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MARRUBIUM ALYSSON L. LEAVE EXTRACT: PHYTOCHEMICAL COMPOSITION, ITS ACTIVITY AGAINST GUT MICROBIOTIC

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ABSTRACT

The present study aimed to identify the Four habitats were selected for collecting plant data, sampling and analysis of the study plant (Marrubium alysson) where The statistical analysis (ANOVA1) detected significant variation in ether extract, crude fiber and hemicellulose organic nutrients content of M. alysson among the study habitats and non-significant variation in carbohydrate and celluose. The highest average of crude fiber and hemicellulose were recorded in orchards and cultivated crops, the lowest of Ether extract were recorded in Agricultural roodsides and Cultivated crops. detected high significant variation in total Flavenoid and total phenolic content among the study habitats.the highest average of total Flavenoid, were recorded in orcards, while the lower, were in Agricultral roadsides. The and lowest highest value of total phenolic content, was recorded in cultivated crops and orchards. in this study is to make four extracts separate of M. allyson in pure form and her activity against gut microbium, were its leaves contained Phytochemical investments of total flavonoids, carbohydrates and total phenolic compounds, this study showed the ethanol extract the highest anti gutbacterial activities, follwed by benzeine extract, methanol and chloroform . where B. copri are the most sensitive to the Ethanol extract, while B. subtilis was the most resistant to all extracts.

Keywords: Gut microbium، Habitats, Marrubium Alysson, Phytochemical,

1. INTRODUCTION

Recently, almost eighty percent of Worldwide uses medicinal plants for their therapeutic activity According to the WHO[1].where 25 to 50% of current pharmaceuticals are derived from plants [2]. However, the botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and The vast antimicrobial effects of medicinal plants are basically dependent on their phytochemical constituents. where, the

phytochemical constituents of plants fall into two categories based on their role in metabolic processes, namely primary and secondary metabolites [3].

Traditional medicine is based largely on many Herbal medicines species to treat different ailments and for their medicinal value in the rural and tribalareas [3-4], where great contributed toward maintaining human health About 80,000 of flowering plants are used for medicinal purposes by the peoples worldwide, The most important of which is the Lamiaceae family, Marrubium alysson (M. alysson) [4-5].

The genus Marrubium L. (Lamiaceae), Family Labiatae, contains 200 genera and 3,000 species. Plants belonging to this family have been used to treat various diseases [6]. wheres that Marrubium is the most popular plant remedies, used as hypoglycemic, expectorant and influence on bile secretion, So White horehound was included in the pharmacopoeias and Merck's Index of Phytotherapy (1910), [7-8], It is used also in the treatment of cold, cough, asthmaand diureticand in the form of decoction with honey syrup [9-8-10]. as atreatment for disorders to skin, liver, gastroprotective, immune system and pulmonary disorders [11-12-13] and antiviral. [14] The Lamiaceae family, Marrubium alysson (M. alysson) is an asterid dicot genus of Old World aromatic perennial herbherbs, [13] It is belong taxonomically as the mint family of flowering plants. [15-16]. is commonly distributed in north africa and its Arabic name is 'Hashisha Rabiah' or Marute" in the Mediterranean herbal region, [13-17] While commonly known as white horehound in Europe,[13] species of this family are Extremely aromatic and produces volatile oil [18] Marrubium alysson is native to the Americas and the main centers of diversity are north- Africa is extensively, where was found on the Mediterranean coastal strip from El-

Sallum to Rafah, as well as the Sinai desert.[8-19] distributed in areas raising sheep, Its height is about one-foot , branched , densely covered with a thick, white, and cottony felt [19]. Also plant grows in waste ground Sometimes. [19-20].

It is noteworthy that M. Alysson for many pharmacological activities such as antihypertensive, anti-oxidant, inflammatory, diabetic, asthmatic, bacterial, and antifungal effects,[21] thes plant has been shown to possess a number of accumulating flavonoids. these flavonoids have important free radical scavenging activities [13].

2. MATERIAL METHODS

2.1 Plant collection

Twelve samples of leaves were collected from the study sites. The whole plant of *M. alysson* L. used in this study was collected once through the growing season of March 2021 from El Hawary farms in Benghazi, Libya of four habitats (Orchards**,** Cultivated plants, Agricultral rood sides, Cultivated crops). air- dried in the shade, ground to a fine powder and stored at low temperature (20ºC).

2.2 Phytochemical study:

Chemical analyses of tested samples were analyzed according to AOAC methods for crude fiber and ether extract as the following:

2.2.1 Firstly: Crude fiber (CF) analysis:

Crude fiber content was determined as follows:

2.2.1.1 First stage

Two grams of sample is put into 250 ml conical flask and 1.25% Sulfuric acid solution was added. The sample was heated for 30 min, filtered and washed. The layer of tissue with 125 micrometer pore size was placed in the Buchner flask.[22]

2.2.1.2 Second stage

The residual material on layer of tissue is transferred into conical flask and 1.25% NaOH solution we are added. The sample is heated for 30 min, filtered and washed with water. The whole residual material is transferred into crucible and dried for 12 h at 105°C and weight of dried crucible was recorded (W1). After that the crucible are placed into muffle oven at 600°C for 3 hrs and weight of crucible are recorded (W2).

% CF ={ W1 – W2} * 100 / weight of sample.[22-23-24]

2.2.2 Secondly: Fat (Ether extract, EE)analysis:

The lipid content is determined by directly extracting the sample with Diethyl ether in an intermittent Soxhlet extractor for 8 h. The residue in round bottom flask after solvent removal represents the lipid content of the sample.

% EE = quantity of fat extraction * 100 / weight of sample.[25]

On the other hand, cell wall constituents (fiber fractions) composed of (ADL, NDF and ADF) determined according to F.Ma, Also, cellulose and hemiceullose was calculated by difference as follows:

Hemicellulose = NDF – ADF

Cellulose = ADF – ADL[26]

Total flavenoids and phenolic combound was according to methods are outline by Allen (1989) [27]

2.2.3 Preparation of extracts

Twenty gram of leaves powder dissolved in 100 ml benzene 99% for 30 min at room temperature. Also filtration with Whatman No.1 filter paper, the Extract was stored In airtight container at 4 C° in refrigerator[28]. Filtrate was Saves In sealed tubes for use later. (Benzene Extract). Dried precipitate and added a100 ml chloroform with stirring for 20 Minute we get to run the (chloroform Extract). Also dried precipitate and added him 100 ml of 70% ethanol with Altharikk for a simple and Then we get to run on (Ethanol Extract) 100 grams of the plant has added 250 ml of distilled water with 250 ml of methanol, the mixture is stirred and then we get to run on (methanol extract). [27]

2.2.4 Method Used for Screening

2.2.4.1 Anti- Microbial activity

Evaluation of the antimicrobial activity, the 'agar holes well method was used for the antimicrobial susceptibility testing following (*Bacillus subtilis, Staphylococcus aureus, Bacteroidetes vulgatus, Bacteroidetes rodentium, Bacteroidetes copri, Escherichia coli lactobacillus acidophilus*), Antimicrobial potentialities were expressed as the diameter of inhibition zones. plant extracts were examined as antimicrobial agent against all microbial isolates. Inoculum suspensions of all bacteria isolates were spread on the surface media. Equidistant (1 cm diameter) holes were made in the agar using sterile Cork borer, Plates were left in a cooled incubator at 4 °C for one hour and then incubated at 37 °C for 24 hour . Inhibition zones developed due to plant [29] active

extracts ingredients were measured after 24-48 hours of incubation. The zone of inhibition was measured with the help of standard scale [30]. The experiments were carried in Faculty of Education, Benghazi university, for Microbiology research Laboratory.

3. RESULTS

3.1 Phytochemical study

The phytochemical screening of M. alysson extracts in four habitats (Alhawary). showed the presence of carbohydrates, flavonoids, amino acids, phenolic compounds, ether extract, crude fiber, cellulose and hemicellulose.

3.1.1 Organic Nutrients

3.1.1.1 *Primary Metabolites*

The statistical analysis (ANOVA1) detected significant variation in ether extract, crude fiber and hemicellulose organic nutrients content of *Marrubium alysson* among the study habitats and non significant variation in carbohydrate and celluose (Table 1). The highest average of crude fiber and hemicellulose (12.4, and 17.6 % respectively) were recorded in orchards and cultivated crops, while the lowest average of crude fiber (5.3 %) was recorded in Cultivated crops.the lowest of Ether extract (1.2 and 2.2% respectively) were recorded in Agricultral roodsides and Cultivated crops. The highest average of carbohydrate (34.63%) was recorded in Orchards, while the lowest average (28.74%) was recorded in Cultivated crops. The lowest and highest value of cellulose (13.3 and 17.0% respectively) was recorded in cultivated crops, Orchards and Cultivated plants.

TABLE 1. MEAN AND STANDARD DEVIATION OF THE ORGANIC NUTRIENTS (%) OF MARRUBIUM ALYSSON (LEAVES) IN DIFFERENT HABITATS, EE: ETHER EXTRACT, CF: CRUDE FIBER, HE: HEMICELLULOSE, CE: CELLULOSE AND CP: CARBOHYDRATE, OR: ORCHARDS, CUP: CULTIVATED PLANTS, AR: AGRICULTRAL ROODSIDES, CUC: CULTIVATED CROPS.

Habitat	Organic nutrients (%)							
	ADF	EE	CF	NDF	HE	CE	ADL	CP
OR	20.5±0.5a	3.9±0.20a	12.4±1.8a	36.9±0.5a	16.3±0.3c	17.0±0.8a	3.5±0.1a	34.63±0.5a
CUP	20.5±0.5a	3.2±2.61b	10.5±6.5b	36.9±0.5a	16.3±0.3c	17.0±0.8a	3.5±0.1a	32.63±0.5ab

AR	20.1±0.3b	1.2±2.6d	10.0±6.2b	36.6±0.3b	16.5±0.2b	16.6±0.4b	3.5±0.1b	34.29±0.7ab
CUC	16.1±0.2c	2.2±2.5c	5.3±3.0c	33.7±0.2c	17.6±0.1a	13.3±0.2a	2.7±0.1a	28.74±0.2b
F-value	0.26	6729.1***	12.75***	1.32	1405.9***	0.5	1.292	1.564

*****: $p < 0.001$**

3.1.1.2 Secondary Metabolites

The statistical analysis (ANOVA1) detected high significant variation in total Flavenoid and total phenolic content of M. Alysson among the study habitats (Table 2). The highest average of total Flavenoid,(12.9 mg g-1) were recorded in orcardes, while the lowest (5.27 mg g-1) were in Agricultral roodsides. The lowest and highest value of total phenolic content (6.33 and 10.26 mg g-1) was recorded in cultivated crops and orchards respectevily.

TABLE 2. AMOUNTS OF TOTAL FLAVENOID AND TOTAL PHENOLIC CONTENT IN DIFFERENT HABITATS OF MARRUBIUM ALYSSON. MMAXIMUM AND MINIMUM VALUES ARE UNDERLINED, CUP: CULTIVATED PLANTS, AR: AGRICULTRAL ROODSIDES, CUC: CULTIVATED CROPS.

Habitat	**Total flavenoids**	**Total Phenolics**
	(mg g^{-1})	
OR	12.9±0.2a	10.26±0.7a
CUP	10.23±0.3b	10.24±1.0a
AR	5.27±0.3d	9.73±0.5a
CUC	6.52±5.8c	6.33±0.3b
F-value	**433.447*****	**35.80*****

*****: $p < 0.001$**

3.2 Anti- Microbial activity

3.2.1 Ethanol extract

The ethanol extract (70%) of Marrubium Alysson had strong antimicrobial activity on the growth of the microbial isolates (Fig 1). The highest antimicrobial activity (47 mm inhibition zone) was attained by

applying the extract from the M. Alysson against P. copri, followed by 36 mm from applying extract against B. rodentium, while the lowest activities (12 and 18 mm) on B. vulgatus and L. acidophilus, respectively. However, Bacillus subtilis, E.coli and Staphylococcus aureus showed no sensitivity towards Ethanol Extract.

3.2.2 Chloroform extract

The chloroform extract of M. Alysson had antimicrobial activity on growth of the microbial isolates (Fig 1). It was found that Marrubium chloroform extract had no effect on L. acidophilus, B. subtilis and S. aureus. The highest effect (31 mm inhibition zone) was that of extract against B. vulgatus, followed by extract on E. coli (28 mm), while the lowest effect (12 mm) was recorded for extract on B. rodentium and P. copri.

3.2.3 Methanol extract

The methanol extract of M. Alysson collected from the different samples had no effect on B. subtilis (Fig 1). The methanol extract had its highest effect on P. copri with inhibition zone of 39 mm, followed by B. vulgatus, B. rodentium, E. coli, L. acidophilus and S. aureus (26, 23, 21 and 19 mm, respectively)

3.2.4 Benzene extract

The results of the biological activity of the benzene extract of Marrubium Alysson indicated that 4 microbial strains of the investigated organisms showed high susceptibility. The largest inhibition zone (45 mm) was recorded due to the effect of extract against P. copri

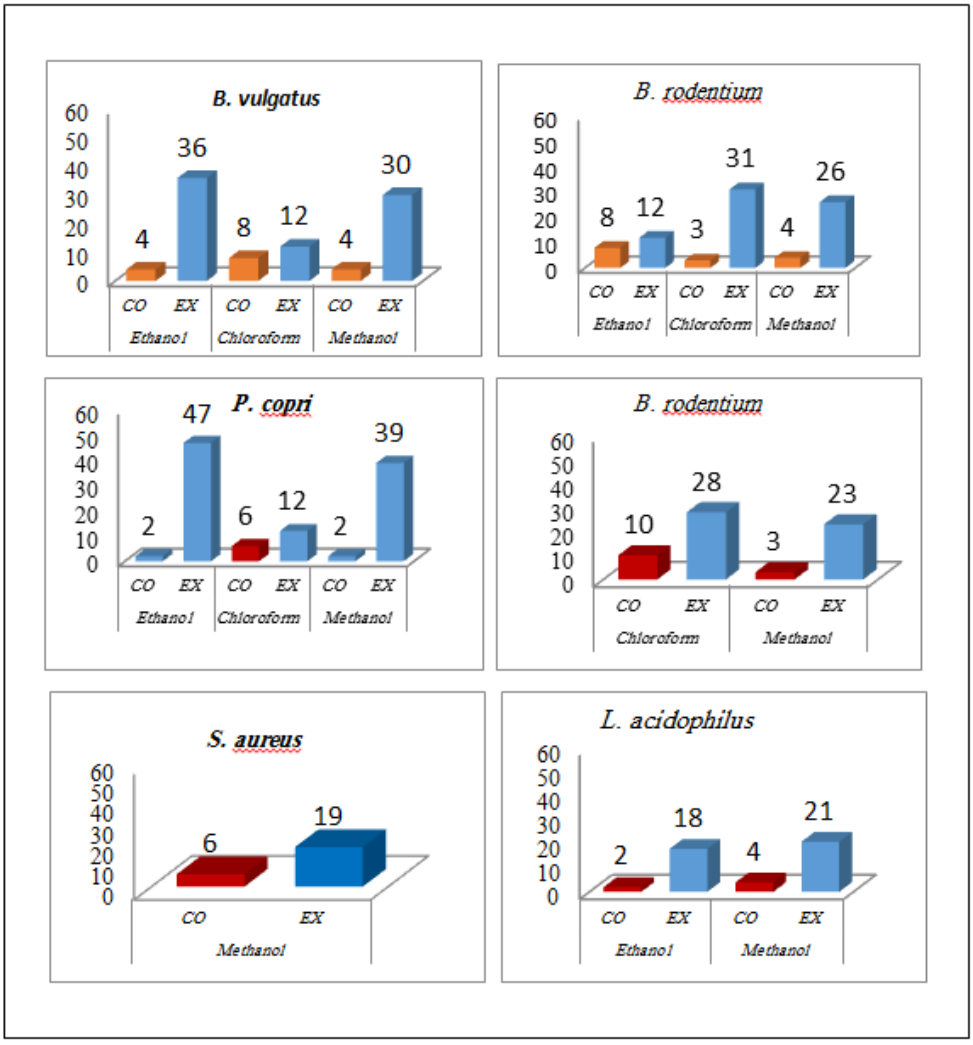

Fig.1 Antibacterial activity of Marrubium alysson extracts and chemicals solvent (control) against bacteria

4. DISCUSSION

Marubium Alysson L. the common name of the species is (White horehound) play an important role in antimicrobial activity diversity Alla [8].The results indicate the existence of anti bacterial compounds in the leaves extracts of M alysson, the most

important were total phenolic, total flavonoids and carbohydrates -14-10] [21 . M. alysson had a Minimum Inhibition zone varied from No effect against Protobacteria and Firmicutes to Maximum effect against Bacteroidetes (P. copri).

This is consistent with Alla and Edziri [8-15]. The affect observed for some of the different extracts in this study is in support for further isolation of the compounds responsible for the observed gut antimicrobial activity which that against B. vulgatus, B. rodentium, P. copri, and E. coli, whil minimum effect against S. aureus and L. acidophilus, it is no effect against B. subtilis. in particular the activity of the Ethanol, Benzine and Methanol Extract of M. alysson against some Bacteroidetes could provide future antibacterium active principles, This is consistent with Alla [8].

5. CONCLUSION

M. alysson L. extracts collected in Benghazi, libya showed antimicrobium activity, This study indicates that the M. alysson is a natural source of Antimicrobium molecules that needs further investigation to isolate its bioactive compounds and to help understanding of mechanism of action.

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