ISOLATION AND BIOLOGICAL ACTIVITY STUDY OF SOME ACTIVE SUBSTANCES AND ELEMENTS QUANTIFICATION OF THE WATER, ALCOHOLIC AND OIL EXTRACTS OF CUMINUM CYMINUM

Omar Hamad,Shehab AL- Obaidi [a], Mohammed Oday Ezzat [b], *Mohd Nizam Mordi [b]

[a]Chemistry Department, Women Education College, Al-Anbar University , Anbar , Iraq

[b]Centre for Drug Research , Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

(*mnizam@usm.my)

ABSTRACT: This article contained a disclosure of certain active substances isolated from the seeds of Cuminum Cyminum such as Tannins and Saponins and volatile oils, and the percentage in the form was ((22.0%) (68.0%, 10.0%)) respectively. As well as the appointment of some mineral elements in the cumin seeds like sodium, calcium and potassium and the concentration in the sample was (207)ppm, (306) ppm, (311) ppm, respectively, measured by using Flame Spectrometer. Completed the study as well as the effective of anti-bacterial for the extracts of water and alcohol by hot and cold procedures for cumin by using two kind of pathogenic bacteria which are Staphylococcus Aurous and Escherichia Coli , The study showed the different ability of inhibition for extracts and different inhibition diameters vary according to different active substances and the concentrations and the kind of the bacteria.

1. INTRODUCTION

Medicinal plants are an important source of effective materials intervention in the preparation of many drugs , where has been proven scientifically that active substance manufacturer laboratory does not perform the same influence physiological role of the active ingredient derived from medicinal plants in addition to side effects left by the material prepared in the body, which may not appear until after a period may be long. Cuminum is annual plant belong to sex of (Cuminum) and type of (Cyminum) and the scientific name is Cuminum Cyminum [1]. Cumin is a herbal plant limited growth with thin dark green color leaves, The plant bears small white flowers and purple fruits are oval rectangular split each of them to dry quickly when it bent and aromatic odor and tastes bitter used for commercial purposes as a spice and savory in the manufacture of some types of pies, pastries, cheese-making industry, drinks, preparation of soups and curries, therapeutic purposes for many diseases as a treatment of indigestion, anti-rot, cure for colic, repelling gas, antiseptic sewage tract, expelling tapeworms, intestinal worms, fragmentation of kidney stones, ureter, treat shortness of breath, asthma, coughs and as an antioxidant and calms the pain of dental aphrodisiac and helps to dissolve cholesterol [2] . Information as is indicated by the U.S. Department of Agriculture to contain the Cuminum plant on the : fat, saturated fat, carbohydrates, fiber, protein and cholesterol, while the active substances: essential oil accounted for 2-5% most of its components (aldehyde cumene 25-35% - Alonathol - Pinyin - Feilandran - Terbanin - Mersin - Careoffill - Alsiamin - limonene) - flavonoids (Ibeginan) . It also contains the chemical installed on many of the biologically active substances and Kaltaninat Alclaicosadat and flavonoids and alkaloids and volatile oils [3] , were selected from each kind of bacteria (Escherichia Coli and Staphylococcus Aurous) and to the medical significance for humans because it is one of the negative and positive bacteria to Gram-causing dye to many diseases.

2. MTHODS AND EXPERMINTAL

Source and classification of plant :

Cumin plant (Cuminum Cyminum) used in this research has been obtained from the local market of Anbar.

1. Materials and working methods of water, alcohol and oil extraction:

Cumin seeds (Cuminum Cyminum) has been collected from one of the shops in the city of Fallujah, seeds dried, grounded and preserved in laboratory temperature until use. They have been diagnosed in the Herbarium of the Iraqi National General Authority for examination and certification of seeds Ministry of Agriculture. For the preparation of aqueous extract were taken (40) g of seed powder and placed in the conical flask containing the (200) cm3 of distilled water with mixed using a blender magnetic for a period of (30) minutes and a centrifuge for (15) minutes then put the solution in stages in the electric furnace and the temperature (35) °C and for a place to extract it and preparation for concentrations (25, 15, 10, 5, 1) %.

Alcohol extraction obtained from the set (50) g of powdered seed in the unit of extraction (Soxhelt) and added to it (350) ml of ethanol with (99.5%) concentration and the process continued extraction for a period of (12) hours at temperature of (40) ^{0}C, using a rotary evaporator (Vacuum Rotary Evaporator) at temperature of (35) ^{0}C [4], preparation of different concentrations done by the same way as preparation of different concentrations of aqueous extraction.

Oil extraction obtained by adding of (350) ml of petroleum ether (60-40) ° C to a continuous extraction and followed the steps above in the preparation of alcoholic extract [5].

2. Isolate the active ingredients:

A – Tannins: Tannins were isolated from Cumin by adding (75) ml of distilled water to (0.5) g of cumin powder and put the mixture in a water bath boiled for (30) minutes, run for a combination centrifugal speed (200 cycle \ minutes) for a period of (20) minutes. Transferred in stages to the flask capacity (100) ml and complete the volume to the mark with distilled water then added to the mix (20) ml of (4%) lead acetate with continuous shaking then nominate the sediment is taken and dried at (70) ° C in the electric furnace [6].

B- Saponins : Weight of (10) g of cumin powder and added to it (50) ml of (20%) ethanol , then heated using a water bath for half an hour (55) ^{0}C with constant stirring, after filtering the solution separated. The filtrate was added to it (100) ml and then heated the solution using a water bath at (90) ^{0}C, the volume of final solution become (40) ml, after that the filtrate transfer and added to it (20) ml of ether in a separating funnel then separated layer of water and neglected layer of ether was added to the layer of water (10) ml of butanol (n-butanol) then evaporation the resulting solution in the water bath and dry the solution to get Saponins [7,8].

C- Volatile Oil : Extraction of volatile oils in cumin done by using (Soxhlet) and using of ether as organic solvent, this is done by mixing of (5) g of cumin powder with (150) ml of ether and complete the process of extraction for a period of (24) hours after that separation of the solvent form volatile oils has been done [9].

D- Qualitative Detection: Detection of Carbohydrates, Flavonoids, Catechol , acidity and Alloquanthusianaedin.

3. Elements Determination :

The Spectrometer Flame technology (Flame photometer) used for elements determination and to estimate the elements done by using GENWAY PFP7 and (UV-visible spectroscopy) from BIOTECH Engineering Spectro Scan 60D England made , In the beginning of preparation of sample for analysis done by taken of (1) g of cumin powder and dissolved in (20) ml of royal aqua (HNO_3 + 3HCL) , leave it for half an hour, after that filtering of the mixture and then complete the filtrate to 100 ml with distilled water. A series of standard solutions prepare then measured the intensity of the emission standard solutions prepared and models solutions [10].

4. Study the effectiveness of anti-bacterial pathogenesis :

Method of deployment drill (Agar-well diffusion method) used depending on using of kirby Baauer process [11], in the measurement of the sensitivity of the bacteria used in the research of various concentrations of substances extracted from cumin to get (Escherichia Coli and Staphylococcus Aurous) bacteria then isolated and diagnosed in a laboratory culture of the children's hospital in Ramadi . After that using of (Mueller Hinton ager) to test the sensitivity of bacteria to extracts from cumin and prepared as instructed by the company processed, then put the dishes in the incubator at a temperature (37) ^{o}C for a period of (24) hours and was then measure the diameter of inhibition (Inhibition Zone) [12,13] in each hole mediated by the ruler and record the results.

5. Preparation of standard solutions of the substances isolated from cumin:

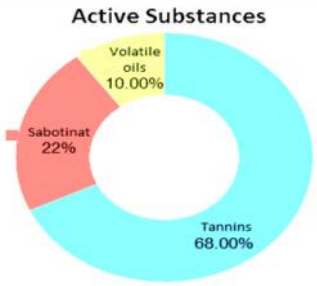
A series of extracts of different solutions has been prepared in concentration of (1%, 5%, 10% 15% 25%) mg / ml.

3. RESULT & DISCUSSION

Table (1) and scheme (1) shows the percentage of active ingredient that has been isolated from cumin (Cuminum Cyminum) ,the percentage of Taninat , Saponins and volatile oils are (68.0% 22.0%, 10.0%), respectively, where it is noted that the Tannin has recorded the highest percentage by weight in the cumin, followed by Saponins and volatile oils. The study showed qualitative statements of the active compounds contained in cumin and the presence of flavonoids, carbohydrates, condensed tannins, catechol, alloquanthusyandin and saponins.

Table (1): the percentage of active ingredient in cumin (Cuminum Cyminum).

Active Substance	Percentages
Tannins	68.0%
Sabotinat	22.0%
Volatile Oils	10.0%

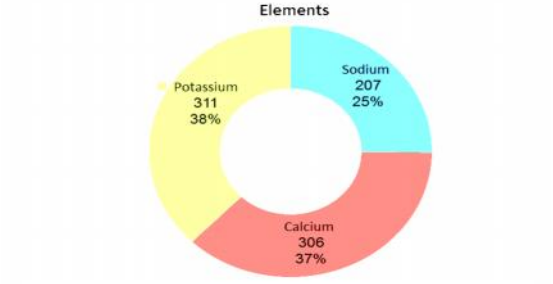


Scheme (1) shows the percentage of extracted material

Table (2) and scheme (2) Show the amount of mineral elements in cumin where the result showed that cumin contains sodium (207) ppm, calcium (306) ppm and potassium (311) ppm, all are important functional and metabolic metals for body. The sodium an important role in maintaining the balance of fluids outside the cell in the body as well as function pH of the fluid and is also involved with the potassium in the organization movement of Involuntary muscle such as heart muscle, Calcium promotes strong bones and teeth and contributes to the transfer of meta neurological and heart beat regulation also magnesium interaction in the building of body tissues and it important component of more than (300) enzyme in the body with important metabolic and biology functions [14].

Table (2): the amount of mineral elements in cumin measured by Flame Spectrometer Technology.

Concentration (ppm)	Symbol	Element
Sodium	Na	207
Calcium	Ca	306
Potassium	K	311



Scheme (2) shows the percentages of elements in the extract of cumin

Tables (3.4) Show the results of anti-bacterial activity of extracts from cumin which have been studying separately in different concentrations by using two types of pathogenic bacteria (Escherichia Coli and Staphylococcus Aurous). The result show that the extracts of water for cumin have the higher activity at (25) mg / ml, where the inhibition zone diameter was (1.4) mm for (Staphylococcus Aurous) and (1) mm for the (Escherichia Coli), followed by the rest of the varying concentrations and rates table (3).

Table (3): The effect of cold aqueous extract of cumin in different concentrations on the growth of bacterial pathogenesis races

Inhibition zone diameter (mm)		Conc.in mg\ml
Escherichia Coli	Staphylococcus Aurous	
10	14	25
6	13	15
5	12	10
8	11	5
7	8	1

Table (4): The effect of cold alcoholic extract of cumin and different concentrations on the growth of bacterial pathogenesis races

Inhibition zone diameter (mm)		Conc.in mg\ml
Escherichia Coli	Staphylococcus Aurous	
1.5	4	25
1.5	3	15
1	2.1	10
1	1.1	5
0.5	1.1	1

The inhibitory action of the extracts of water is due to that it contained Tannins, which include some phenolic compounds such as Gallic acid and Tannic acid, both have biological influence against many races of bacteria due to the presence of aggregates hydroxyl (-OH), which have the ability to form bonds of hydrogen between the hydroxyl group in these compounds and the water molecules in the bacterial cell and that the water (90%) of the weight which disables dynamic process in the bacterial cell [15], as these compounds (Gallic acid, Tannic acid) as they are phenolic compounds have the ability to coagulation of proteins in the bacterial cell and destroy the enzymes involved in the manufacture of the necessary amino acids to increase cell division [16].

Generally, from tables (3.4) for all extracts and all prepared concentrations the effect against (Staphylococcus Aurous) bacteria is higher than against (*Escherichia Coli*) bacteria. It is found that the inhibition zone diameter of the cold water extract is bigger than for all other extracts. This is due to the varying proportion of active substances in extracts of different terms, we found by searching the Tannins and different phenolic compounds is largely responsible for the opposite effect of microorganisms compared to other components in the cumin.

Water is one of the best solvents used in extracting of Tannin from its own sources of plant exclusive of other solvents [17], is well known that there are two types of Tannins , biodegradable and extensive Tannins . The advantage of first type for analyzing to the components of the original when exposed to high temperatures [18], or polymerase at temperatures higher than 60 °C [19]. For this reason, attributed of effectiveness of the warm aqueous extract comparing with cold aqueous extract.

As for the alcoholic extract of the weakness of relative biological activity compering with cold aqueous extract was due to the disintegration of Tannin when exposed to alcohol solvent so it's best to exclude alcohol in extracting of Tannin [18].

4. ACKNOWLEDGEMENT

M. O. Ezzat thanks The Academy of Sciences for The Developing World (TWAS) and Universiti Sains Malaysia (USM) for financial support under TWAS-USM Fellowship.

5. REFERENCE

1. A. Al-Rawi, ”Medicinal Plants of Iraq” Baghdad Second Eddition , 22-25, (1988).
2. S. Hashim and M. Gamil, " plants and herbs between the Iraqi folk medicine and scientific research" Baghdad, Dar Al-Thaorao for Press and Publication, 67, (1988).
3. A. M. Rizk, ”The Phyto Chemistry of Flora of Qatar”, King Print Of Richmond , Great Britian , 132, (1986).
4. T.B. Harborne, Phytochemical methods. Halasted press. John wiely & Sons, New York, p. 178, (1973).
5. B. Angood, S.A. Ermel, H. Schmutterer, *J. of Natural and APL. Sci.*, **1**,13-25, (1996).
6. J. Smith , F. John, British Journal of Nutrition, , 88, 587–605, (2002).
7. F.George, K. Zohar, P.S. Harinder , B. Makkar, *Chem. Eur. J.* , **15** , 4373 – 4381, (2009).
8. R. Mohammed, J. Peng , T. Kelly , *J. Nat. Prod*, 69 ,**12** , 1739-1744, (2006).
9. J. E. Bartmess, J. A. McIver, R. T. , *J. J. P. J. Am. Chem. Soc.* , **107**, 3902, (1990).
10. D.L. Pavia, G.M. Lampman, & G.S. Kriz, , *Organic chemistry Mason*, **53** , 653, (2004).
11. J. Vandpitte , K. Engback , P. Piot and C.C. Heuck *Chem. Eur. J.* , **4** ,78-110 , (1991).
12. R. Brown and I.R. Poxton , *Proc. Natl. Acad. Sci.* , **44 (2)** , 98–104, (1996).
13. Y. M. Sageska , T. Uemura, Yaugaku zasshi, Mar., 116 , **3**, 238, (1997).
14. M, Vishwanath, D. Saradesai, New York, Marcel Dekker,INC., 210-212, (1997).
15. Faittin S., "The Complete book of Minerals for Health",pennsalnania, 72-80, (1981).
16. Allan G. , Robert A. , Denis St. J. Michael J. S. and James , 2ed ,UK, 106 -114, (1999).
17. J. Grimshow, Coffeys.(ed), Vol. 111, Part D. Amsterdam: Elsevier Science Publishing Co, 92-102, (1976).
18. J. D. Reed, *J. Animal Society*, **73**, 1516-1528, (1995).
19. E. Haslam, Chemistry of Vegetable Tannins. London: Academic Press INC, 32-44, (1966)