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REVIEW ARTICLE

QUANTITATIVE ASSESSMENT OF CHEMICAL COMPOSITION, ANTIMICROBIAL EFFICACY AND SOME ACTIVE COMPOUNDS OF MERREMIA DISSECTA

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ABSTRACT

The current search targeted the type *Merremia dissecta* (JACQ) from the family Convolvulacea. It is one of the plants that have not been studied in Iraq previously and saw a rapid spread after 2003 which were seen developing in the fields and spaces of the Faculty of Agriculture at the University of Baghdad in,), so the study aimed to documentation the plant locally and conducted chemical analysis of Merremia leaves showed presence of fat, protein, carbohydrate, ash, fiber and dry matter with percentage 0.57, 24.25, 22.74, 5.69, 10.28and, 94.31%respectively. By qualitative detection were indicated the presence of tannins, alkaloids and with greater extend glycosides and flavonoids. The percentage of phenols 52.98 and 87.7, while for flavonoids were 2.65 and 7.5 mg / 100mg for aqueous and Ethanolic extract respectively. The aqueous extract has an inhibition activity against negative bacteria P. aeruginosa and gram-positive Staphylococcus aureus with a rate of inhibition diameter 10 and 7 mm respectively. It also has an inhibition effect toward Aspergillus oryzae with inhibition percentage 26.56, 29.68 and 39.06 % at concentration 1000, 2000, 300 ppm respectively and with inhibition percentage reached 27.5, 30.0 and 45.0 % toward Penicillium. spp at the same concentrations also noted increasing of inhibition percentage with increased concentration against two types of mold. Identification some active groups by FTIR technology and existence of: O-H, C-C, C=C, C-O, C=O, C-H and N-H with different stretching frequencies. The Ethanolic extract showed only a stretching frequency between 3000-3250 cm-1 indicating group N-H.

KEYWORDS

Merremia dissecta, Antimicrobial activity, Active Compounds, Phenols, Flavonoids

1. INTRODUCTION

The current search targeted the type registration *Merremia dissecta* (JACQ) from the family Convolvulacea for the first time which were seen developing in the fields and spaces of the Faculty of Agriculture at the University of Baghdad in 2013, Their herbal samples preserved in the Iraqi National herbarium (Baghdad-Ibu-Ghraib) *Merremia dissecta* was first discovered in the Caribbean, then Florida, Mexico, Historically and currently, the species has been used as a condiment, medicine, ornamental by an array of cultures , it is perennial climbing plant with slender twinning stem growing from long, thin taproot , the stems can be 3-6 meters long and up to 2 cm in diameter, scrambling over the ground and twining into nearby vegetation for support, the plant is harvested from the wild for local use as a medicine [1] .

Growth habitat as open grasslands and along roadsides, at elevations from sea-level up to 300 meters, the plant is sometimes cultivated as an ornamental, it has often escaped from cultivation and become naturalized, spreading by means such as shattering capsules that disperse seeds around the parent plant; plants spreading by rooting at nodes; and fragmentation [2]. The leaves smell like bitter almonds and are used in India for making liquor , an infusion of the leaves is taken as a sedative in the treatment of chest complaints, cold infusion , is a remedy for giddiness, snake bites or, a hot infusion is taken to relieve urinary infection, a decoction of the whole plant, used as a wash, is an effective remedy for scabies and itch, a poultice of crushed fresh leaves is applied as a resolute and sedative for treating inflammations, the leaf contains cyanogenetic heteroside, this vine has escaped from gardens and taken over roadsides, disturbed sites, riparian areas and coastal vine thickets in northern Australia intoxication [3].

2. MATERIALS & METHODS

2.1 Samples Collection and Preparing

Merremia plants collected from college of Agriculture / University of Baghdad (Ibu-Graib), the samples are dried in the shade; and grinding at chemical tests.

2.2 Extraction

Extraction by water to get aqueous extract (AE) according to the method mentioned by a researcher, While Ethanolic Extract (EE) gained by according to the method described by another researcher, then extract dried and sterilized by Millipore (0.45um) for antimicrobial tests [4,5].

2.3 Determination of chemical composition of Merremia leaves

The estimation included protein, fat, fiber, ash and moisture [6].

2.4 Carbohydrate was estimated according to the following equation

(Total Carbohydrate % = 100 – (protein % + fat % + fiber % + ash + moisture)

2.5 Phoytochemical Tests

Effective plant compounds were detected, glycosides as mentioned in a research according to method of a researcher [7,8]. Phenols, flavonoids,

Tannins [9,10]. Alkaloids, according to method of a researcher Saponins following the method of another researcher [11].

2.6 Quantitative Assessment for Some Active Compounds

2.6.1 Total phenols

Added 0.5 ml extract aqueous or ethanolic (1mg/ml) to 2-5 ml folin – ciocatteu reagent, then added 2ml (Na2 CO3 7.5%) mixture left 30 min, absorption was measured at 760 nm depending gallic acid as reference [12].

2.6.2 Flavonoids

Added 5ml distilled water to 1ml extract aqueous or ethanolic (1mg/ml) in volumetric flask (10ml) then added 0.3 ml ((NaNo2 5%), after 5 min added 0.6 ml (Alcl3), after that added 2ml (NaOh 1ml), five minutes later, complete the size the mark. Absorption was measured at 510 ml depending rutin as reference [13].

2.7 Determination of antimicrobial activity

Estimated antibacterial activity by filter paper discs diffusion speared 0.1ml of bacterial suspension with age (18 hr) by shape rod on nutrient agar plate and potato dextrose Agar for yeasts, distributed discs were in petites regularly Download each disc (10 ul- micro-liter) extract plate put in incubator 37c, 24hr for bacteria 28c, 48hr for Yeats, The diameter of the inhibition zone around the disc was measured to determine the ability of extract inhibition of growth [14].

2.8 The inhibition effect of extract

Prepared spore suspension (2.03 x107) spore/ml for Angier then epilated 3 arrival 104 ×2.01 spore /ml Penicillium spp its suspension (2.1 × 104) spore/ml save, prepared concentrations from extract :1000,2000,3000 g/ml mixed with medium PDA (sterilized and cooled) 45-50 cº. put one deep from suspension in the middle to plate for each concentration by repeating, left one plate control (without extract) then incubation plates for 7 days at 28 c [15]. Calculated inhibition percentage on the following equation:

percentage of Inhibition % = (growth rate of mould colony(control)-growth rate of mold treatment×100)/(growth rate of mould colony(control))

2.9 Analytical Study

Spectroscopy of forrier transmition Infrared maked tablets from samples to analyzed 40 mg (sample)with 120 mg potassium bromide, mixed well for 10 min, then pulled 40 mg and pressed hydraulics in forrier transmission infrared system (with 8 bar) for 60 sec, after that put pressed tablets in discator 80cº for 16 hour analyzed with frequency (500-4000) cm -1 [16].

3. RESULT AND DISCUSSION

The table (1) shows approximate chemical analysis of Merremia leaves, the percentage of fat 0.57, while the same table shows a high percentage of carbohydrate and protein; proteins play an important role in human health, especially in developing countries, since the average protein in less than what is required. It is necessary to find sources of new edible protein [17]. Plant proteins remain a very important source of food and raw materials for human and animal consumption [18]. Proportions of proteins in Merremia was 24.22%. Carbohydrates which are important food elements because of the content of sugars and their derivatives are the main source in the processing of the body energy, it was 22.74%. Also, the table shows the percentage of ash 5.69%, the percentage of fiber was 10.28 %. not a few dry matters in Merremia leaves was 94.31%.

Table 1: The percentage of basic components in Merremia leaves.

Component	Percentage %
Fat	0.57
Protein	24.25
Carbohydrate	22.74
Ash	5.69
Fiber	10.28
Dry matter	94.31

The active compounds of *Merremia dissecta* were detected in its leaves and observed alkaloids, glycosides, tannins, saponins, phenols and flavonoids, as shown in the table (2) for both aqueous and alcoholic extract, the idea of this study come after a broad appearance in Iraq after 2013, which has attracted our attention especially since the plant is a medicinal plant known and has multiple benefits (vital effectiveness).

The first step was to collect it and send a sample to Iraqi national herbarium for verification that was confirmed.

Table 2: Quality Chemical Detection of Active Compounds in Merremia Leaves.

	compound	Detection used	Detection Guide	Detection Result
1-	Tannin	1- lead acetate 1% 2-ferric chloride 1%	Gelatinous residue Blue- green color	+
2-	Glycosides	1-Fehling reagent A 2- Fehling reagent B	Red residue	+ +
3-	Alkaloids	1-Mayer reagent 2-Picric acid	White residue White residue	+
4-	Flavonoids	Ethyl alcohol 95% + KoH50%	Yellow Color	+ +
5-	Saponin	With agitation	Stalde bubbles for two minutes	+
	phenols	Folin reagent	blue color	+

Some of these active compounds were quantified, phenols and flavonoids were 52.98, and 2.65 mg /100 gm respectively in aqueous extract while reaching 87.7 and, 7.5, mg /100 gm respectively in alcoholic extract, the amount of phenols and flavonoids are higher in the alcoholic extract than water extract, Table (3). The extraction method and the type of solvent used in the extraction as well as the extraction temperature and the time,

also polarity of phenol compounds extracted all these factors are influence the process of extracting compounds [19]. The absolute solvents characterized by low solubility of phenolic and polyflavonoids compounds [20].

Table 3: Quantification of Phenol, Flavonoids in Merremia leaves mg/100gm

Extract	Phenols	Flavonoids
Aqueous extract	52.98	2.65
Alcoholic extract	87.7	7.5

3.1 Antimicrobial activity

The test of determination phenols and flavonoids gave an indication of the presence of phenols have a number of bioactivities including: antimicrobial activity, which it studied. The aqueous extract *of Merremia dissecta* do not show inhibition effect towards bacterial isolates, excepting only one gram positive bacteria Staphylococcus aureus and also one gram negative bacteria Pseudomonas aeruginosa with rate of diametrer7 and 10 mm respectively, table (4) while the same extract showed a disincentive effect toward mould Aspergillus oryzae and Penicillium spp. With inhibition percentage reached 26.56, 29.68 and 39.06% for the first and more than for the second 27.5, 30.0 and 45.0 % from lower concentration 1000 ppm to 2000 ppm and 3000 ppm respectively (table 5). Also observed increasing of inhibitory percentage with increased concentration.

Table 4: The Inhibitory effect of Merremia leaves in growth of some species of Microorganisms

Microorganisms	Diameter Rate of Inhibition zone (mm)
	Aqueous extract
Bacillus cereus	-
Escherichia coli	-
Pseudomonas flourescens	-
Pseudomonas aeruginosa	10 mm
Staphylococcus aureus	7 mm
Salmonella typhimurium	-
Saccharomyces cerevisiae	-
Candida albicans	-

- No Inhibition Effect

∗ With disk diameter (4 mm)

Table 5: Effect of the water extract of Merremia leaves in inhibition the growth of Mold Aspergillus oryzae and Penicillium spp

Extract	Concentration ppm	*A. oryzae*		*Penicillium spp*	
		Growth rate (cm)	Inhibition percentage %	Growth rate (cm)	Inhibition percentage %
Aqueous	1000	0.47	26.56	0.58	27.5
	2000	0.45	29.68	0.56	30.0
	3000	0.39	39.06	0.44	45
	Control	0.64	-	0.80	

3.2 The identification of active group by "FTIR"

It was shown for both aqueous and ethanolic extract of *Merremia dissecta* bonds frequencies for active groups, as characteristic in table (6) and its figures 1 and 2 about the appearance of adsorption bonds refer to (OH) group at stretching frequencies 3371 and 3379 cm-1for aqueous and ethanolic extract respectively, back to phenolic OH groups [21]. Bonds frequencies for (CH) group ranged from 2931 to 2961 cm-1 for aqueous extract and between 2864-2927 cm-1for ethanolic extract, represented by a group CH3group [22]. The extracts gave frequencies between 1327-1381 to 1408-1446 cm-1 for aqueous extract refer to C=C group, while ranged from 1377,1411 and 1450 cm-1for ethanolic extract, may be back to benzene ring, these results are agreeable with was found by a researcher in waste of squeezed grape extract [23,24]. We found (C-O) group with vibration frequencies1203-1276 and 1273cm-1 for aqueous ethanolic extract respectively, also other vibration frequencies near to it, this was mentioned by a researcher absorption bonds near from 1159cm-1 refer to C-O group [25]. The extracts gave bonds with frequencies 1608, 1612 and 1669 cm back to C=O group. The regions in which functional groups absorb are show bonds illustrate in figures.

Table 6: Absorption Bonds by "FTIR" for Merremia leaves.

Extract	Stretching frequency /functional group cm^{-1}								
	H	OH	C-H	C-O	C-C	C=C	C=O	H	N-H
Aqueous	3372	2931 2961	1203 1276	1203 1276	1056 1076	1327 1381	1612 1608	771 810	
Ethanolic	3377	2864 2927	1273	1273	1022 1080	1377 1411 1450	1669	713 767 817	3000-3250

(C-C) group appearance its frequency between 1022-1080 cm-1 for aqueous extract and 1056-1076 cm-1 for Ethanolic extract, and appearance of different absorption bonds of intensity at the frequency region 700 to above 800 cm-1.The stretching vibrations of single bonds to hydrogen give rise to the absorption at the high frequency end of the spectrum as a result of the low mass of the hydrogen atom [26].

The ethanolic extract showed only a stretching frequency between 3000-3250 cm-1 indicating a group of (N-H).

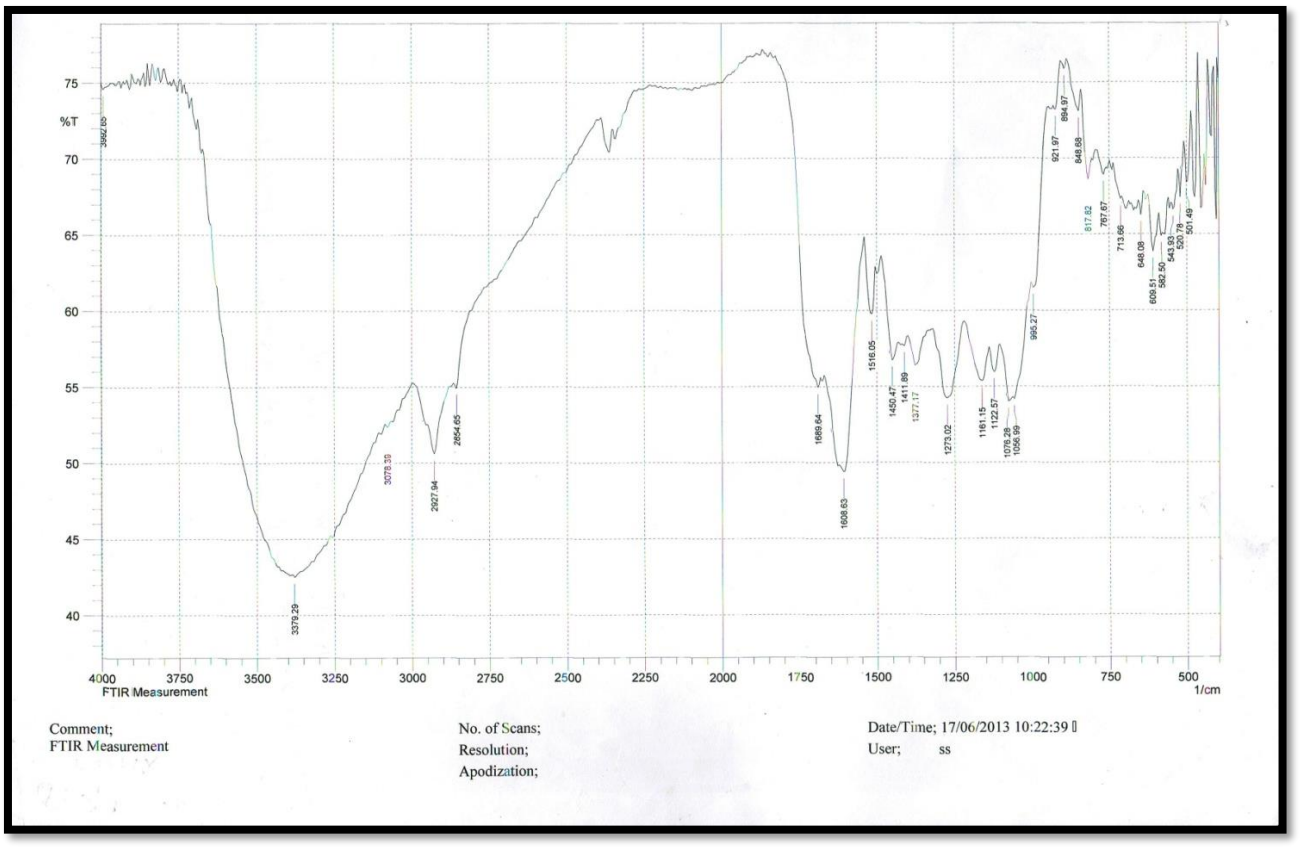

Figure 1: Analysis by spectroscopy FTIR for Ethanolic Extract of Merremia leaves

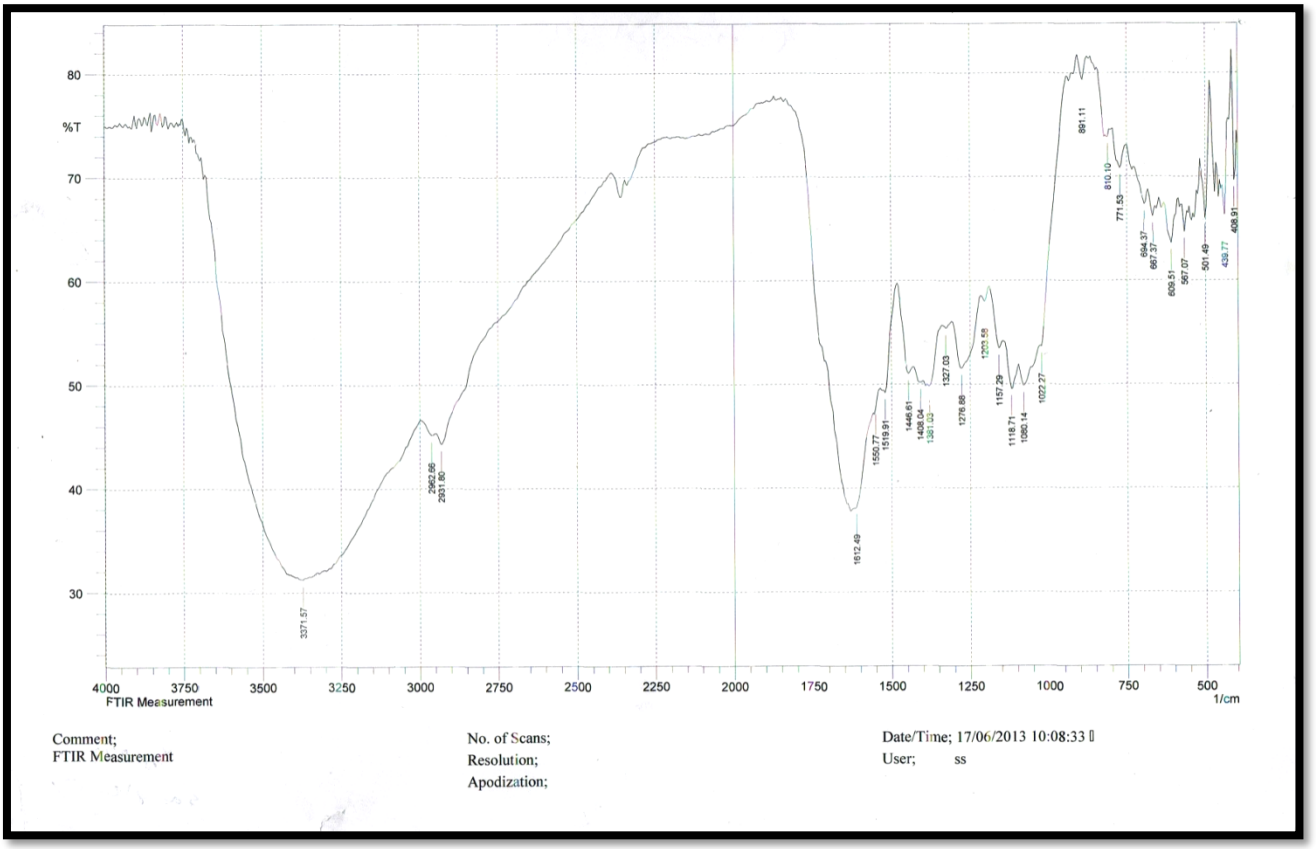

Figure 2: Analysis by spectroscopy FTIR for Aqueous extract of Merremia leaves.

4. CONCLUSION

Each country has a diversified plant wealth depending on the nature of its geographical and climatic diversity. Merremia plants are a rich source of active compounds that have a variety of activities that can be exploited in different applications, including the pharmaceutical industry m the biological treatment of plant disease and food applications by adopting the effectiveness of compounds in the inhibition of pathogenic microorganisms that cause food poisoning and vector disease by food. The effectiveness of compounds in delaying and inhibition of lipid oxidation,

then preservation of food and prolonging the life of food.

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