC value.

Many natural products have attracted much attention as potential antimycobacterial agents (Kinghorn, 2001; Gupta et al., 2010; Guzman et al., 2010). In recent years, there are pleny of researches on phenolics and their biological activities involved in the literature. Phenolic compounds obtained from plant extracts show great variety, with at least 8000 different structures (Bravo, 1998). Estevinho et al. (2008) showed that differences in the profiles of phenolic compounds are dependent of the flora predominance. Chun et al. (2005) reported that high phenolic and antioxidant activity was related to high antimicrobial activity against ulcer-associated *H. pylori*. Cinnamic acid is a naturally occurring phenolic compound that shows antimicrobial activity. Chen et al. (2011) showed that cis-cinnamic acid that was transformed from trans-cinnamic acid showed higher synergistic effect with INH or RIF against tuberculosis than trans-cinnamic acid.

Siedel & Taylor (2004) investigated plants, *Pelargonium reniforme* and *P. sidoides* (Geraniaceae) fractionation of n-hexane extracts against *M. aurum, M. smegmatis, M. fortuitum, M. abscessus* and *M. phlei*. They reported that linoleic acid was the most potent compound (MIC of 2 mg/l) against *M. aurum*. Koysomboon et al. (2006) isolated flavonoids from the stems and roots of the mangrove plant *Derris indica*. They reported antimycobacterial activity at MIC values between 6.25 and 200 µg/mL, except in two of ten known compounds. Askun et al. (2009) indicated that *Origanum minutiflorum* and *Thymbra spicata* var. *spicata* methanol extracts have antimycobacterial activity against *M. tuberculosis.*

T. spicata var. *spicata* was more effective (MIC 196 µg/ml) than *O. minutiflorum* (MIC 392 µg/ml). They suggested that a high quantity of rosmarinic acid might be one of the responsible constituent for the observed antimycobacterial activity. Gordien et al. (2009) studied two terpenes, sesquiterpene and longifolene; and two diterpenes, totarol and trans-communic acid, obtained from the aerial parts and roots of *Juniperus communis*. They reported that totarol showed the highest activity against *Mycobacterium tuberculosis* H37Rv and that longifolene and totarol exhibited the most activity against rifampicin-resistant variants. These results supported the ethnomedicinal use of this species as a traditional anti-TB remedy. Kuete et al. (2010) investigated the antimycobacterial activity of five flavonoids (isobachalcone, kanzanol C, 4-hydroxylonchocarpin, stipulin, amentoflavone) and determined their effects on preventing the growth of mycobacteria with MIC < 10 µg/ml on *M. tuberculosis*. In addition, isobachalcone and stipulin showed total inhibition of *M. tuberculosis* strain H37Rv. Bernard et al. (1997) mentioned that rutin showed antibacterial activity on *E. coli* by inhibited topoisomerase IV-dependent decatenation activity and caused *E. coli* topoisomerase IV which is essential for cell survival, dependent DNA cleavage (Bernard et al., 1997; Normark et al., 1969; Cushnie& Lamb., 2005). Huang et al. (2008) indicated that evidence that vanillic acid might be helpful to prevent of the development of the development of diabetic neuropathy by blocking the methylglyoxal-mediated glycation system.

Mandalari et al. (2007) also reported that, pair-wise combinations of eriodictyol, naringenin and hesperidin showed both synergistic and indifferent interactions that were dependent on the test indicator organism and their cell wall structure. Parekh and Chanda (2007) reported that the crude methanol extract of *Woodfordia fruticosa* contains certain constituents, such as tannins, with significant antibacterial properties, which enables the extract to overcome the Gram-negative cell wall barrier.

Kamatou et al. (2007) studied 16 South African *Salvia* species that are used in traditional medicine to treat microbial infection. They identified three species, *S. verbenaca, S. radula* and *S. dolomitica*, which exhibited MIC value at 0.10 mg/mL and which also showed antibacterial activity. Green et al. (2010) reported on the activities of acetone extracts of four plants, while *Berchemia discolor* showed efficacy at MIC 12. 5µg/mL, on H37Ra and 10.5µg/mL on the clinical isolate; the others (*Bridelia micrantha*, *Warbugia salutaris*, and *Terminalia sericea*) showed efficacy at 25µg/mL on both H37Ra and clinical isolate. The authors validated that these plants include mycobactericidal compounds that are effective against multidrug-resistant *M. tuberculosis.* Graham et al. (2003) presented an antimycobacterial evaluation of 216 species of Peruvian plants (in 63 families). Dichloromethane extracts from slightly more than half of the samples tested showed MIC value at 50 µg/ml concentration against *M. tuberculosis.* Billo et al. (2005) reported that methanolic extract of *Amborella trichopoda* fruits shows MIC value between 1 and 2.5 µg/ml, which was better than pyrazynamide and ethambutol in the same conditions.

Fabryet et al. (1998) reported that solvent extracts of plants with MIC values less than 8 mg/mL may be considered as antimicrobially effective. Gautam et al., (2007), shows that extracts of plant species from wide range of families and genera have exhibited significant in vitro antimycobacterial activities and this efficacy is interestingly compatible with the ethnomedicinal knowledge on plants.

Lechner et al. (2008) showed that myricetin was the most efficient intensifier of INH susceptibility in all tested strains by decreasing the MIC value of INH by as much as 64-fold; the second most effective compound was quercetin. Huang et al. (1980) tested two benzenoid compounds isolated from *Ardisia japonica* in-vivo on 201 patients infected with *M. tuberculosis* (Okunade et al., 2004). They reported that both compounds showed over 80% efficacy.

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6. Conclusion

In order to test the plant extracts, a potential drug resistant *M. tuberculosis* isolates was obtained from pulmonary tuberculosis hospital patients. The strains and isolates were then treated with plant extracts that are used for ethnopharmacological purposes. The level of the phenolic compounds and some flavonoids extracts were determined by liquid chromatography–mass spectrometry (LC-MS). The evaluation of results included the plants efficacy, their major phenolics, flavonoids and antimycobacterial activities. All plants extract showed antimycobacterial activity.

O. acutidens, S. microstegia, and *T. syriacus* were exhibited the lowest MIC value at 0.4 mg/mL against *M. tuberculosis* H37 Ra. *S. boissieri* and *T. syriacus* showed activity at MIC 0,4 mg/mL against *M. tuberculosis* H37 Rv. The prominent MIC and MBC values against *M. tuberculosis* H37 Rv were determined at 0,8 mg/mL for *S. boissieri* and 3,1 mg/mL for *S. cretica* subsp. *smyrnaea. S. boissieri* and *T. cilicicus* were effective against two TB-positive isolates.

The present work provides a preliminary insight into the effects of phenolics against *M. tuberculosis.* Plants of the Lamiaceae family have been shown to include new and effective constituents against *Mycobacterium tuberculosis*. Examination of these species, reported above, shows that rutin hydrate and vanillic acid were plentiful in all three extracts for these genera in Lamiaceae. All extracts of the *Origanum* species, *Salvia*, *Satureja, Stachys* and *Thymus* genera were rich in rosmarinic acid. With the exception of *S. viridis*, these species did not contain gallic acid.

We suggest that phenolics and naturally occurring flavonoids (polyphenols) are mainly responsible for antimycobacterial, cytotoxicological and mutagenic activity against *M. tuberculosis*. In some plants, (*O. acutidens*, *O. sipyleum, S. microstegia,* and *Stachys byzantine)* MIC and MBC values of CL extracts were in the same concentrations. These results might be due to several factors, such as a toxic effect caused by some compounds in the extracts. Liu et al. (2010) showed that a high concentration of cinnamic acid has toxic effects on soil bacteria. The other reason might be that the primary targets of the flavonoids have not been studied as widely in bacteria as in eukaryotes. While flavonoids affect enzyme systems such as prostaglandin, cyclooxygenase and lipoxygenase in eukaryotic cells, the bacteriocidal effect of the flavonoids might have caused the metabolic disorders on metalloenzymes by which their heavy metal atoms combine with flavonoids as ligand complexes in bacteria.

These strong complexes might disrupt the metabolism of organism (Havsteen, 2002). Flavonoids are also known to have mutagenic and antitumor activities (Hodec et al., 2002; Havsteen, 2002). Quercetin affects bacteria by inhibiting the catalytic activity of DNA topoisomerase I and II (Constantinou et al., 1995; Hodec et al., 2002). Quercetin was also reported by Xu et al. (2000) and Spedding et al. (1989) to have inhibitory effects on HIV1-protease and reverse transcriptase.

It is imperative to investigate the use of new, cheaper and efficient compounds to control *Mycobacteria tuberculosis*. Recent studies have examined plants and the effectiveness of their different types of extracts on *M. tuberculosis*. Advanced research into the structure and activity relationships among naturally occurring flavonoids will yield greater understanding of their pharmacokinetics and effects on mycobacteria metabolism according to their structure. It is of great importance to determine the mechanisms of action of flavonoids on *M. tuberculosis* .

7. References

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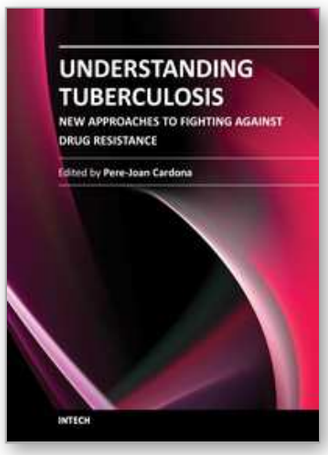

Understanding Tuberculosis - New Approaches to Fighting Against Drug Resistance

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In 1957, a Streptomyces strain, the ME/83 (S.mediterranei), was isolated in the Lepetit Research Laboratories from a soil sample collected at a pine arboretum near Saint Raphael, France. This drug was the base for the chemotherapy with Streptomicine. The euphoria generated by the success of this regimen lead to the idea that TB eradication would be possible by the year 2000. Thus, any further drug development against TB was stopped. Unfortunately, the lack of an accurate administration of these drugs originated the irruption of the drug resistance in Mycobacterium tuberculosis. Once the global emergency was declared in 1993, seeking out new drugs became urgent. In this book, diverse authors focus on the development and the activity of the new drug families.

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