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Phenolic content, antioxidant and antimicrobial activities of *Trachyspermum ammi* aerial parts growing wild in the north of Tunisia

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Abstract - Phenolic compounds make up one of the major families of secondary metabolites widely distributed in the plant kingdom, and they are found in foods of vegetable origin, constituting an integral part of our daily diet and in wild species as the major bioactive components that has shown to exhibit anti-carcinogenic, anti-microbial, antioxidant and anti-viral activities. The present work is devoted to the study of antiradical and antimicrobial activities of aqueous and organic extracts (Ethanol 70%) of *Trachyspermum ammi (T.ammi)* aerial parts. In order to compare its antioxidant activities, samples were extracted with 100% water or 70% ethanol. Their total phenolic content (TPC), flavonoid (TFC) and condensed tannin (CT) contents as well as DPPH and ABTS assays were measured. In addition, the antibacterial and antifungal activities of all extracts were also investigated against six human pathogenic bacteria and one fungal strain (*Candida albicans*). Our result has shown that, aqueous extract has demonstrated the best concentration in TPC (162.17 mg GAE.g^{-1} dw) and TFC (91.22 mg RE.g^{-1} dw) as compared to the ethanolic fraction. However, no significant difference (p>0.05) was observed in term of condensed tannin content (14.85 vs 14.23 mg CE.g^{-1} dw). Furthermore, the aqueous extract has shown the best antioxidant activity with IC_{50} values of 4.26 and 4.89 µg.ml^{-1} for ABTS and DPPH assays, respectively. The assessment of antimicrobial activity has shown that the remarkable inhibition of the bacterial and fungi growth was observed almost against all strains notably for the ethanolic extract for which all tested microbial strains was highly sensitive with an inhibition zone that significantly exceed that of the antibiotic tested under the same conditions. The results of our current study has demonstrated that extracts from *T.ammi* aerial parts can be used as a practical food additive or preservative in various food products and as a potential source of antimicrobial components.

Keywords: Antimicrobial activity, Flavonoids, Free radicals, Organic extract, polyphenols, *Trachyspermum ammi aerial parts*, Condensed tannin.

1. Introduction

The widespread resistance of microorganisms to antibiotics is emerging as a global health issue (Levy et al. 2002). The rate of resistance to these drugs is higher in developing countries as compared to developed countries because of extensive and indiscriminate use of antibiotics over the last few decades and people's ability to self-medicate without a prescription from a physician (Akram et al. 2007). Many efforts have, therefore, been made to discover new antimicrobial compounds from various kinds of sources such as plants, animals and microorganisms. A large number of plant products have long been used as a source of therapeutic agents worldwide (Gottlieb et al. 2002 ; Singh and Bhat 2003) and Herbal medicine have shown promising efficacy against multi-drug resistant strains of pathogens (Khan et al. 2009). Thousands of herbal secondary metabolites have been identified and it is estimated that other thousands are yet to be discovered. Since secondary metabolites from natural sources have been elaborated within living systems, they are often perceived as biological friendliness than totally synthetic molecules and have showing more "drug – likeness'' (Koehn and Carter 2005), making them good candidates for further drug development. Former studies (Mahran et al. 1991; Helle et al. 2004; Dash et al. 2011) have demonstrated species from *apiaceae* family are endowed with high biological activities and reported the importance of the investigation of non studied ones. *Trachyspermum ammi* L. is one of the *apiaceae* species; named also *Carum copticum* L. and known as ajwain (Bairwa et al. 2012). It is a highly reputable plant as a source of constituents with promising bioactivity to be exploited at pharmaceutical level. It is an annual herb up to 90 cm tall, native to arid and semiarid regions of the Mediterranean zone notably Egypt (Ashrafi 2002). It is also widely distributed and cultivated in the middle east (Iraq and Iran), Asia (Afghanistan, Pakistan) and India. The plant is a valuable herbal that has been used by human in a variety of ways. The fruits, are used to flavor and preserve foods, in perfumery and in medicine (Pruthi 1992). They possess, antispasmodic and carminative properties, and are used traditionally in the treatment of flatulence, diarrhoea, abdominal pains, and bronchial problems (Bairwa et al. 2012). *T.ammi* seeds are widely studied and former works have reported that they contain 2–5% of a essential oil, with thymol as the major component (35–60%), which is a strong germicide, antispasmodic and fungicide agent (Zarshenas et al. 2014). However, to the best of our knowledge, no study has been investigated the anti-radical and antimicrobial activities of Tunisian *T.ammi* extracts. Therefore, in the present study, extracts of *T. ammi*, which were prepared using solvents with different polarities, were tested to screen their antioxidant and antimicrobial activities against multi-drug resistant Gram negative (*Escherichia coli* (*E. coli); Salmonella thyphimurium (S.thyphimurium) , Pseudomonas aeruginosa (P. aeruginosa) , Klebsiella pneumonia (K., pneumoni) Enterobacter*), *and a gram positif strain (Staphylococus aureus) and a fungui : Candida albicans* (*C. albicans*), which is a promising new target for developing novel antibiotics. Each activity was evaluated by using two different tests: DPPH and ABTS for antioxidant potential and, agar disc-diffusion and micro-dilution methods for antibacterial and antifungual activities.

2. Materials and methods

2.1. Chemicals and reagents

All reagents were obtained from Sigma–Aldrich (St.Louis, MO, USA). The solvents were of appropriate purity. Peptone, NaCl, yeast exract, malt extract and agar were purchased from BIO-RAD (France).

2.2. Plant material

Aerial parts of *Trachyspermum ammi* was collected from Mateur-Bizerte (Tunisia, 36°42'45" N; 9°10'53" E; 340 m) in 2014. The plant samples were identified by Pr. Youssef Ammari from the Ecology forest laboratory, INRGREF-Tunisia. The voucher specimens of *Trachyspermum ammi* are deposited (TAMO 2014) at the INRGREF. The plant materials were washed under running tap water and air dried.

2.3. Extraction of the Phenolic Compounds

2.5 grams of *T. ammi* was then homogenized to fine powder in an analysis blender IKA A10, and extracted sequentially with ethanol (70%) and water for one hour. Each extract was filtered through Whatman No. 1 filter paper. It was then evaporated to dryness under vacuum at 40 °C using rotary

evaporator (Büchi, Switzerland). The residue was dissolved in 4 mL of ethanol (70%) and water, then filtered on a 0.45 µm filter (Gelman GHP) for the quantification of phenolic compounds and the determination of biological activities. Each extraction was performed in quadruplicate.

2.4. Determination of total phenolic content

The level of total phenolic content (TPC) was determined in a triplicate via the Folin–Ciocalteu reagent by using the method of Housseinian et al (2009). In brief, 200 µl of each extract were mixed with 1.9 mL of Folin–Ciocalteu reagent and were incubated for 5 min at 20 °C in the dark. Then, 1.9 mL of 7% sodium carbonate (w/v) solution was added. The mixture was allowed to stand in the dark at 20 °C for 2 h before measuring the absorbance at 760 nm using an UV– visible spectrophotometer. TPC values were determined from a calibration curve prepared with a series of gallic acid standards. Results are expressed as mg of the gallic acid equivalent per g of dry weight.

2.5. Determination of total flavonoid content

Flavonoid quantification was performed following the reported method of Rigane et al (2011, 2013) with some modifications. Briefly, 200 µL of aqueous or ethanolic extract were added to 75 µL of 7% $NaNO_2$ solution. After 6 min, 150 µl of 10 % $AlCl_3$ was added. After incubation at room temperature for 5 min, 500 µl of NaOH (1 M) was added to the mixture. The final volume was adjusted to 2.5 mL with water. The mixture absorbance was read at 510 nm in a UV-Vis Spectrophotometer. Rutin was used as a reference standard and results were expressed as mg of the rutin equivalent per g of dry weight. All determinations were performed in triplicates.

2.6. Determination of condensed tannins

The condensed tannins were assayed colorimetrically by the method proposed by Wissem et al (2009) with some modification. To 50 µl of each extract, 3 ml of 4% vanillin reagent, a 1.5 ml volume of 4% concentrated H_2SO_4 were added. The absorbances of samples were read at 500 nm after standing for 15 min at room temperature. Catechin (0-50 $\mu g.ml^{-1}$) was used as a standard in these experiments. The content of condensed tannins in the ethanolic and water *T. ammi* extracts was expressed as mg catechin equivalents per g of dry weight.

2.7. Quantification of antioxidant activity

To assess the antioxidant potential of bioactive compounds, the application of at least two different assays varying in their mechanisms of antioxidant action has been recommended (Schlesier et al. 2002; Rigane et al. 2011, 2013). The antioxidant capacity of the studied samples was determined applying the DPPH and ABTS assays.

2.7.1. DPPH scavenging activity

Organic extract samples were analyzed for their capacity to scavenge the stable DPPH radical according to Rigane et al (2011; 2013). The Inhibition (IC_{50}) of free radical DPPH was calculated in percentage: $IC_{50}= [(A_{blank} - A_{sample})/A_{blank}] \times 100$, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test extract), and A_{sample} is the absorbance of the test extract. The concentration of the test extract providing 50% inhibition (EC_{50}, expressed in $\mu g.mL^{-1}$) was calculated from the graph plotted with inhibition percentage against the extract concentration.

2.7.2. ABTS activity

The free-radical scavenging capacity was measured using the ABTS discoloration method according to the method of Thaipong (2006) and Rigane et al. (2011).. The radical scavenging activity (RSA) was calculated as a percentage of ABTS discoloration using the equation : $\%\ RSA=[(A_{ABTS}-A_S)/A_{ABTS}] *100$, where A_{ABTS} is the absorbance of the ABTS solution and A_S is the absorbance of the solution containing the extract. The result was expressed as EC_{50} value in µg/ml calculated from the graph of ABTS scavenging percentage activity against extract concentrations.

2.8. Antimicrobial activity

2.8.1. Test Microorganisms

Ajwain aqueous and organic extracts was tested against a panel of pathogenic microorganisms including *Staphylococcus aureus* ATCC 2319 (American Type Culture Collection, Rockville, MD, USA), *Escherichia coli* (ATCC8739), *Salmonella thyphimurium* (NCTC 6059) and *Pseudomonas aeruginosa* (ATCC 27850) as a reference strains and two clinical strains *Enterobacter* and *Klebsiella pneumonia* supplied by the microbiology laboratory of the regional hospital of Béja (Tunisia). The former strains were isolated, identified and characterized by conventional biochemical methods (Khan et al. 2009). A fungi Candida albicans were also included in the study. Bacterial strains were cultured overnight at 37 °C in blood agar plates (Oxoid, Basingstoke, UK). C. albicans was grown in Sabouraud Dextrose Agar (Oxoid). Tests were performed following the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2009). For paper disc diffusion method, a suspension of the tested microorganism ($1–2 \cdot 10^7$ cells per ml in saline and 10^6 per ml for Candida) was spread on the solid media plates using a sterile cotton swab. Sterile paper discs (6 mm in diameter) were placed on the surface of inoculated plates and spotted with 60 µl of each extract. The plates were incubated 24 h at 35±2 °C for bacteria and 48±5h for C. albicans. The diameters of zone inhibition (including the 6 mm disc) were measured with a calliper. A reading of more than 6 mm indicated growth inhibition. Tetracycline (10µg disc) was used as a positive control and discs with water and 70% ethanol as negative control. Each experiment was performed in quadruplicate.

2.8.2 Determination of MIC and MBC/MFC

The minimum inhibitory concentration (MIC) and the minimum bactericidal/fungicidal concentration (MBC/MFC) were determined using a broth micro-dilution method. Stock standard solutions at10 mg/mL in ethanol and water were prepared for organic and aqueous extracts respectively. Working solutions were prepared by dilution in micro-tubes at concentrations between 1000 µg/mL and 15.6µg/mL using nutrient medium as the diluents. Ethanol (50µl) was used as control and did not show any inhibitory activity. The bacterial suspensions were added in the micro-tubes at the concentration of 10^5-10^6 cfu/mL (colony forming units/mL). Each inoculum was prepared in its respective medium and density was adjusted to 0.5 Mcfarland standard (10^8 CFU/mL) and diluted to 1:100 for the broth microdilution procedure. The micro-tubes were incubated aerobically (37° C for 24 h and 30°C over 24h) . Bacterial and fungal growth was shown by the presence of turbidity in the micro-tubes. MICs were determined as the first tube in ascending order that did not show any turbidity. To confirm MIC and establish MBC/MFC, 20 µL of broth was removed from each well and inoculated on nutrient agar for bacteria and YPD plates for fungal strain. After aerobic incubation at 37° C overnight, the highest dilution that yielded no bacterial/fungal growth on solid medium was taken as MBC/MFC. Each experiment was performed in triplicate.

2.9. Statistical analysis

Statistical analysis of all data was performed using a Tukey's test followed by Dunn's post –hoc multiple comparison test (SPSS. v15). All data was expressed as mean ± standard deviation. A value of $p \leq 0.05$ was considered as statistically significant.

3. Results and discussion

3.1 . Yield of Prepared Extracts

Extracts of *T.ammi* aerial parts were obtained following a maceration solvent extraction procedure and yield of extracts obtained for each solvent was calculated separately (Figure 1). They varied over a range of 6.22 %–10.98 % (dry weight basis). The highest yield was obtained for water extract (10.98 ±0.874 %) followed by ethanolic (6.22±1.31%). A significant difference was observed among the solvents ($p < 0.05$). Such a wide variation is due to the different extraction solvent ratio and polarities. Previous reports have shown that the type of extraction solvent as well as the isolation procedures may have a significant impact on the yield of extraction from plants material (Pellegrini et al. 2007).

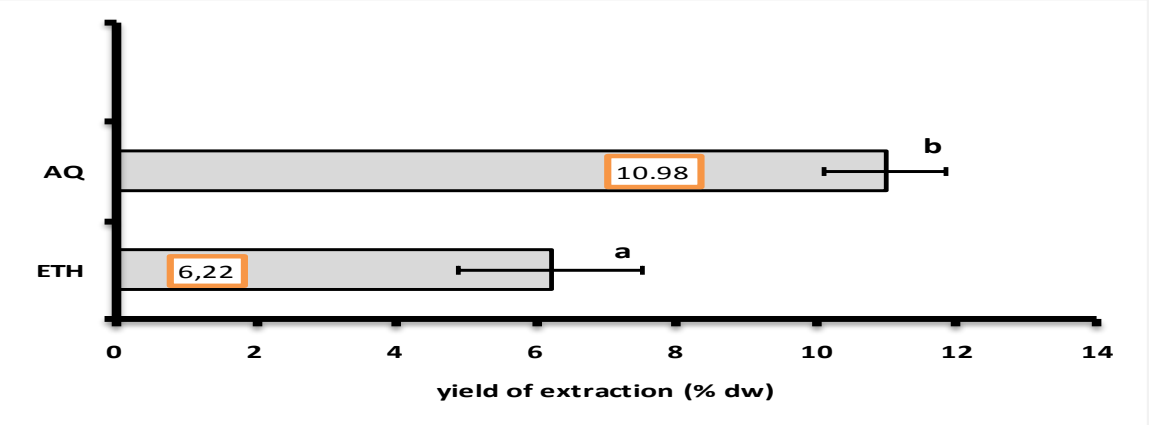

Figure 1. Extraction yield (% dry weight basis) of T.ammi aerial part. AQ, Aqueous extract; ETH, Ethanolic extract . Mean were significantly different at p<0.05

3.2. Colorimetric quantification of antioxidant compounds

Concerning the *T. ammi* growing in Tunisia, no data were available for their total phenol contents and for the first time, the *T. ammi* different extracts were studied regarding their total phenol, total flavonoid and condensed tannin contents.

3.2.1. Total polyphenol contents

Result examination showed significant differences in *T. ammi* total polyphenols when comparing the two extracts (Figure 2). The total phenolic content in aqueous extract of the *T. ammi*, as estimated by the Folin-Ciocalteu method, had the highest level (162.17 mg GAE.g^{-1} DW) followed by the ethanolic extract (89.9 mg GAE.g^{-1} DW). This important variability pointed out the solvent influence on the extractability of antioxidant compounds, in particular for phenolics. Indeed, several studies indicated that phenols are moderately polar compounds, so they tend to accumulate in the fraction of high-medium polarity such as ethyl acetate (Saada et al., 2014). On the other hand, Mahboba et al (2010) mentioned that water is almost universally shown as the practical solvent used to extract bioactive substances. In this regard, many reports declared that organic solvents (used in single or mixed forms) especially polar ones are most preferable for extraction of biologically active plant ingredients (Ferrero et al. 2007).

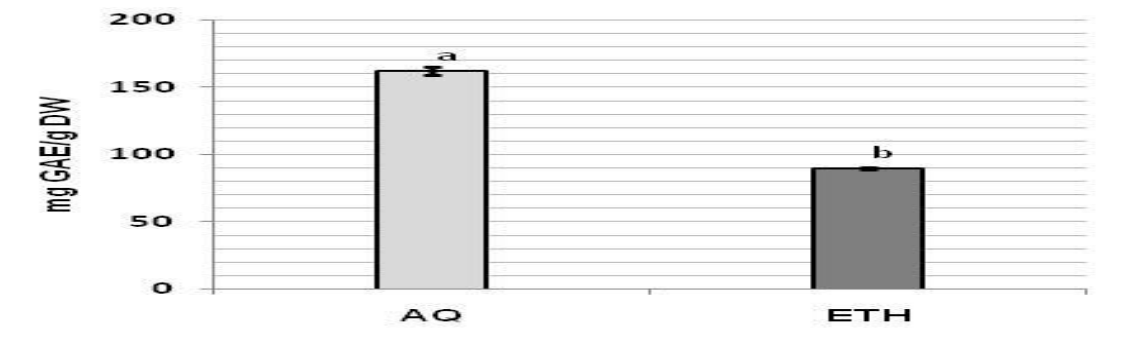

Figure 2. Total Phenolic content expressed in mg GAE.g^{-1} DW. Results are expressed as mean± standard deviation of 3 determinations. Means with different letters were significantly different at p<0.05.

3.2.2. Total flavonoid and condensed tannin contents

The result analysis exhibited significant differences in content of total flavonoid according to the solvent extracting power (Figure 3A). Yet, flavonoids represented a larger part of total polyphenol content in aqueous *T. ammi* extract (~ 57 %) than in the ethanolic one (~ 44 %). Interestingly, condensed tannin contents, which were in lower abundance than flavonoids, showed the same pattern

with non significant differences between the two extractable solvents; i.e; the aqueous extract as the richest one (14.85 mg RE.g^{-1} DW) as compared to the ethanolic fractions (14.23 mg RE.g^{-1} DW) (Figure 3B). Likewise, the significant variability between the fractions, in the phenolic compound contents, may be attributed to the extracting power of the solvent used and its chemical nature (organic or aqueous), structure, degree of polymerization and the interaction of these compounds with each other (Mahboba et al. 2010; Saada et al. 2014).

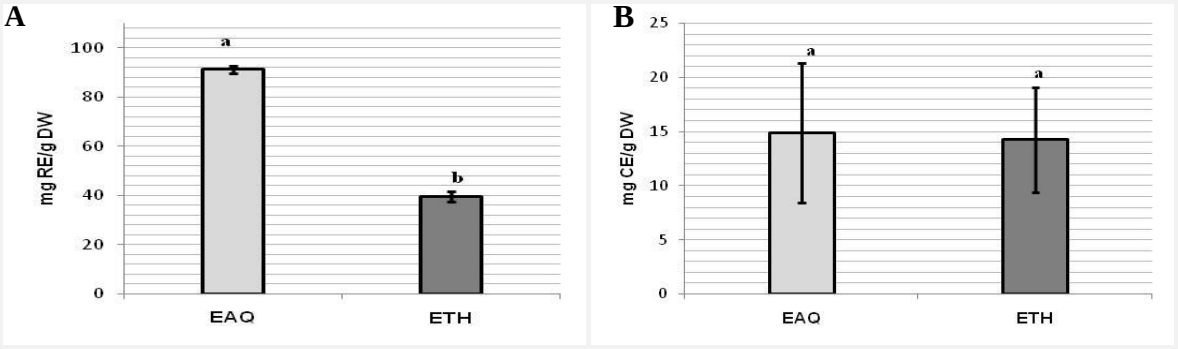

Figure 3. Total Falconoid (A) and condensed tannin content (B) expressed in mg RE.g^{-1} DW and mg CE.g$^{-1,}$ respectively. Results are expressed as mean± standard deviation of 3 determinations. Means with different letters were significantly different at p<0.05

3.3. Antioxidant activities (ABTS$^{\cdot+}$ and DPPH assays)

Several antioxidant methods have been proposed to evaluate the free radical scavenging capability of plant materials and to explain antioxidant mechanisms and actions. Among these, free synthetic radical scavenging like DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ are most commonly used for the evaluation of the total antioxidant behavior of extracts (Hajlaoui et al. 2010).

The results of scavenging activity on DPPH radicals and ABTS$^{\cdot+}$ are shown in Table 1. All the plant extracts of *T. ammi* showed a propensity to quench the free radicals. According to the DPPH assay, the lowest IC_{50} value was observed in AQ extract (4.89 ± 0.16 µg.ml^{-1}) and thus presenting the highest radical scavenging activity. Ethanolic extract presented higher IC_{50} value (128.82 ± 0.62 µg.ml^{-1}) and so having the lowest antioxidant activity. According to ABTS test, the higher value was observed for aqueous extract (4.26 ± 0.00 µg.ml^{-1}) while ethanolic extract of *T. ammi* exhibit lower level (3.63 ± 0.00 µg.ml^{-1}).

Table 1. Antioxidant activity of the extracts obtained from dried powder formulation of *T.ammi* aerial part extracts assessed using DPPH and ABTS assays and expressed as IC_{50} values (±SD)

	ABTS assay	**DPPH test**
Aqueous extact	4.26±1.52^{a}	4.89±0.16^{b}
Ethanolic extract	3.63±0.4^{c}	128.82 ±0.62^{d}

The results are presented in IC_{50} values (µg/ml), meaning that higher values correspond to lower radical scavenging activity. IC_{50} is the concentration of the extract that corresponds to 50% of of antioxidant activity for the ABTS and DPPH assays. Different letters (a-d) in the same line represent significant differences between samples (p<0.05).

Recently, Phenolics or polyphenols have received considerable attention because of their physiological function, including antioxidant, antimutagenic and antitumour activities (Othman et al. 2007). Phenolic compounds such as flavonoids, phenolic acids and tannins are widely distributed in plants (Li et al. 2006), which have gained much attention, due to their antioxidant activities and free radical-

scavenging abilities, that potentially have beneficial implications for human health (Govindarajan et al. 2007). It is worth noting that the antioxidant activity of the phenolic compounds were attributed to its redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers and have also metal chelating properties (Rice-Evans et al., 1995, Wissem et al., 2010; Rigane et al., 2011; 2013). Thus, it has been reported that free radical-scavenging activity is greatly influenced by the phenolic composition of the sample (Cheung et al. 2003). Our results corroborate those of Amensour et al (2009) who studied the total phenolic content and antioxidant activity of methanolic, ethanolic and aqueous extracts of leaves and fruits from Moroccan myrtle. These authors mentioned that leaf extracts showed higher antioxidant activities than berry extracts, while the overall antioxidant strength was in the order methanol > water > ethanol in leaf extracts and methanol > ethanol > water in berry extracts.

3.4. Antimicrobial activity

The antibacterial potential of *T.ammi* organic and aqueous extracts against six pathogenic bacteria and one fungi was assessed by the presence or absence of inhibition zones, the MIC and the MBC values (Table 2). The obtained results showed that both organic and aqueous extracts had great potential for antibacterial against the most evaluated bacteria and a anti-fungal potential against *candida albicans*. The maximal inhibition zones diameters for tested microorganisms were in the range of 15±1.1-22.75±3.25 mm for ethanolic extract (and 7.8±1.2-20±1 mm for aqueous extract. Blank discs (disc containing only solvent) produced no zone of inhibition indicating that the solvents themselves did not possess any antimicrobial effect. During the assay, Ethanol extract was found to have maximum zone of inhibition against both gram positive and gram negative strains including E-coli (21±2.16mm), k.pneumonia (13.25±3.04 mm) and staph aureus (18.25±3.94mm). Furthermore, c. albicans has shown a high sensitivity (Figure 4) to the former fraction with an inhibition zone of 22.75±3.25 mm which highly exceed that of the standard antibiotic (tetracycline). On the other hand, the pure aqueous fraction was selective and showed no effectiveness against S. aureus and enterobacter . Nevertheless, water extract was significantly (p<0.05) effective against five strains notably E-coli with an inhibition diameter of 19±4.32 mm and the multiresistant p.aeroginosa (Jian et al. 2005) that shown the best sentivity with an inhibition diameter 1.4 fold higher than that of ethanolic fraction and was significantly effective than that of tetracycline (18mm).

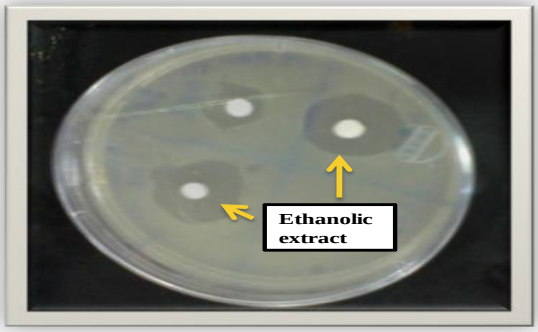

Figure 4. Zone of antimicrobial activity of T.ammi ethanolic extract against *C.albicans*

The aqueous fraction was also able to inhibit the growth of *candida albicans* in a dose dependent manner with a MIC value of 87.5µg.ml^{-1} and MBC of 175µg.ml^{-1}. It is therefore clear that the polarity of the extraction solvent highly modulate the effectiveness of the extract against pathogenic strains. The antimicrobial potential of aqueous extract may be related to the high level of phenolic components in *T.ammi* aerial parts. Cowan et al (1999) showed that several classes of polyphenols such as phenolic acids, flavonoids and tannins serve as plant defense mechanism against pathogenic microorganisms, insects, and herbivores. In fact, the site and the number of hydroxyl groups on the phenol components increased the toxicity against the microorganisms. However, for the ethanolic fraction, that showed less phenolic and total flavonoid content then the water fraction, the significant antimicrobial activity could be attributed to the action of antibiotic compounds or to the presence of metabolic toxins more soluble in ethanol than in water. Our result is in accordance with that of Dash et al (2011) , who

studied the antimicrobial activity of several extracts of the aerial parts of *centella asiatica* L. (Apiaceae) including polar and non polar solvents. These authors mentioned that ethanolic fraction was found to be most active and significant than corresponding organic extracts. However, according to Khan et al (2010) who reported that The petroleum ether fraction of *T. ammi* fruits showed best activity when compared to its other fractions including ethanol, petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone and methanol. Based on the previous work (Khan et al. 2010) the highly biological activities of *T.ammi* could be due to a synergistic effect of the different plant parts (leaves , stems and seeds). Our result is promising since both fractions are able to inhibit gram positive and gram negative bacteria. Indeed With the rise in the emergence of various multidrug resistant microorganisms and the changing patterns of susceptibility and the availability of new antimicrobial agents, continuous updating of knowledge concerning treatment of disease caused by such pathogens is required. Further studies will be necessary to understand the mechanisms of action underlying the effects of the extract and their active compounds.

Table 2 : Antimicrobial activity expressed as the diameter of the inhibition zone, minimum inhibitory (MIC) and bactericiidial (MBC) / Fungicidial (FBC) concentration against a yeast and Gram positive and Gram negative bacteria of T.ammi organic and water extracts.

		Ethanolic extract			**Aqueous extract**			**Tétracyclin[c] (10µg / disc)**
	Strains	**[d]ZI (mm)**	**MIC[a] (µg/ml)**	**BMC/FMC[b] (µg/ml)**	**ZI (mm)**	**MIC (µg/ml)**	**MBC/FMC (µg/ml)**	**D (mm)**
	E Coli	21±2.16[d]	87.5	260	19±4.32[d]	175	350	30
Gram negative bacilli	***Entérobacter***	18.75±2.06[e]	175	350	7.8±1.2[f]	175	350	28
	K.Pneumonia	13.25±3.04	175	350	na[m]	na	na	11
	S.typhimurium	18±1.29[g]	43.75	87.5	16±0.7[h]	43.75	87.5	25
	P. aeruginosa	15±1.1[i]	87.5	175	20±1[j]	43.75	87.5	18
Gram positive cocci	***S. aureus***	18.25±3.94	87.5	175	na	na	na	15
Fungi	***C. albicans***	22.75±3.25[k]	43.75	87.5	11±3.19[l]	87.5	350	17

E Coli: *Echerichia Coli;* ***S.typhimurium****: Salmonella thyphimurium,* ***P. aeruginosa*** *:Pseudomonas aeruginosa* , ***K.Pneumonia*** *:Klebsiella pneumonia ;* ***S. aureus*** *Staphylococus aureus,* ***C. albicans:*** *Candida albicans* . [d]ZI, disc diffusion method as recommended by NCCLS. Diameter of zone of inhibition (mm) including disk diameter of 6 m [a]MIC was considered as the lowest concentration of each extract showing a clear zone of inhibition. [b] No bacterial/fungal growth on solid medium was taken as MBC/MFC. [C]Tetracyclin was used as a positive control. [m]ma, not active . Means (±SD) within the same line are significantly different at <5%.

4. Conclusion

The present study provides the first investigation on phenolic content and biological activities of *T.ammi* aerial parts growing in Tunisia. We demonstrated that the significant antioxidant and antimicrobial activities of both extracts are highly dependent on the solvent polarity and these activities are due to the synergistic effect of different parts of the plant. Ethanolic fraction was able to inhibit all tested bacteria and fungi while water extract show a selective antibacterial activity based on their gram coloration with a significant inhibition of *p.aeroginosa* growth. This indicate that *T.ammi* is a new botanical source that could provide useful compounds for multidrug resistant microorganism and oxidative stress related diseases and suggest its use in the food industry as a potential natural source of antioxidants and in the pharmaceutical domain for the production of new antibiotics. The resulting information can also provide scientific support upon the increase of the used of biomass from herb in order to isolate new biologically active compounds.

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