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Wild Chamomile *(Matricaria recutita* L*)* from the Taounate Province, Morocco: Extraction and Valorisation of the Antibacterial Activity of its Essential Oils

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ABSTRACT

The resistance of micro-organisms, especially bacteria, to antibiotics available in the market is a worldwide problem. In view of this resistance, the main purpose of this research is to extract and study the antibacterial activity of *Matricaria recutita* essential oils (EOs) of the Taounate region. EOs of *M. recutita* was extracted by hydrodistillation of the Clevenger type (HC) and microwave-assisted hydrodistillation (MAH). EOs was extracted by MAH was analysed by gas chromatography coupled with mass spectrometry (GC-MS) in order to determine its chemical composition, and it was tested against four bacterial strains (*Escherichia coli, Staphylococcus aureus, Bacillus subtilus and pseudomonas aeruginosa*) using the disk diffusion method and the microdilution method. The MAH method provided the highest yield (0.75±0.013%). Furthermore, the results obtained revealed that *M. recutita*'s EOs contains 24 chemical compounds representing 98.49% of the total oils. The majority compounds were Chamazulene (26.11%) and Cis beta farnesene (11.64%). For the evaluation of antibacterial activity, EOs of *M. recutita* showed significant activity against *E. coli* (13.27±0.18mm), *S. aureus* (14.13±0.11mm), *B. subtilis* (15.2±0.13mm) and *P. aeruginosa* (13.07±0.09 mm) and with MICs of 8.33, 8.33, 6.25 and 8.33 µL/mL, respectively. The current results suggest that *M. recutita's* EOs contain certain antimicrobial properties, and can be used as antimicrobial agents in drugs for the treatment of infectious diseases.

Keywords: Extraction, Essential oils, *Matricaria recutita*, Antibacterial activity.

Introduction

Currently, in light of the resistance of microorganisms against the antibiotics available on the market, the most important means of treating several bacterial infections are medicinal and aromatic plants. These have various antibacterial compounds and do not cause harmful side effects.[1] Moreover, medicines based on plants are an essential source for combating serious diseases, especially in developing countries, and approximately 60-80% of the world's population still rely on these traditional medicines to treat common diseases.[2,3]
In Morocco, a large number of people use medicinal plants for their healthcare.[4] Thanks to its geographical position, topography, geology and climate, Morocco is a significant floristic area in North Africa.[5] Particularly because of its composite and differentiated environment, Morocco has a rich endemic flora with 978 taxa, which represent more than half of the endemic species of North African countries.[6]
Chamomile is originally distributed from Europe. It is used as a dietary supplement and antibacterial agent in Africa, Europe and Asia.[2] Also, *M. recutita* known as Asteraceae or Chamomile, is an annual plant and part of the Asteraceae family.[7]

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This species is considered to be one of the most famous medicinal plants commonly consumed as herbal teas and teas. Moreover, some statistics show that every day more than a million glasses of camomile tea are drunk.[8] The properties of *M. recutita* are numerous; therapeutic, cosmetic, agri-food, etc.[9] From one hand, this plant is used for the treatment of various diseases, including those related to inflammatory conditions,[2] bacterial and gastrointestinal infections,[10] muscle spasms,[11] etc. On the other hand, the oils extracted from chamomile can be used in cosmetics and perfumery.[2] This oil is known for its best quality and is generally known as "blue oil" due to the presence of certain chemical compounds, such as terpenoids, in particular chamazulene and bisabolol.[12] Chamomile is an economic asset. However, recent evidence has shown that the emergence of resistant microorganisms is increasingly restricting the effectiveness and potency of commercially available drugs.[13] This can lead to failure in the treatment and management of infections.[14] In addition to this, it has been estimated that the steadily increasing resistance to antibiotics will lead to around 10 million deaths every year by 2050.[15] To this effect, medicinal plants have proven to be more effective and cheaper than conventional drugs in the treatment of various diseases. Furthermore, they have shown less and even none of the side effects.[16]
In spite of all these studies and scientific literature, chamomile planted in Taounate area of Morocco has so far not been the subject of such a detailed study in terms of phytochemicals and antibacterial activities. We have therefore oriented our study in this direction with the aims of **(i)** extracting the essential oils from the aerial parts of *M. recutita* by two methods and then comparing these two methods, **(ii)** separating and identifying the chemical compounds by the GC-MS method, and **(iii)** evaluating *in vitro* the antibacterial activity of the essential oils of *M. recutita* against four pathogenic bacterial strains (*Escherichia coli* ATB: 57) B6N, *Staphylococcus aureus, Bacillus subtills* and *Pseudomonas aeruginosa*).

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Materials and Methods

Study region presentation

The study area, Tahar Souk (50 km from the town of Taounate, 35°1′22″ N, 4°8′27″ W), is one of the 28 rural communes of Taounate. It is situated in the south of the Moroccan Rif, and is part of the region of Fez-Meknes. It is bordered to the north by the province of Chefchaouen, to the south by Fez city, to the east by Taza city and to the west by Sidi Kacem city. The climate of the study area is Mediterranean, characterised by its humidity in winter and semi-aridity in summer.[17]

Plant material used

The aerial parts of *M. recutita* were collected from the Tahar Souk, Taounate, northern Morocco in the early morning of 1st April 2019. The plants were identified by the Botanist Amina Bari and given the voucher specimen no. 05-21-TT0015 before being deposited at the Herbarium of the Department of Biology, Laboratory of Biotechnology, Environment, Agri-Food and Health, Faculty of Sciences Dhar El Mahraz, Sidi Mohammed Ben Abdellah University, Fez, Morocco. The aerial part of *M. recutita* was dried in a shady place in a well-ventilated room at ambient temperature (25±3°C) before being ground into powder (Figure 1).

Extraction methods of the M. recutita's EOs

The extraction of the EOs from the aerial parts of *M. recutita* was carried out using two methods, hydrodistillation of the Clevenger type (HC) and microwave-assisted hydrodistillation (MAH).[18] In the first step, the results obtained were calculated and expressed as a percentage by weight of the dried plant material. In the second step, the essential oils obtained by microwaves were subjected to phytochemical profiling by gas chromatography coupled with mass spectrometry (GC-MS).

Hydrodistillation of the Clevenger type

This method consists of applying hydrodistillation to the dried aerial parts by means of a Clevenger-type apparatus. The distillation was based on the boiling of a mixture of water and 100g of the dried parts of *M. recutita* in a 1 L flask topped by a 60 cm long graduated column connected to a refrigerant.[19] The oils-laden vapours pass through the refrigerant and there are condensed, then recovered in a burette. The distillate contains the hydrolate and EOs. The EOs were collected with a propette and stored in an Eppendorf tube at 4°C and protected from light.

Microwave-assisted hydrodistillation

This method relies on a microwave oven as a power source. This MWDc (119 WH, 20 L, 2.45 GHz, Whirlpool, China) was connected directly to Clevenger appliance and a cooling system for continuous condensation of the distillate. First, the procedure consisted of mixing 100 g of samples of the dried aerial parts of *M. recutita* with water (200 ml) in a flask (2 L). Subsequently, the mixture is heated in a microwave oven at a power of 600 W and an extraction time of 20 min. The water and EOs vapour are then continuously condensed in a cooling system outside the microwave cavity and collected in a Clevenger receiving device. The excess condensed water was pumped back into the extraction flask in order to provide an environment of constant humidity.[20] As for the EOs extracted, it was dehydrated on anhydrous sodium sulphate and stored in a freezer until it was used for gas chromatography coupled with mass spectrometry (GC-MS).

Gas chromatography–mass spectrometry (GC–MS)

The EOs obtained by microwave-assisted hydrodistillation were subjected to chromatographic analysis using a TRACE GC ULTRA equipped with a non-polar VB5 (95% methyl, 5% phenyl), a capillary column (30 m x 0.25 mm diameter and 0.25 µm film thickness), directly linked to a mass spectrometer (Polaris Q) (EI 70 eV). The temperature of the injector and detector has been adapted to 250 and 300°C. As for the oven temperature, it has been programmed between 40 and 180°C at 4°C/min and at 20 °C/min for 180-300°C. The carrier gas was helium with a flow rate of 1 ml/min; the sample (1 µl) was injected in a splitless mode.

Antibacterial activity of EOs extracted from M. recutita

Bacteria strains

The antibacterial activity of the EOs extracted from *M. recutita* was evaluated against 4 Bacteria strains (*Escherichia coli (ATB:57) B6N*, *Staphylococcus aureus*, *Bacillus subtils* and *pseudomonas aeruginosa*) which were provided by the Laboratory of bacteriology Hassan II University Hospital Center of Fez, Morocco.

Disk diffusion method

The evaluation of antibacterial activity of the EOs extracted from *M. recutita* was performed by the disk diffusion method.[21] Petri dishes containing the NB (nutrient broth) medium have been sown with the four bacteria strains. Wattman paper disks (6 mm in diameter) were deposited on the surface of the inoculated media and impregnated with 20 µL EOs extracted from *M. recutita*.[22] The inoculated petri dishes were incubated at 37 °C in the dark. The inhibition diameter was determined after 24 hrs of incubation.[23]

Determination of the minimum inhibitory concentration (MIC)

The determination of the MIC of the *M. recutita*'s EOs against the four bacterial strains (*Escherichia coli* (ATB:57) B6N, *Staphylococcus aureus*, *Bacillus subtills* and *pseudomonas aeruginosa*) was carried out using the microdilution method in accordance with the technique described by Balouiri et al.[21] After 24 hrs incubation at 37°C.[23] The MIC is determined using the colorimetric method (TTC 0.2% (w/v)).[24]

Statistical analysis

Each experiment was done in triplicates. The results were designed and processed using GraphPad Prism 8.0.1 software. The statistical analysis of the results obtained was carried out using SPSS 20 software on the basis of an analysis of the mean (Student *t-test*) and an analysis of variance ANOVA at the threshold of $\alpha = 5\%$.[25]

Figure 1: The flowers of *M. recutita* during the drying process.

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Results and Discussion

Yield rate of M. recutita's EOs

Once the EOs had been extracted from the aerial parts of *M. recutita* by HC and MAH, the yield obtained was calculated according to the dry matter of the aerial parts of the plant used. The results obtained (Figure 2) showed that the yield obtained by MAH (0.75 ± 0.013%) was higher than that obtained by HC (0.3 ± 0.005%). Also, we can deduce that the yield of EOs extracted from the aerial parts of *M. recutita* changes values as a function of time and type of the extraction method used. We also noticed that a certain speed in reaching the extraction temperature of 100 °C for the first droplet of EOs, a high yield of EOs, lower energy requirements and a high purity of the EOs.[26] Therefore, MAH offers many advantages, such as lower energy costs, shorter extraction time and it is a cleaner method because it is not based on solvents and doesn't generate residues.[27]

Studies that have been carried out with different research projects have revealed that our yield rate is higher than that of Hajjaj *et al*[28] who have obtained a yield of 0.4%, and lower than that of Kazemi[29] who has obtained a yield of 0.82% and Mahdavi *et al*[30] who has achieved a yield of 1.27%. The difference in yield, especially with the results of Mahdavi et al[30] is explained by the fact that the EOs were extracted from the flowers. Consequently, we found that the EOs are mainly concentrated in the flowers. Indeed, the yield of EOs from the same plant varies according to a number of factors, including geographical and climatic conditions, the collection period, the parts of the plant studied, the techniques and the conditions of EOs extraction, etc.[31]

Chemical Composition of EOs

The chromatographic analysis provided information on different components of the EOs extracted from *M. recutita* and enabled the obtained levels to be compared with the standard ranges available. These components were listed according to retention times (RT) in ascending order. The EOs of the aerial parts of *M. recutita* obtained by microwaves consists of 24 compounds (Table 1 and Figure 3), representing 98.49% of the total oils. These oils are dominated by Chamazulene (26.11%), followed by Cis beta farnesene (11.64%), Eucalyptol (8.19%), Coumarine (6.01%), Trans-caryophyllene (5.95%), Galaxolide (5.31%), *α*-Cedrol (4.81%), Camphor (4.33%), Thymol (3.49%), 2-*α*-pinene (3.31%), Benzoic acid (2.92%), Lucenin 2 (2.82%), Camphene (2.62%). In addition, there are other constituents with very low levels such as *β*-Pinene (0.31%) and Acide *α*-linolenique (0.14%).

However, these results are similar to those of some previous studies from worldwide on *M. recutita's* EOs. In fact, the main component of all these oils is sesquiterpenes. As far as the Moroccan *M. recutita's* EO is concerned, it is dominated by Chamazulene, Cis-*β*-farnesene, Eucalyptol and Coumarin.[28] As for Egyptian *M. recutita*, the majority constituents of its EOs were α-Bisabololol oxide A, B, *α* -bisabolol, and *β*-farnesene.[32] As for the Spanish *M. recutita's* EO, it is rich in Bisabolol A oxide, *α*-bisabolol, *α*-bisabolol B oxide, and E-*β*-farnesene.[33] For the Ethiopian *M. recutita*, its EO is marked by the abundant presence of Bisabolol B oxide, Chamazulene and Indicycloether.[34] Concerning *M. recutita's* EO from southern Iran, it is mainly composed of α-bisabolone A oxide, α-bisabololol A oxide and Chamazolene.[35] According to Stanojevic *et al*,[36] the EO of chamomile from Bosnia and Herzegovina is generally composed of *β*-farnesene, *α*-farnesene, chamazulene, α-bisabololol and its oxide. According to Formisano et al,[37] the majority compounds of the EO of *M. recutita* from south-central Italy are Chamazulene, Cis-tonghaosu, Spathulenol, α-bisabolol oxide B and α-bisabololol oxide A. According to the studies of Rezaeih *et al*[38] on the vegetable oil of Turkish *M. recutita*, bisabolone oxide, bisabololol oxide A, en-yn dicycloether and bisabolol oxide B took precedence over all other components. Lastly, the results of this study focused on the EO of *M. recutita* from certain European countries, showed that the predominant components are bisabolol A oxide, *α*-bisabolol, bisabolol B oxide, cis-enynebicycloether, bisabolon A oxide and chamazulene.[39] Thus, the chemical composition of the same variety of plant varies from one country to another. In fact, several factors affect the chemical properties of the plant, among others such as, genetic diversity, the parts used for the isolation of EOs, climatic conditions, etc.[29,40] Furthermore, the bioactive compounds present in EOs in general can vary qualitatively and depend quantitatively on the seasonal sampling period, growing conditions, environmental factors and geographical location.[41]

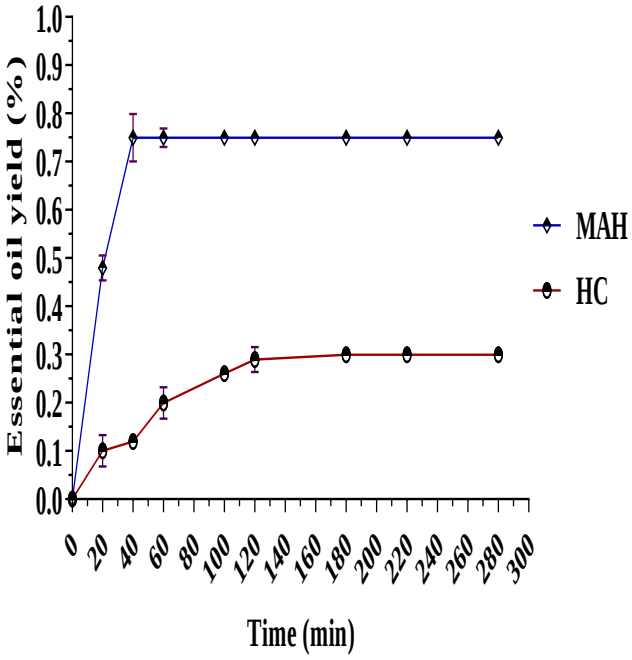

Figure 2: Yields of EOs extracted from the *M. recutita* aerial part by HC and MAH.

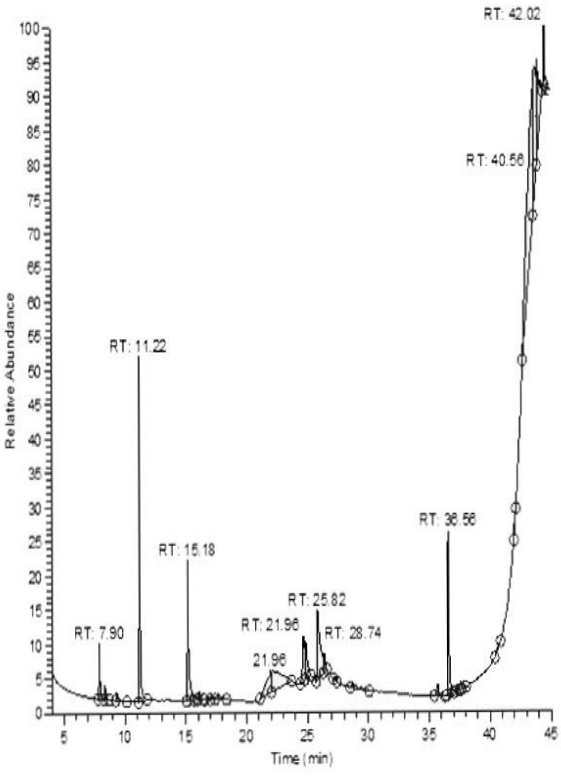

Figure 3: Chromatogram of EOs extracted from *M. recutita* by MAH.

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Table 1: Chemical composition of *M. recutita's* EOs

No	Compounds	RT	Percentage (%)
1	Cis-Ocimene	7.90	1.55
2	Camphène	8.37	2.62
3	2-*α*-pinène	9.33	3 .31
4	Eucalyptol	11.22	8.19
5	Camphre	15.18	4.33
6	Quercetine	15.74	1.19
7	Pregnane	16.10	0.45
8	Acide octadecadienoïque	16.79	1.43
9	Lucenin 2	17.65	2.82
10	Thymol	21.96	3.49
11	Galaxolide	22.11	5.31
12	Acide benzoïque	24.65	2.92
13	Acide salicyque	24.86	2.3
14	Trans-caryophyllène	25.82	5.95
15	Terpinene-4-ol	26.38	0.92
16	Naphtalene	27.32	1.58
17	*α*-Cedrol	28.74	4.81
18	Anisaldehyde	35.67	0.79
19	Coumarine	36.56	6.01
20	Acide α-linolenique	37.15	0.14
21	*β*-Pinene	37.24	0.31
22	Acide alpha-linolenique	37.76	0.32
23	Chamazulene	40.56	26.11
24	Cis beta farnesene	42.02	11.64
	Total	-	98.49

Antibacterial activity of EOs extracted from M. recutita

The results of the antibacterial activity of the *M. recutita's* EOs are presented in Figure 4 shows the inhibition zone of the EOs determined for the four bacterial strains using the disk diffusion method. The results showed that the EOs have a substantial inhibitory effect against the four bacterial strains tested, noted by significant growth inhibition halos. The results indicated that *B. subtilis* was the most sensitive strain tested with *M. recutita's* EOs with the strongest inhibition zone (15.2±0.13mm). also, the EOs showed strong antimicrobial activity against *E. coli* (13.27±0.18 mm), *S. aureus* (14.13±0.11 mm) and *P. aeruginosa* (13.07±0.09 mm). The MIC results of *M. recutita's* EOs against the four bacterial strains are shown in Figure 5. The data indicates that *M. recutita's* EOs displayed varying levels of antimicrobial activity. The results obtained show that the MIC values presented by the *M. recutita's* EOs had a variable level of antimicrobial activity. the MIC were between 6.25 and 8.33 µl/ml.

Several scientific research projects have focused on the valorisation of EOs of medicinal and aromatic plants in general and the EOs of *M. recutita* in particular to fight against pathogenic microorganisms. Kazemi[29] has shown that EOs of *M. recutita* have significant antibacterial activity against *S. aureus* (30mm), *B. subtilis* (32mm) and *P. aeruginosa* (19mm). Soković et al[42] have also shown that *M. recutita's* EOs have significant antibacterial activity against *B. subtilis* (12mm), *S. aureus* (10mm), *E. coli* (9mm) and *P. aeruginosa* (0mm) with MICs of 7, 8, 10 and 10 µg/mL respectively. Also, Chouia et al[43] have shown that EOs of *M. recutita* have significant antibacterial activity against *E. coli* ATCC 25922 (14.33 ± 0.57), *S. aureus* ATCC 25923 (10.67 ± 0.57) and *P. aeruginosa* ATCC 27853 (18.33 ± 1.15). The growth of the bacterial strains tested in our study and in other studies reacted differently to the EOs and their components, indicating that the different components may have different modes of action or that the metabolism of some bacteria is able to better overcome or adapt to the effect of the EOs.[42] Chalchat *et al*[44] have shown that the antimicrobial activity of EOs is highly dependent on their chemical composition, particularly their main constituents. In addition, Kazemi[29] has shown that Gram-positive bacteria are particularly sensitive to the EOs of *M. recutita*, this may be due to the bacteria outside membrane.Numerous studies concerning the antimicrobial effectiveness of EOs in foods suggest that the use of EOs can improve food safety.[27] Therefore; there is a growing demand for accurate knowledge minimum effective inhibitory concentrations of EOs in order to enable a balance between sensory acceptability and antimicrobial efficacy in the food matrix.[45] This suggests that the EOs extracted from *M. recutita* from the region of Taounate, Morocco can be valorised in food safety thanks to its high antibacterial activity and its low MIC value against pathogenic bacteria.

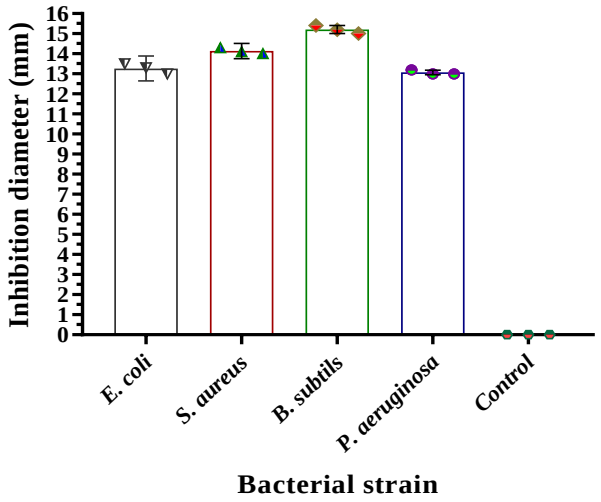

Figure 4: *Antibacterial activity of EOs extracted from M. recutita*

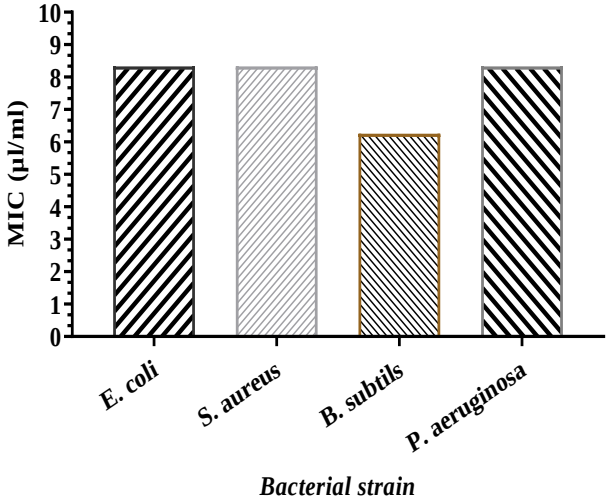

Figure 5: MICs of EOs extracted from *M. recutita*

Conclusion

To the best of our knowledge, there is no detailed report for valorisation of *M. recutita's* EOs from the region of Taounate, Morocco. *M. recutita*'s EOs of the Taounate region, Morocco has significant antimicrobial activity against pathogenic bacteria compared to *M. recutita*'s EOs from other worldwide regions described in recent studies. The current results suggest that *M. recutita's* EOs contain certain antimicrobial properties, which can be used as antimicrobial agents in drugs for the treatment of infectious diseases. However, further research is needed to obtain more information about the modes of action, safety and toxicity of *M. recutita's* EOs.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the study presented in the current article is original and that any liability for claims relating to the content of this article will be borne by them.

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