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COMPARATIVE STUDY EVALUATING PHYTOCHEMICAL SCREENING, FUNCTIONAL GROUPS ANALYSIS, AND ANTIMICROBIAL ACTIVITY OF *TROPAEOLUM MAJUS* L. LEAVES, FLOWERS, AND FRUITS

Leila AILANE[1*], Abdelghani DJAHOUDI[2] and Salima BENNADJA[3]

[1]Department of Biochemistry, Laboratory of Biochemistry and Environmental Toxicology, Faculty of Sciences, BadjiMokhtar University of Annaba, PO Box 12 Annaba 23000, Algeria.
[2]Department of Pharmacy, Microbiology Laboratory, Faculty of Medicine, BadjiMokhtar University of Annaba, PO Box 205 Annaba 23000, Algeria.
[3]Department of Pharmacy, Plant Biology Laboratory, Faculty of Medicine, BadjiMokhtar University of Annaba, PO Box 205 Annaba 23000, Algeria.
** Correspondence author: Leila AILANE, Email: leila.mohammed.ailane@gmail.com;*
ORCID: https://orcid.org/0000-0001-5103-8963; Lindekin: www.linkedin.com/in/leila-ailane-84381490
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ABSTRACT

Tropaeolum majus is a herbaceous plant that belongs to the Tropaeolaceae family. It is native to the Andes mountain ranges, where it is considered as an important herb in their traditional medicine. However, this plant has known, over time, a wide distribution all over the world, including north Africa. Indeed, *Tropaeolum majus* has shown in recent years a noteworthy flowering in northeast Algeria, where reigns a *Mediterranean climate*. The aim of this comparative study was to conduct a preliminary assessment of the phytochemical composition and antimicrobial activity of *Tropaeolum majus* that grows under the Mediterranean climatic conditions of Annaba city in northeast Algeria. Thus, a phytochemical screening of 20 metabolites families was performed on free-air dried and pulverized leaves, flowers, and fruits of this plant using standard qualitative analysis methods based on colour / precipitation reactions. Afterwards, methanolic leaves and flowers extracts, and fruits fixed oil were prepared and served for the functional groups analysis using *Fourier Transform Infra Red spectroscopy* (4000-400 cm^{-1}), as well as the antimicrobial screening using the disc-diffusion method. Apart from narcotics and free anthracene derivatives; that are absent in the three organs, the phytochemical screening revealed the presence, in *Tropaeolum majus*, of the sought metabolites, mainly phenolic compounds, coumarins, sterols, triterpens, iridoids, and alkaloids. However, the nature and predominance of these metabolites differ between organs, with a unique presence of saponosides in fruits, and that of free quinones in flowers. Likewise, FTIR analysis revealed absence of infrared radiation absorption in the region between 2260-2220 cm^{-1}, which indicated absence of toxic cyanide groups; but showed distinct peak values, with a specific infrared radiation absorption in the region located between "1700-700 cm^{-1}", revealing the presence of various functional groups in the three extracts, including aromatic, nitrogen, and sulfur compounds. *Tropaeolum majus extracts were found to exert a good antimicrobial activity against tested* $Gram_{(+)}$ and $Gram_{(-)}$ *germs, especially against a troublesome* food poisoning germ: *Bacilluscereus, and the antibiotic-resistant genera: Pseudomonas*, *Acinetobacter*, *Staphylococcus*, *Enterococcus*, and *Klebsiella. These findings show that* Annaba City Mediterranean climate factors improved *Tropaeolum majus* leaves, flowers, and to a second degree fruits nutritional and medicinal qualities enhancing their phytochemical composition as well as antimicrobial properties. Hence, this study suggests that this plant could be a potential source for different nutraceutical products that can be used as food additives or dietary supplements.

Keywords **:** Antimicrobial activity, *Tropaeolum majus* L., Phytochemical screening, Mediterranean climate, FTIR.

Introduction

Tropaeolum majus L. (*T. majus*), Known as "Nasturtium", is an important medicinal plant from the order Brassicales, Tropaeolaceae family. It is an annual, bushy, flowery plant; distinguished by a fleshy trailing stem, veined green orbicular leaves with slightly wavy edges peltated on a long petiole, as well as yellow, red or orange beautiful flowers in the form of an open funnels that carries a spur. All the parts of this herb, mainly leaves and flowers, are edible; and are characterized by a pungent peppery flavor. Besides, this species is one of the most popular source of edible flowers (Garzón *et al.*, 2015; Ailane *et al.*, 2019).

T. majus is native to the Andes mountain range in South America. These mountains are distinguished by their very impressive height (the peak is about 6962 m), continuity (extending through seven countries) and length (7000 km); and are characterized by a very contrasting climatic conditions that change radically throughout the chain (Garreaud, 2009). This species was first introduced in Europe in the XVIth century and elsewhere thereafter (Ailane *et al.*, 2019). Its firmness and its adaptability offer this plant the ability to grow in many regions of the world including Algeria. Indeed, *T. majus* has shown, in recent years, a remarkable flowering in Northeast Algeria, which is a

temperate zone with a Mediterranean climate. Annaba is one of the most important cities of this zone. It is located on a coastal plain with an average altitude of about 5 m above sea level, slightly inclined towards the sea. Indeed, the city is bordered on its east-northeast by the Mediterranean Sea, and on its west-southwest by the Edough mountain range (about 1000 m altitude). This city is one of the wettest regions in Algeria; the average annual rainfall is about 671 mm; humidity is relatively constant throughout the year, and winds, especially maritime winds, contribute to the maintenance of a high level of humidity (> 70 %) (Dahech and Saihia, 2019).

Previous phytochemical studies on *T. majus* have shown the presence of a variety of bioactive compounds; including phenols; with antioxidant, antimicrobial, and anti-inflammatory properties. Furthermore, pharmacological studies on this species have proven its effectiveness in the treatment of genitourinary and urinary tract infections, upper respiratory tract disorders such as bronchitis, and asthma, and many other disorders (Duke *et al.*, 2009; Bazylko *et al.*, 2013; Ailane *et al.*, 2019). However, in our country *T. majus* is only used for ornamental purposes; its culinary and medicinal uses are little known and uncommon. Therefore, in order to put the light on the nutraceutical and medicinal qualities of this plant, as influenced by the Mediterranean climate of Northeast Algeria, the present comparative study was conducted with the aim of a prelimenary qualitative characterization of the phytochemical composition, using phytochemical screening and Fourier transform infrared spectroscopy (FTIR) functional groups analysis; as well as the antimicrobial activity of leaves, flowers, and fruits of *T. majus* growing under the Mediterranean climatic conditions of Annaba city, in Northeast Algeria.

Material and Methods

Technical material

The following equipment were used: coffee grinder (SAYONA, PRC), precision balance (KERN & SOHN, Germany), rotary evaporator (BUCHI-RII, Germany), bain-marie (Memmert, Germany), and FTIR spectrometer (PerkinElmer, France). Most of reagents were supplied by Sigma-Aldrich, with the exception of hydrochloric acid (HCl) supplied by BDH PROLABO (South Africa), iron chloride by Merck (Germany), and magnesium chips provided by METALLPULVER24 (Germany). The culture media specific to tested microbial strains and to the aromatogram were purchased previously prepared for direct use from a local supplier.

Plant material

T. majus leaves, orange flowers, and fruits were harvested during the period between November (2016) and April (2017) in the region of "Caroube" (latitude: 36°93342, longitude: 7°76308), located in the North on the gulf of ANNABA city, northeast Algeria. The identification of the plant species was carried out at the Laboratory of Plant Biology of Medicine Faculty of BadjiMokhtar University of Annaba - Algeria. The harvesting of the plant was conducted according to the World Health Organization (WHO) guidelines on good agricultural and collection practices for medicinal plants (WHO, 2003). After the harvest, the plant material was free-air shade dried for 9-15 days, then pulverized.

Phytochemical screening

In our study, we were able to screen 20 phytochemicals families using several solvents of different polarities. Detection of phytochemicals in *T. majus* pulverized leaves, flowers, and fruits is based on assays of constituents' solubility, precipitation reactions, and / or a specific colour change. The presence of a phytochemical is indicated by a "+"; where the number of "+" is a function of colour and / or precipitate intensity, which is proportional to the concentration of phytochemicals. The phytochemical screening was conducted using different standard qualitative analytical methods as follows:

Detection of phenolic compounds

1 ml of 1% ferric chloride ($FeCl_3$) aqueous solution was added to 5 ml of 5 % infusion of each organ. The presence of phenolic compounds is indicated by the development of a greenish-brown or blackish-blue precipitate (Diallo, 2005).

Detection of flavonoids

- **Sodium hydroxide (NaOH) test:** 2 ml of 30 % NaOH solution was added to 5 ml of 5 % infusion of each organ. The development of yellow colour indicates the presence of flavonoids (Diallo, 2005).
- **Cyanidin reaction:** 5 ml of hydrochloric alcohol was added to 5 ml of each organ's 5 % infusion; then, few magnesium chips were introduced. The development of a cherry-red colour indicates the presence of flavonols; an orange colour reveals the presence of flavones; and a purplish-red colour shows flavanones presence (Diallo, 2005).

Detection of tannins

- **Condensed tannins:** 2 ml of Stiasny's reagent was added to 5 ml of each organ's 5 % infusion. After few minutes of heating in a bain-marie at 90°C, the formation of a pink or more or less brown precipitate indicates the presence of condensed tannins (Faugeras and Lavenir, 1965).
- **Hydrolysable tannins:** few drops of 2 % $FeCl_3$ aqueous solution were added to 5 ml of each organ's 5 % infusion. Hydrolysable tannins presence is detected by the development of a greenish-brown precipitate (Rizk, 1982).

Detection of anthocyanins

1 ml of hydrochloric acid (HCl) was added to 5 ml of each organ's 5 % infusion. Afterwards, 1 ml of ammonium hydroxid (NH_4OH) concentrated solution was added drop-wise. Anthocyanins presence is revealed by the appearance of a colour that turns blue-green (Diallo, 2005).

Detection of leucoanthocyanins

5 ml of 5 % infusion of each organ was added to 5 ml of hydrochloric alcohol. After heating in a bain-marie at 50 ° C for a few minutes, the appearance of a cherry red or purplish-red colour indicates the presence of leucoanthocyanins (Diallo, 2005).

Detection of coumarins

3ml of 10% NaOH solution was added to 2ml of the 5 % infusion of each organ; then, the mixture was vortexed.

The presence of coumarinsis illustrated by the formation of yellow colour (Diallo, 2005).

Detection of anthracene derivatives

Free anthracene derivatives : 1 g of the pulverized plant material was added to 10 ml of chloroform. The mixture was heated in bain-marie for 3 min, then, was filtered while hot. The obtained filtrate volume was adjusted to 10 ml. Afterwards, 1 ml of dilute NH_4OH was added to 1 ml of the chloroform filtrate. The appearance of a more or less red colour after vortexing indicates the presence of free anthracene derivatives (Badiaga, 2011).

Combined anthracene derivatives: 10 ml of distilled water and 1 ml of HCl were added to the previous chloroform-depleted residue. The mixture was heated in bain-marie for 15 minutes; then, was cooled under running cold water and filtered. 5 ml of chloroform are added to 5 ml of the filtrate. After stirring (Badiaga, 2011):

- The organic phase was separated and 1 ml of NH_4OH solution was added to it. The appearance of a more or less red colour indicates the presence of O-heterosides.
- 10 ml of distilled water and 1 ml of 10% $FeCl_3$ were added to the aqueous phase, and the mixture was heated for 3 minutes in a bain-marie; then cooled under running cold water. After stirring, the organic phase was separated and 5 ml of chloroform with 1 ml of NH_4OH were added to it. The appearance of a more or less red colour indicates the presence of C-heterosides (Badiaga, 2011).

Detection of mucilage

5 ml of diethyl ether was added to 10 ml of the 10% decoction of each organ, the mixture was then vortexed. Appearance of a fluffy precipitate in the ethereal phase reveals mucilage presence (Diallo, 2005).

Detection of Alkaloids

5 g of the pulverized plant material was macerated, for 24 hours, in 100 ml of 20 % sulfuric acid (H_2SO_4) solution. Afterwards, 15 ml of filtrate was divided, equally, in 3 test tubes. Few drops of Mayer's reagent were added in the first tube, of Bouchardat's reagent in the second, and of Dragendorff's reagent in the third. The development of a yellowish precipitate after adding Mayer's reagent, a brown precipitate after adding Bouchardat's reagent, and / or an orange precipitate after adding Dragendorff's reagent indicate the presence of alkaloids (Diallo, 2005).

Detection of reducing compounds

2 ml of Fehling's liquor was added to 2 ml of each organ's 5% infusion. The mixture was heated in a boiling bain-marie for 8 minutes. Reducing compounds presence is revealed by the appearance of a red brick precipitate (Azzi, 2013).

Detection of narcotics

0.5 g of the pulverized plant material was macerated, for 15 minutes under continuous stirring, in 5 ml of petroleum ether. The filtrate was subsequently evaporated to dryness in a bain-marie; then, 3 to 4 drops of 5 % alcoholic potassium hydroxide (KOH) solution were added. The appearance of a purple colour reveals the presence of narcotics (Diallo, 2000).

Detection of starch

5 ml of the 5 % infusion of each organ was heated until boiling, in a bain-marie, with 10 ml of a saturated sodium chloride (NaCl) solution. Then, few drops of the starch reagent were added. The appearance of a purplish blue colour illustrates the presence of starch (Bruneton, 1999).

Detection of saponosides

A 1 % decoction of each organ was prepared by boiling 2.5 g of plant material in 250 ml of distilled water for 10min. After filtration, the decoction was left to cool then the volume was adjusted to 250ml with distilled water. Subsequently, 10 ml of the decoction was introduced into a test tube. The latter was sealed with the thumb and then agitated for 15 seconds at the rate of two agitations per second. The formation of a foam of at least 1 cm that persists after 15 min indicates the presence of saponosides (Karumi *et al.*, 2004).

Foam index determination: the decoction was introduced into a series of 10 test tubes, the volumes of which are calibrated according to Table 1.

- If the foam height is less than 1 cm in all the tubes, the foam index is less than 100, which indicates absence or very low concentration of saponosides.
- If the foam height is higher than 1cm in all the tubes, the foam index is higher than 1000. In this case, the decoction must be diluted and the test is carried out again.
- If the foam height is 1 cm in one of the tubes, the foam index is determined using the following equation:

$$\text{Foam index} = \frac{10 \times 1}{(\text{tube decoction volume} \times 2.5)/250}$$

Detection of triterpenes, sterols and carotenoids

1g of the pulverized plant material was macerated, for 24 hours at 4°C, in 20 ml of diethyl ether. The macerate was filtered; then, the filtrate volume was adjusted to 20 ml using diethyl ether. The filtrate was divided into three tubes as follows (Faugeras and Lavenir, 1965):

- **First tube :** 0.5 ml of chloroform was added to 5 ml of the filtrate, followed by 3 ml of concentrated H_2SO_4 solution. The appearance of a brownish-red or purple ring at the contact zone of the two liquids reveals the presence of triterpenes.
- **Second tube:** 3 drops of concentrated H_2SO_4 solution was added to 3 ml of the filtrate. There may be a development of several colours, of which red colour indicates the presence of terpenes, and the blue colour reveals carotenoids presence.
- **Third tube:** 3 drops of concentrated H_2SO_4 solution was added to 3 ml of the filtrate, followed by 6 drops of acetic anhydride. The development of a purple colour that turns green illustrates the presence of sterols.

Detection of iridoids

They are detected by the Lieberman-Burchard reaction, which consists on adding 1ml of concentrated HCl solution on the filtrate of the pulverized plant material decoction, followed by a heating in bain-marie for few minutes. The development of a black precipitate indicates the presence of iridoids (Faugeras and Lavenir, 1965).

Detection of free quinones

Few milligrams of pulverized leaves, flowers and fruits were moistened with 20 % HCl solution, then macerated in 3 ml of chloroform-ether (V / V) for 24 hours. The filtrate was made basic by adding 10% NaOH solution. The appearance of a purplish red colour indicates the presence of free quinones (Fournet, 1979).

Detection of cardiac glycosides

5 ml of acetic acid containing traces of $FeCl_3$ was added to few milligrams of pulverized plant material followed by 5 ml of H_2SO_4 containing traces of $FeCl_3$. The presence of cardiac glycosides is confirmed by the formation of 2 phases, one coloured in a reddish brown (acetic acid) and the second in a blue-green (H_2SO_4) (Edeoga *et al.*, 2005).

Detection of amino acids

On a Whattman filter paper, a drop of each organ's 5 % infusion was deposited, followed by a drop of ninhydrin solution. After heating in a laboratory oven at 90°C for 5 min, the appearance of a blue or purple tint indicates the presence of amino acids (Mavian, 2012).

Extracts preparation

Methanolic extracts preparation

1 g of the pulverized leaves and flowers was soaked for 24 hours in 20 ml of absolute methanol (≥ 99.7%). After filtration, methanolic solutions were evaporated to dryness at 60 °C under reduced pressure in a rotary evaporator. Extracts were kept in 4°C for further studies.

Fruits fixed oil extraction

Fruits fixed oil was obtained using Soxhlet and hexane following the method of Despiau(1978). Briefly, 10 g of powdered *T. majus* fruits was introduced into cellulose Soxhlet thimble, was covered with cotton, then was placed in the extractor chamber. 150 ml of hexane was poured into the flask, then all Soxhlet parts were put together over heating. At the end of the extraction (about 6 hours), the hexane containing the oil was evaporated to dryness at 60 °C under reduced pressure in a rotary evaporator. In order to remove the last traces of hexane from the oil residue, the flask was heated for 20 minutes at 103 °C.

Fourier Transform InfraRed spectroscopy (FTIR) analysis

Before FTIR analysis, the resultant methanolic leaves and flowers residues were diluted in 1 ml of dichloromethane. Afterwards, leaves and flowers residues dilutions as well as fruits fixed oil were placed in a crystal diamond, then were exposed to an infrared beam with wavelengths ranging from 2.5 µm to 25 µm (wave number range: 4000 - 400 cm^{-1}) to record the characteristic peaks values for each extract.

Antimicrobial screening

Tested Microbial strains

The antimicrobial screening was conducted on 16 microbial strains that were provided by the Microbiology Laboratory of Medicine Faculty at BadjiMokhtar University of Annaba, Algeria. These strains are as follows:

- Eleven clinical strains, isolated from the University Hospital: *Escherichia coli* BLSE, *Klebsiella pneumoniae* Scy, *Klebsiella pneumonia* $C^{(+)}$, *Klebsiella pneumonia* $C^{(-)}$, *Serratia marcesens, Bacillus cereus*, *Pseudomonas aeruginosa* VIM-II, *Acinetobacter baumannii*, *Acinetobacter baumannii* (IMP®), *Acinetobacter baumannii* NDM1, *Staphylococcus aureus*
- Four reference strains: *Staphylococcus aureus* ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922;
- A fungi: *Candida albicans*.

Screening Method

In vitro inhibition of microbial growth was examined using aromatogram method, also called disc-diffusion in agar medium method. This technique is based on the diffusion of *T. majus* extracts, using discs impregnated with different concentrations of each extract (crude extracts with 5 dilution in dimethyl sulphoxide: 1/2, 1/4, 1/8, 1/16, and 1/32), on agar medium (MH: Mueller-Hinton) previously seeded with selected germs. After 18 to 24 hours of incubation at 37 °C, qinhibition zones diameters were measured. According to Duraffourd *et al.* (1990) classification, extract is considered inactive if inhibition diameters is less than or equal to 8 mm; with intermediate activity for diameters between 8-14 mm; moderately effective for diameters between 14-20 mm; and very effective if diameters are surpassing or equal to 20 mm.

Results

Phytochemical screening

T. majus leaves, flowers and fruits screening data is shown in Table 2. These results show the presence of a variety of phytochemical compounds in the studied plant. In addition, apart from narcotics and free anthracene derivatives which are absent in the three organs; all sought metabolites were detected; though the nature and the presence intensity of these compounds differ from one organ to another. Indeed, the three organs of *T. majus* have in common the hegemonic presence of coumarins, sterols and triterpenes. Similarly, combined anthracene derivatives (O- and C-heterosides), phenolic compounds, and iridoids are predominant in *T. majus* leaves and flowers. Conversly, only C-heterosides combined anthracene derivatives predominate in fruits; whereas O-heterosides combined anthracene derivatives are completely absent. On another note, *T. majus* leaves are also characterised by flavone-type flavonoids, alkaloids, reducing compounds, and cardiac glycosides supremacy; while flowers are distinguished by the hegemony of anthocyanins, and leucoanthocyanins. Furthermore, *T. majus* leaves and fruits share the absence of starch and free quinones, which are moderately present in flowers. We also note the unique predominance of saponosides in *T. majus* fruits.

FTIR analysis

Leaves-MeOH FTIR spectrum (Figure 1) is characterized by a broad and intense band, which appears at 3377.57 cm^{-1}. It is also characterized by the appearance of eight more or less thin and intense bands at: 1738.87 cm^{-1}, 1633.83 cm^{-1}, 1516.31 cm^{-1}, 1454.81 cm^{-1}, 1414.36 cm^{-1}, 1272.91 cm^{-1}, 1135.21 cm^{-1}, and 1056.17 cm^{-1}.

Likewise, a broad and intense band, which appears at 3379.91 cm^{-1}, characterizes Flowers-MeOH infrared spectrum (Figure 2). However, only six other more or less thin and intense bands distinguished this extract; these bands

appear at: 1634.67 cm^{-1}, 1513.39 cm^{-1}, 1454.74 cm^{-1}, 1414.93 cm^{-1}, 1278.74 cm^{-1}, and at 1057.26 cm^{-1}. On the other hand, *T. majus* fruits fixed oil FTIR spectrum (Figure 3) shows seven more or less thin and intense characteristic bands at: 3004.85 cm^{-1}, 2924.74 cm^{-1}, 2854.11 cm^{-1}, 1746.29 cm^{-1}, 1711.99 cm^{-1}, 1465.32 cm^{-1}, and at 1165.65 cm^{-1}. The other bands that appear in *T. majus* extracts FTIR spectrums are less intense and of different amplitudes. Wavenumber assignment of each band in the three extracts spectrums, with the corresponding functional group are shown in Table 3.

Antimicrobial screening

Obtained inhibition diameters (Table 4) show that among the three tested extracts, Flowers-MeOH was the most efficient against the majority of studied germs, particularly antibiotic-resistant germs. Indeed, apart from *P. aeruginosa* VIM-II, against which Flowers-MeOH was moderately effective with the crude (10 mm) and 1/2 dilution (9 mm); this extract showed the most powerful activity against all the other strains at 1/32 dilution: *Escherichia coli* ESBL (11 mm), *Klebsiella. pneumoniae* C-(13 mm), *Klebsiella. pneumoniae* C+ (10 mm), *Serratia marcescens* (15 mm), *Pseudomonas aeruginosa* ATCC 27853 (10 mm), *Acinetobacter baumannii* (11 mm), *Acinetobacter baumannii* IMP (12 mm), *Acinetobacter baumannii* NDM-1 (10 mm), *Staphylococcus aureus* ATCC (14 mm), *Enterococcus faecalis* (13 mm) *Klebsiella pneumonia Scy* (9 mm) and *Candida albicans* (13 mm). We note that *Bacillus cereus* was most sensitive to all *T. majus* extracts in the following descending order: fruits fixed oil (10-39 mm), Flowers-MeOH (9 - 19 mm), and Leaves-MeOH (7-14 mm). *Escherichia coli* ATCC 25922 showed a similar reaction to the three extracts with inhibition diameters ranging from 7 to 16 mm. Leaves-MeOH crude extract was particularly effective against two antibiotic-resistant strains at 1/2 dilution: *Klebsiella pneumonia Scy* (16-8 mm) and *Pseudomonas aeruginosa* VIM-II (18-8 mm). Moreover, fruits fixed oil, in addition to its high activity on *Bacillus cereus*, it was efficient against *Pseudomonas aeruginosa* VIM-II (7-17 mm), and moderately effective on *Staphylococcus aureus* MRSA (8-12 mm).

Discussion

T. majus leaves, flowers and fruits preliminary phytochemicalscreening data show a total absence of narcotics as well as free anthracene derivatives in the three organs. Narcotics absence being logical because the plant is known for its common culinary use (Ailane *et al.*, 2019); Moreover, their absence is consistent with the absence of *T. majus* toxicity reported after the subchronic use of different oral doses of its extracts on Wistar rats (Gomes *et al.*, 2012).

For the rest of the sought phytochemicals, screening results vary from an organ to another. For instance, amino-acids have shown a moderate presence in the three organs; however, starch had a moderate presence in flowers, but a total absence in leaves and fruits. Presence of these primary metabolites is consistent with reports on researches conducted on the same species. Indeed, Mizrahi *et al.* (1970) reported the presence of amino-acids in *T. majus* leaves; while Wilkinson and Douglas (2003) reported their presence in *T. majus* nectar. However, da Silva *et al.* (2012) reported that starch was the minor non-structural carbohydrate found in *T. majus* flowers, which undergoes a drop during flower development. For their part, Hoth *et al.* (1986) reported that starch is formed during *T. majus* seed development, but is absent in mature seeds.

Our study also revealed the absence of saponosides in leaves and flowers, but their presence in fruits. A similar pattern of results was also obtained from other researches. Effectively, in their study, Scio *et al.* (2012) and Hayat *et al.* (2017) reported saponosides absence in *T. majus* flowers and seeds methanolic extracts; while Valsalam*et al.* (2019) reported their absence in leaves ethanolic extract, but their presence in leaves methanolic extract. Saponosides absence in leaves and flowers can be explained by the fact that qualitative and quantitative content of saponosides differs in the organs of the same plant, which is probably due to separate biosynthesis (Défago, 1977). Besides, saponosides presence in *T. majus* fruits explains starch absence in this same organ, because the water-soluble elements, including sugars, are precursors of saponosides synthesis (Varma and Shulka, 2015). In addition to saponosides, *T. majus* was found to be rich in other types of terpenes. Indeed, a strong presence of triterpenes and sterols was detected in the three organs of this plant; while carotenoids and iridoids are in higher concentration in leaves and flowers. Scio *et al.* (2012) also detected triterpenes presence in *T. majus*, but the same study reported sterols absence. Our results are also consistent with studies by Butnariu and Boston (2011), Carvalho *et al.* (2015), and Valsalam *et al.* (2019) who reported presence of steroids in *T. majus*, knowing that steroids are sterols derivatives (Goad and Akihisa, 2012). As for carotenoids, their presence as well as their identification have been reported in various studies (Lim, 2014).

Terpenes are not the only type of secondary metabolites found in this species; our study also revealed the presence of a heterogeneous variety of phenolic classes in *T. majus* leaves, flowers and fruits. Indeed, coumarins and hydrolysable tannins presence was detected in the three organs; however, condensed tannins are more prominent in leaves and flowers while anthocyanins and leucoanthocyanins predominate in flowers. As for flavonoids, high concentrations of flavone were detected in leaves and fruits, and flavonol, in flowers. Free quinones, on the other hand, were found only in *T. majus* flowers. This variety of phenolic compounds explains the high concentration of total phenolic compounds detected in leaves and flowers, and their moderate concentration in fruits. Our results are in agreement with those reported by the study of Valsalam *et al.* (2019), in which presence of tannins and flavonoids, as well as quinones' absence were detected in *T. majus* leaves extract. However, according to Scio *et al.* (2012) this plant is devoid of tannins, and provided with flavonoids. Conversely, according to Carvalho *et al.* (2015) *T. majus* is devoid of flavonoids, and provided with tannins. Furthermore, anthocyanins' presence in this plant has been reported in other studies (Brondani *et al.*, 2016).

Alkaloids, another class of secondary metabolites, that predominate in leaves compared to flowers and fruits. These results are agonistic to phytochemical screening results carried out by other studies that indicated the presence of alkaloids in *T. majus* leaves, flowers, and seeds (Butnariu and Boston, 2011; Scio *et al.*, 2012; Valsalam *et al.*, 2019; Bawazeer *et al.*, 2021). Likewise, the presence of cardiac glycosides was observed in *T. majus* flowers and fruits in a lower concentration compared to leaves. This result is in agreement with those reported by Valsalam *et al.* (2019) and

Bawazeer *et al.* (2021) studies, which revealed cardiac glycosides presence in *T. majus* leaves; but in disagreement with those reported by Hayat *et al.* (2017) that revealed their absence. Reducing compounds are also more prominent in leaves compared to flowers and fruits; which seems logical, because the screening revealed the presence, in the three organs, of amino-acids, starch, various phenolic compounds, and carotenoids, that are themselves reducing compounds (Siddiqi and Husen, 2016).Other secondary metabolites have also been detected; these are anthracene C-heterosides that are in abundance in the three organs; O-heterosides, and mucilages that appear to have moderate concentration.

Beside phytechemical screening, FTIR spectroscopy enabled us to determine another qualitative aspect of *T. majus* leaves, flowers and fruits chemical composition; more precisely the functional groups of their extracts. It should be noted that FTIR spectrum of the three extracts was recorded between 3500–700 cm^{-1}. Undeniably, FTIR spectroscopy results are congruent with those of *T. majus* leaves, flowers and fruits phytochemical screening. Indeed, the first thing that can be noticed when observing the transmission diagrams is that none of *T. majus* extracts could absorb infrared radiation in the region between 2260 -2220 cm^{-1}. The non-absorption of infrared radiation in this region indicates the absence of cyanide groups (Ragavendran *et al.*, 2011); therefore, *T. majus* extracts do not contain any toxic substances. This is consistent with the absence of narcotics detected by the phytochemical screening. The second thing that may attract our attention when observing the three FTIR spectrums is the characteristic absorption of infrared radiation of each extract located in the region between 1700-00 cm^{-1}; with ten, eleven, and thirteen absorption bands of different width and intensity for *T. majus* Leaves-MeOH, Flowers-MeOH, and fruits fixed oil respectively. It is accepted that absorption bands, which appear in this so-called "fingerprint region", accurately characterize chemical compounds as they generally reflect very complicated absorption series that are mainly due to the interaction between vibrations within a molecule, and which are unique to each compound (Ramírez-Hernández *et al.*, 2019). This is in favour of the previous phytochemical screening, as the concordance between *T. majus* extracts FTIR spectrums and the complexity as well as the phytochemical heterogeneity of the detected metabolic substances in this species, mainly secondary metabolites, is clearly visible.

In any case, previous researches on plants metabolic substances confirm the presence of primary and secondary metabolites in *T. majus* extracts. For example, Beraka *et al.* (2020) reported that infrared absorbance at 3010 cm^{-1} corresponds to an aromatic "C-H" bond, and that at 1247 cm^{-1} matches to an aromatic "C-O-C" ether. They have also reported that phenyl rings absorb at around 1513.38-1400.11 cm^{-1}. Furthermore, Belboukhari *et al.* (2013) were able to distinguish, in their study, the absorbance of terpene compounds such as pentanone which absorb infrared light at 2924 cm^{-1} and at 1711 cm^{-1}, heptanol at 1727 cm^{-1}, pinene at 1645 cm^{-1}, canphor around 1460 cm^{-1}, terpinolene at 1449 cm^{-1} and at 1165 cm^{-1}, myrcene and octanal at 1378 cm^{-1}, eucaliptol at 1460 cm^{-1} and at 1274 cm^{-1}, and camphene at 1121 cm^{-1}. On the other hand, the study by Hayat *et al.* (2020) indicated that the absorbance between 3000-2800 cm^{-1} accords to the region of "C-H" vibrations of lipids, and that between 1480-600 cm^{-1} corresponds to secondary amides embedded in tissue proteins; while phenyl rings absorb infrared light at around 1500 cm^{-1}. For their part, Sculz and Baranska (2007) reported that iridoids absorb infrared light in the region between 1700-1600 cm^{-1}, that absorbance between 1500-700 cm^{-1} may correspond to vibrations of alkaloid rings, and the one between 1630-1601 cm^{-1} may correspond to benzene rings of phenolic compounds. They added that, in addition to the characteristic vibrations at 3000-2850 cm^{-1}, triglycerides also absorb infrared light at 1746 cm^{-1}, while free fatty acids absorb it at 1711 cm^{-1}. The same study also reported that the structural differences of amylose and amylopectin, which are both starch derivatives, can be revealed in the "C-H" elongation region between 3100–2700 cm^{-1}. Moreover, *T. majus* has been reported to contain sulfur compounds (Bazylko *et al.*, 2013), which tends to be consistent with The FTIR analysis in the three organs.

Comparing our results with those of other studies conducted on the same species, some similarities as well as some differences in *T. majus* extracts FTIR spectra can be observed. For instance, Singh *et al.* (2021) study revealed *T. majus* yellow and orange flowers ethanolic extracts FTIR spectra wavenumbers, recorded between 3700 - 700 cm^{-1}, as follows: 3700 cm^{-1}, 3000 cm^{-1}, 2927 cm^{-1}, 2853 cm^{-1}, 1743 cm^{-1}, 1625 cm^{-1}, 1596 cm^{-1}, 1456 cm^{-1}, 1383 cm^{-1}, 1075 cm^{-1}, and 722 cm^{-1}. In contrast, *T. majus* leaves aqueous extract, used in Bawazeer *et al.* (2021)study, recorded a FTIR spectrum which starts at 3400 cm^{-1} and extends to about 400 cm^{-1}. Indeed, the characteristic wavenumbers of this extract are: 3400 cm^{-1}, 2947.23 cm^{-1}, 2831.50 cm^{-1}, 2592.33 cm^{-1}, 2522.89 cm^{-1}, 2214.28 cm^{-1}, 2044.54 cm^{-1}, 1651.07 cm^{-1}, 1411.89 cm^{-1}, 1099.28 cm^{-1}, 1018.41 cm^{-1}, 648.68 cm^{-1}, and 439.77 cm^{-1}.

Overall, the difference in the phytochemical composition of *T. majus* leaves, flowers, and fruits, as well as the difference between our results and those of other researches conducted on the same species, can be explained by the fact that the presence of plants metabolites varies, not only from one plant to another, but also in different parts of the same species (Achakzai *et al.*, 2009). Indeed, Wink (2013) explained that phytochemicals and their concentration are not static; they vary from an organ to another, within a plant's development cycle as well as within and between populations; this variation results in a wide variety of complex mixtures of plant metabolites. In addition, these differences can also be due to factors which influence the biosynthesis of metabolites; in particular harvest period, and climatic conditions such as high temperature, solar exposure, drought, and salinity (Belfekih *et al.*, 2017).

T. majus rich phytochemical composition justifies its recurrent use in folkloric herbal medicine approved by the Andes populationsinthe treatment of several health conditions, endowing this plant with several curative properties such as antibiotic, anti-inflammatory, antithrombin, antihypertensive, digestive, and febrifuge activities (Duke *et al.*, 2009; Garzón G.A. and Wrolstad R.E., 2009). Indeed, the therapeutic quality of medicinal plants is attributed to their diverse content of bioactive substances, including secondary metabolites. For instance, phenolic compounds are recognized to influence the nonspecific immune response, mainly through improving phagocytosis and proliferation of macrophages and neutrophils. They have also been reported to possess the ability to reduce edema in lung tissue (Cornélio-Favarin *et al.*, 2013); therefore,

abundance of phenolic compounds in *T majus* leaves, flowers, and fruits confer, to this plant, the property to be a potent natural anti-inflammatory; which, more precisely, helps in the treatment of respiratory tract inflammation conditions. Saponosides, on the other hand, are known to have hypotensive and cardiodepressant effects; whereas glycosides are considered as natural cardioactive substances that are used to treat congestive heart failure and arrhythmias (Bhandary *et al.*, 2012). Hence, the presence of cardiac glycosides in leaves, flowers and fruits, along with the unique presence of saponosides in fruits may contribute to *T. majus* cardio / cardiovascular protective properties. The latter could be even more accentuated by the presence of coumarins, sulfur compounds, tannins and anthocyanins. Indeed, *T. majus* has been reported to contain benzyl isothiocyanate, a sulfur compound, which endows this plant with antithrombin activity (Medeiros *et al.*, 2000; Santo *et al.*, 2007). Likewise, coumarins are known, since long time, to possess powerful anticoagulant activity; as they are potent vitamin K antagonists used for the prevention as well as the treatment of thromboembolism (Voora *et al.*, 2005). Tannins and anthocyanins are also reported to have hemodynamic effects that lead to the improvement of vasodilatation in patients with coronary artery diseases (Diebolt *et al.*, 2001). Alkaloids, a nitrogen compounds that are found in abundance in *T. majus* leaves, flowers and fruits; are admitted to possess a large number of healing properties including febrifuge, analgesic, antispasmodic, and bactericidal effects (Roy, 2017).

In fact, our results of the conducted preliminary antimicrobial screening further confirm the folkloric use of *T. majus* as a powerful antibacterial and antifungal component. Indeed, results show that, *Bacillus cereus* was most sensitive to all the three extracts. This strain is well-known for causing food poisoning, and *Bacillus* as well as similar genera have long been a source of concern for food producers due to their endospores' resistance (Tewari and Abdullah, 2015). Many efforts have been made to protect food from infection by these sorts of microbes through the use of various types of preservatives; however, *T. majus* extracts, particularly fruits fixed oil, could be a good natural alternative for food preservation. Moreover, the three *T. majus* extracts gave, in general, a good and a noteworthy antimicrobial activity against the rest of the tested strains.*T. majus* bacteriostatic effect against a wide spectrum of Gram(+) and Gram(-) bacteria, such as *Proteus*, *Klebsiella* and *Pseudomonas* genera, was reported (Ghedira and Goetz, 2013). Additionally, bactericidal and fungicidal effects of *T. majus* extracts towards *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* have already been demonstrated (Valsalam *et al.*, 2019; Butnariu and Boston, 2011). However, all germs, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, were resistant to *T. majus* extracts tested by Bazylko *et al.* (2013).

It should be noted that mechanisms of action of active plant substances are not only dependent on the type of extract, its extraction method, and its concentration; but also on the $Gram_{(+)}$ or $Gram_{(-)}$ germ's nature (Basli *et al.*, 2012; Fertout-Mouri *et al.*, 2016). Indeed, it is known that $Gram_{(-)}$ germs are more resistant than $Gram_{(+)}$ germs. This is due to the difference in the outer walls structure of these germs, as $Gram_{(-)}$ germs have a layer of peptidoglycan anchored between plasma membrane and the outer layer composed of lipopolysaccharides and proteins, thus constituting an impermeable barrier to active antimicrobial substances that may diffuse into these germs in order to prevent their growth. Conversely, the peptidoglycan layer in $Gram_{(+)}$ germs is exposed to the outside which allows diffusion of active antimicrobial agents (Fertout-Mouri *et al.*, 2016). These data seem consistent with the susceptibility of $Gram_{(+)}$ germs to *T. majus* extracts. However, despite the potential protection provided by their wall, $Gram_{(-)}$ germs, in our study, showed sensitivity to *T. majus* extracts. This sensitivity could be attributed to the richness of this species in secondary metabolites. Indeed, it has been reported that a large number of chemical compounds, including phenols and their derivatives, fatty acids, anthocyanins, and others, can affect multiple target sites against germ cells. In addition, these compounds adopt several mechanisms; for example, phenolic compounds can bind to membrane proteins through hydrophobic and hydrogen bonds, thereby altering membrane permeability of $Gram_{(+)}$ and $Gram_{(-)}$ germs (Gonelimali *et al.* 2018). Flavonoids, on the other hand, have potent inhibitory effects on several enzyme systems, and can thus inhibit the cell growth of germs (Narayana *et al.*, 2001). Their antimicrobial activity can also be manifested through various other mechanisms such as inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function and energy metabolism (Das *et al.*, 2013). Moreover, the majority of researches conducted on antimicrobial activity of carotenoids have reported their effectiveness on *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus,* and *Candida albicans* (Moreira *et al.*, 2018). Besides, terpenes are also known for their ability to disrupt microbial membranes (Toure, 2015).

Furthermore, plant extracts cause a significant decrease in internal cytoplasmic pH in $Gram_{(+)}$ and $Gram_{(-)}$ germs. This drop in pH leads to cell membrane damage of these germs that is reflected in changes in membrane potential, of which hyperpolarisation is one of the main indicators (Gonelimali *et al.*, 2018). Additionally, plant phytochemicals can act solely as they can combine and act synergistically. Indeed, the synergistic interactions of these compounds enhance their antimicrobial and antioxidant activities by improving their bioavailability as well as the benefits of synergy; which can, by enhancing each other's effect, affect multiple biochemical processes in germs; hence, overcoming their drug resistance mechanisms (Sharma *et al.*, 2020).

Conclusion

The presented results clearly demonstrate that leaves, flowers, and to second degree fruits of *T. majus*, growing in Annaba City in northeast Algeria, are rich in different plant active metabolic substances, mostly secondary metabolites, which could be partly related to the environmental factors of the Mediterranean climate of the city; as well as screening, extraction, and analyzing methods. These valuable components explain the good antimicrobial activity of this plant, especially against food poisoning bacterium "*Bacilluscereus*", and the other antibiotic-resistant germs. Our results confirm the merits of the reputation that this plant enjoys in terms of richness in bioactive molecules; prompting that, locally and globally, this plant should become part of eating habit for people considering only its ornamental use. Moreover, complexity and heterogeneity of the metabolic substances contained in *T. majus* let foresee good antioxidant

and therapeutic properties that are of use, mainly, in medicinal and food industry fields. More generally, our study put the light on this species as a potential source for different nutraceutical products that can be used as dietary supplements or food additives, which promotes this species to be a potential candidate that should be explored by pharmaceutical industry, thus deserving further *in vitro* and *in vivo* studies.

Table 1 : Decoction tubes calibration used for saponosides foam index determination.

Tube number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Diluted decoction volume (ml)	1	2	3	4	5	6	7	8	9	10
Distilled water volume (ml)	9	8	7	6	5	4	3	2	1	0

Table 2 : Results of phytochemical screening performed on the three organs of *T. majus*.

Metabolits			**Leaves**	**Flowers**	**Fruits**
Phenolic compounds			+++	+++	++
Flavonoids			+++	++	++
			Flavones	Flavonols	Flavones
Tannins	**Condensed tannins**		++	++	+
	Hydrolysable tannins		++	++	++
Anthocyanins			+	+++	++
Leucoanthocyanins			+	+++	+
Coumarins			+++	+++	+++
Anthracene derivatives	**Free anthracene derivatives**		-	-	-
	Combined anthracene derivatives	**O-heterosids**	+++	+++	+
		C-heterosids	+++	+++	+++
Mucilage			++	++	++
Alkaloids			+++	++	++
Reducing compounds			+++	++	+
Narcotics			-	-	-
Starch			-	++	-
Saponosides			-	-	125
Sterols			+++	+++	+++
Triterpenes			+++	+++	+++
Carotenoids			++	++	+
Iridoids			+++	+++	++
Free quinones			-	++	-
Cardiac glycosides			+++	++	++
Aminoacids			++	++	++

Table 3 : General Assignment of T.majus extracts FTIR spectra wavenumbers' (cm^{-1}) with the correspondent functional group.

Functional group		**Wave number (cm^{-1})**		
		Leaves-MeOH	**Flowers-MeOH**	**Fruits fixed oil**
Alkynes	RC≡CH	3377.57	3379.91	-
		2123.42	2127.37	-
	Aromatic C≡C	1516.31	1513.39	-
		2925.83	2935.91	2924.47
Alkenes	$RCH=CH_2$ / RR ` $C=CH_2$	1414.36	1414.93	1417.17
	Aromatic =C-H	-	871.66	-
Nitrogenous compounds	Aromatic C-NO_2	1516.31	1513.39	-
	O-NO_2	1633.83	1634.67	-
		1272.91	1278.74	-
	• R-N=C=S / R-N-C	2123.42	2127.37	-
Phenols (OH)		3377.57	3379.91	-
		1414.36	1414.93	1417.17
Carboxylic acids	C=O	-	-	1711.99
		1738.87	-	1746.29
	-OH	3377.57	3379.91	-
Amide	Primary	3377.57	3379.91	-
	Secondary	1516.31	1513.39	-
Amine	Secondary	1272.91	1278.74	-
		-	-	3004.85
	Aromatic (C-N)	1272.91	1278.74	1233.27

		-	-	1367.21
		-	-	1377.96
Sulfur compounds	S=O	1414.36	1414.93	1417.17
		-	-	1233.27
		1056.17	1057.26	-
	RS-H	-	-	2678.69
	C=S	1135.21	-	1165.66
		1056.17	1057.26	-
Alkanes	CH2	1454.81	1454.74	1465.32
		-	-	722.15
	$-CH_2-$	2925.83	2935.91	2924.47
		-	-	722.15
	$=CH_2-$	2853.64	-	2854.11
	Carbonskeleton (C-C)	1272.91	1278.74	1233.27
		1135.21	-	1100.87
		-	-	1118.64
		-	-	1165.66
		-	-	1065
	Aromatic C-H	-	-	3004.85
		925.82	926.15	-
		813.4	871.66	-
		-	818.35	-
		706.27	705.25	722.15
Lipids	Free fatty acids	-	-	1711.99
	Triglycerides	-	-	3004.85
		2853.64	-	2854.11
		1738.87	-	1746.29
Alcohol (C-O)	Primary	1056.17	1057.26	1065
	Secondary	-	-	1118.64
		-	-	1100.87
	Aromatic (C-OH)	1272.91	1278.26	1233.27
		1135.21	-	-
Carbonyl compounds (C=O)	Aliphatic aldehyde / cyclic ketone	1738.87	-	1746.29
	Aromaticaldehyde / ketone	-	-	1711.99
Associated hydroxyl (-OH) (intermolecular H-bond)		3377.57	3379.91	-
		-	-	2678.79

Table 4 : *T. majus* extracts inhibition zones diameters (mm).

Microbial strains	**Leaves-MeOH**						**Flowers-MeOH**						**Fruits fixed oil**					
	Crude	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**	**Crude**	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**	**Crude**	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**
Escherichia coli ATCC 25922	16	10	12	10	10	7	15	14	12	9	9	9	15	10	10	11	11	9
Escherichia coli BLSE	7	10	14	9	<6	<6	<6	8	8	9	11	11	<6	7	8	8	8	9
Klebsiella pneumoniae Scy	16	14	13	9	8	8	7	7	9	9	9	9	<6	8	10	9	9	9
Klebsiella pneumoniae C-	7	11	11	13	13	7	9	9	11	11	13	13	<6	7	8	8	8	8
Klebsiella pneumoniae C+	7	7	8	8	8	10	8	8	8	10	9	10	<6	<6	7	7	8	9
Serratia marcescens	9	11	11	10	10	9	<6	8	8	9	16	15	<6	7	8	10	9	9
*Pseudomonas aeruginosa*ATCC 27853	9	10	10	8	7	7	8	9	9	10	10	10	7	9	9	8	7	7
Pseudomonas aeruginosa VIM II	18	22	11	9	8	8	10	9	<6	<6	<6	<6	17	13	12	8	8	7
Acinetobacter baumannii	7	8	10	10	10	9	9	9	9	9	11	11	<6	7	7	8	8	11
Acinetobacter baumannii IMP®	7	9	12	12	10	8	9	10	10	11	12	12	<6	7	7	7	8	10
*Acinetobacter baumannii*NDM-1	7	9	11	11	10	10	8	10	13	13	12	10	<6	7	8	8	9	10
*Staphylococcus aureus*ATCC 43300	7	9	10	10	8	7	10	10	13	13	14	14	<6	8	10	10	11	10
Staphylococcus aureus MRSA	9	10	9	7	13	9	8	8	10	10	10	10	8	9	12	12	11	9
Bacillus cereus	14	10	8	8	7	7	19	15	10	10	9	9	39	29	22	19	12	10
Enterococcus faecali ATCC 29212	<6	8	9	8	<6	<6	8	9	10	10	11	13	<6	8	8	9	9	9
Candida albicans	7	8	11	11	7	8	8	9	9	11	11	13	<6	7	7	9	9	9

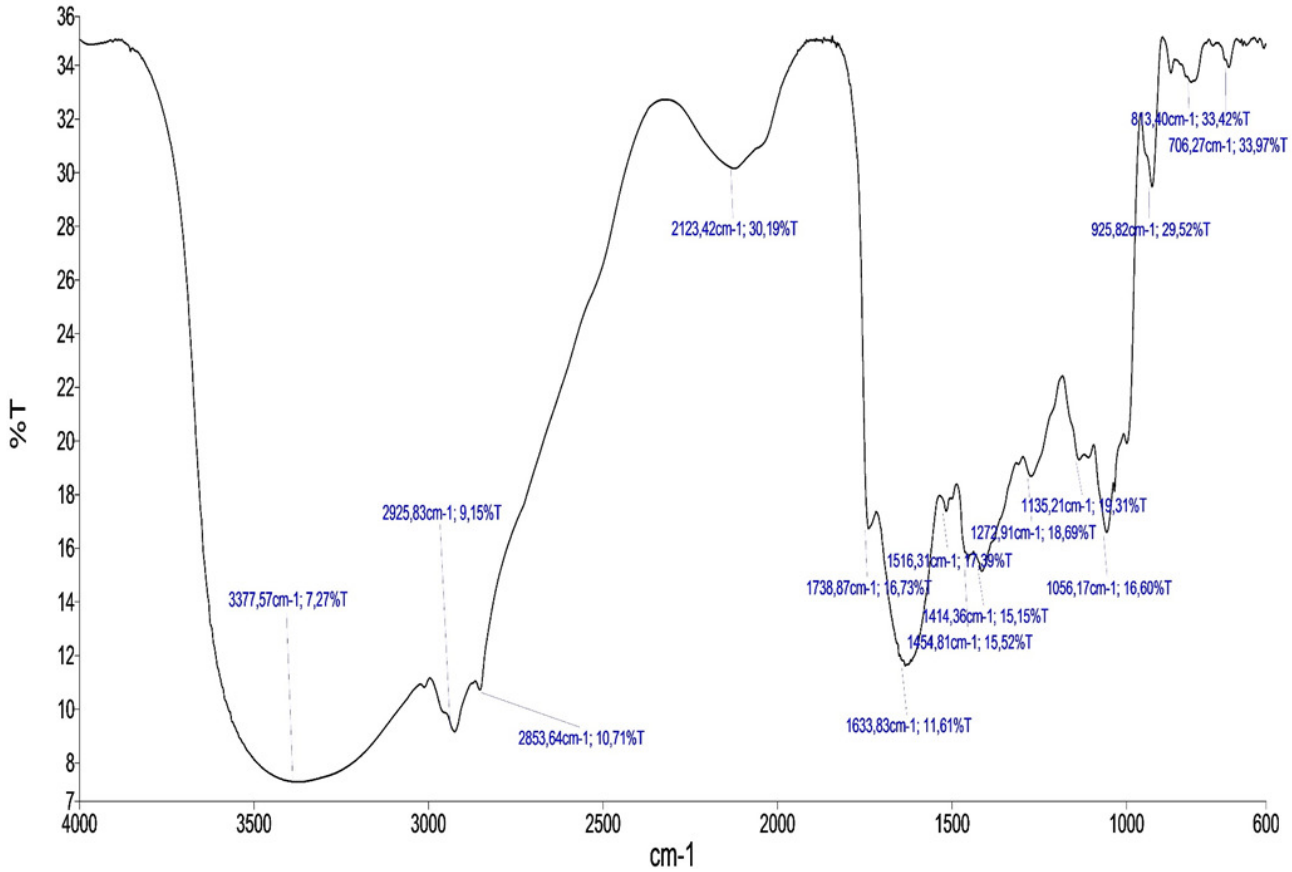

Fig. 1 : FTIR spectrum obtained by infrared analysis of *T. majus* Leaves-MeOH extract.

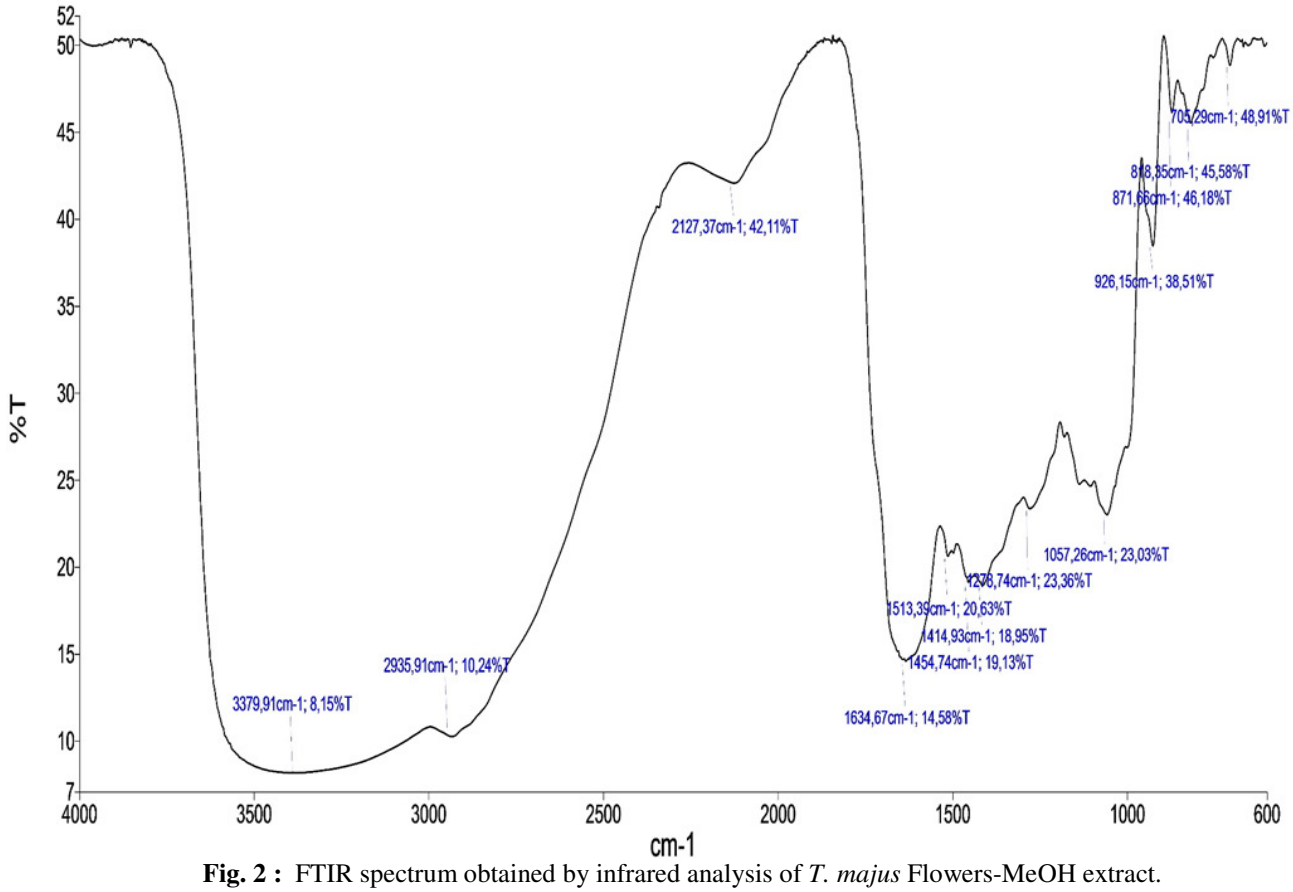

Fig. 2 : FTIR spectrum obtained by infrared analysis of *T. majus* Flowers-MeOH extract.

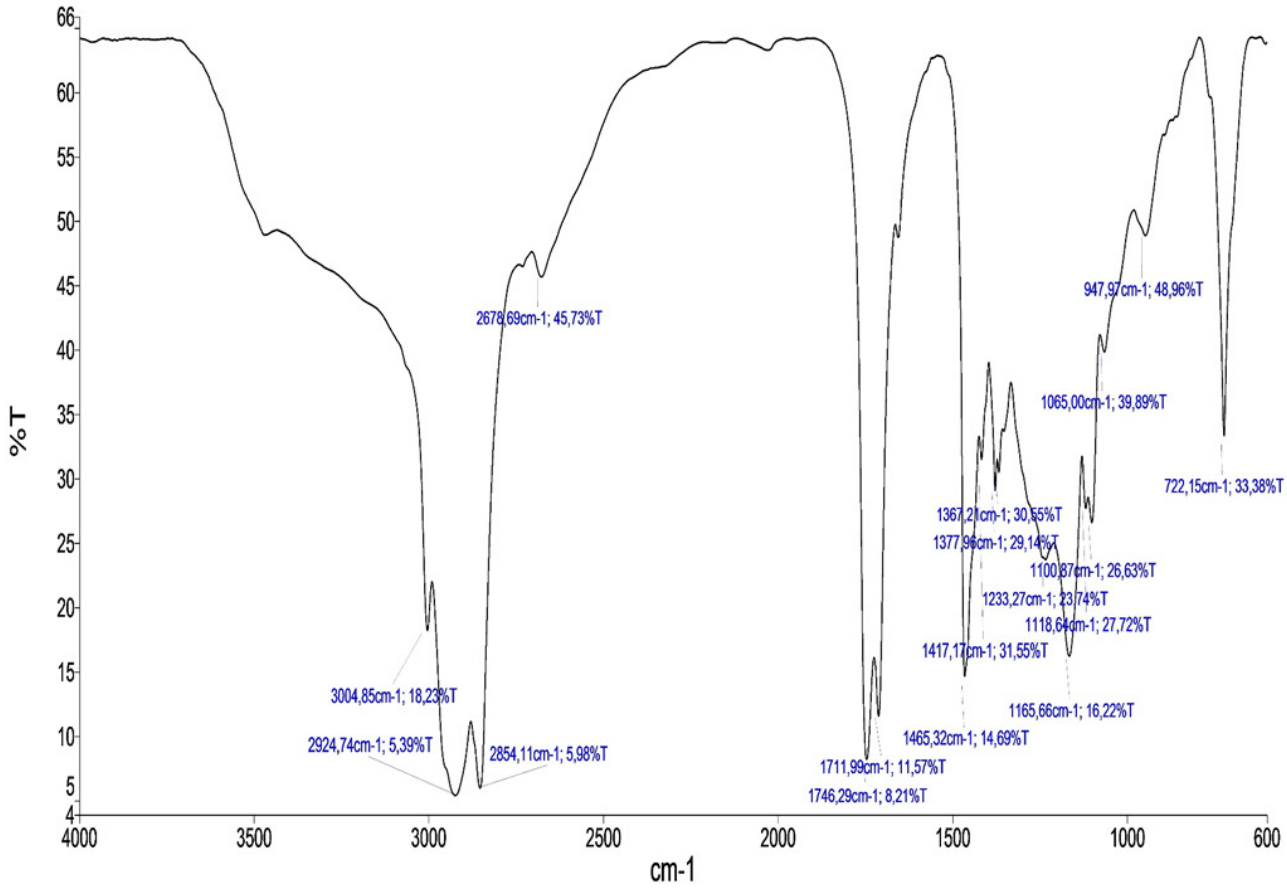

Fig. 3 : FTIR spectrum obtained by infrared analysis of *T. majus* fruits fixed oil.

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