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RESEARCH ARTICLE

Chemical Composition and biological Activity of aqueous and methanolic extracts of Thyme (*Thymus vulgaris*)

Wafaa A. Helmy, A.A. Farrag, A.A. Hasaballah

1. Department of Chemistry of Natural and Microbial Products, National Research Center

2. Microbiology Department, Faculty of Science, Al-Azher University, Cairo, Egypt.

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Corresponding Author

A.A. Hasaballah

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Abstract

This study aimed to investigate the possible biological activity of aqueous, methanolic and sulfated extracts of tow medicinal plants thyme (Thymus vulgaris) and Ginger (Zingiber officinale) at 85 °C under acidic, neutral and alkaline conditions. Fibrinolytic, anticoagulation, antioxidant, antitumor and antimicrobial activities were investigated. The Biochemical studies of the chosen two fungi showed the presence of varying amounts and different forms of total ash, protein , lipid, and total carbohydrates. However, TLC chromatography examination showed that the output of the acid hydrolysis to the plants varying from monosaccharaides. Anticoagulation and fibrinolytic properties of the crude extracts as well as of their sulfated derivatives, at 2 mg/ml of extract were tested and show that the sulfated acidic, sulfated neutral and sulfated alkaline extract of T. vulagaris and sulfated neutral, alkaline extract , sulfated alkaline and methanolic extract of Z. officinale exhibited fibrinolytic activities equivalent to double of the same amount of standard "Hemoclar" . On the other hand, results also showed a promising anticoagulant activities of the sulphated extracts at different concentrations compared with the corresponding native extracts. Also, their antimicrobial activity of the aqueous extracts against both tested Gram positive and negative bacteria, yeast and fungal strains was determined. The chemical modification of the aqueous sulfation can increase significantly its anticoagulation and fibrinolytic activities. And also their extracts show antioxidant activity reached up to 68% comparative by standard. However, the antitumor activity is high for both the native and the sulfated aqueous extracts.

INTRODUCTION

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals.

Thymus vulgaris L. (thyme), locally known “zaatar” a member of the family Lamiaceae, is widely used in Egyptian folk medicine for its expectorant, antitussive, antibroncholitic, antispasmodic, anthelmintic, carminative and diuretic properties. The aromatic and medicinal properties of the genus Thymus have made it one of the most popular plants all over the world. Thymus species are commonly used as herbal tea, flavoring agents (condiment & spice) and medicinal plants (**Stahl-Biskup and Saez, 2002**). The published results reveal that major volatile constituents

obtained from the aerial parts of the plant are geranial, linalool, γ - terpineol, carvacrol, thymol and trans-thujan-4-ol /terpinen-4-ol (**Piccaglia *et al.*, 1993**).
The leaves of thyme (*Thymus vulgaris*) can be used fresh or dried as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals and cosmetics **(Javanmardi *et al.*, 2002)**. Thyme also possesses various beneficial effects, e.g. antiseptic, carminative, antimicrobial and antioxidative properties (**Baranauskiene *et al.*, 2003**). Ginger (*Zingiber officinale* Roscoe) has phenolic compounds such as shogaols, gingerols, sesquiterpenes, bisapolene, zingiberene, zingiberol, sesquiphellandrene and curcurmene. The active ingredients in ginger are thought to reside in its volatile oils, which comprise approximately 1–3% of its weight (**Newall *et al.*, 1996**). Ginger's active ingredients have a variety of physiologic effects. For example, the gingerols have analgesic, sedative, antipyretic and antibacterial effects *in vitro* and in animals **(Mascolo *et al.*, 1989).** Although an intravenous bolus of gingerol had a half-life of 7.23 min in rats (**Ding *et al.*, 1991),** it is not clear how this relates to pharmacokinetics after oral administration in humans. In human aortic endothelial cells, zingerone demonstrated significant antioxidant effects on low density lipoproteins **(Pearson *et al.*, 1997; Zhou and Xu, 1992).**

In human erythrocyte membranes, ginger extracts inhibited lipid peroxidation by 72% **(Sujatha and Srinivas, 1995)**. In addition, in rats fed with a high fat diet, supplementation with ginger provided significant antioxidant effects, raising tissue concentrations of superoxide dismutase and catalase and reduced gluatathione **(Jeyakumar *et al.*, 1999).** So, this study is concerned with the role of water extracts of thyme and ginger to detoxify the injuries of alcohol abuse on liver and brain.

Thyme showed broad antibacterial activity by inhibiting the growth of both gram-positive and gram-negative bacteria. However, gram positive bacteria *Clostridium botulinum* and *Clostridium perfringens* appeared to be more sensitive than the gram-negative organisms **(Nevas *et al.*, 2004).** The alcohol and ethanol extracts of thyme, thyme essential oil, thymol and carvacrol were found to have strong inhibition activity against *Bacillus subtilis*, *S. sonnei*, *E. coli* **(Fan and Chen, 2001).** Aqueous extracts of thyme significantly inhibited the growth of *H. pylori*, reducing its growth **(Tabak *et al.*, 1996).** The essential oil of thyme, or its constituent thymol, decreased viable counts of *S. typhimurium* on nutrient agar (NA) **(Juven *et al.*, 1994).** Thymol showed antagonistic effect against *S. sonnei* in anaerobic conditions in vitro **(Juven *et al.*, 1994).** Carvacrol, a compound present in the essential oil fraction of oreganum and thyme showed a dose-related inhibition of growth of the pathogen *Bacillus cereus* **(Ultee *et al.*, 2000).** The lowest minimum inhibitory concentrations were 0.03% (v/v) thyme oil against *C. albicans* and *E. coli* **(Hammer *et al.*, 1999).** Thyme extracts exerted no microbicidal activity against *Porphyromous aeruginosa* (**Thuille *et al.*, 2003).**

Thyme essential oil exhibited bacteriostatic and bactericidal properties against the non-toxigenic strain of *E. coli* O157:H7 in a broad temperature range. In an *in vitro* antibacterial study, thyme showed greatest inhibition against *A. hydrophila* compare to other psycrotrophic food-borne bacteria such as *Aeromonas hydrophila*, *Listeria monocytogenes* and *Yersinia enterocolitica.* Inhibition of growth was tested by using the paper disc agar diffusion method, while the MIC was determined by the broth microdilution method **(Fabio *et al.*, 2003)**. Thyme oil was tested for its antibacterial activity against *Campylobacter jejuni* (*C. jejuni*), *E. coli* O157:H7, *Listeria monocytogenes*, and *S. enterica* obtained from food and clinical sources and was found most effective against *E. coli, L monocytogenes S. enterica,*and *C. jejuni* **(Friedman *et al.*, 2002).**
On the other hand, different screenings focused on the essential oil of *T. vulgaris* and its effect on food spoiling yeasts **(Conner and Beuchat, 1984; Ismaiel and Pierson, 1990),** especially Aspergillas **(Farag *et al.*, 1989),** on various dermatophytes **(Janssen *et al.*, 1988),** and on some phytopathogenic fungi, e.g. *Rhizoctonzd solani*, *Pythium ultimum*, *Fwarium solani*, and *Calletotrichum lindemthianunz* **(Zambonelli *et al.*, 1996).**

Recent studies have shown that Thymus species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities **(Stahl-Biskup and Saez, 2002).** Aim of the present work was to determine the essential oil composition of eastern Moroccan thyme oil composition. These results will allow deduction of which components are likely to contribute to the antimicrobial activity and determination of any relationships between the components and their antibacterial activity.

Ginger (*Zingiber officinale* Roscoe) has phenolic compounds such as shogaols, gingerols, sesquiterpenes, bisapolene, zingiberene, zingiberol, sesquiphellandrene and curcurmene. The active ingredients in ginger are thought to reside in its volatile oils, which comprise approximately 1–3% of its weight **(Newall *et al.*, 1996).**

Ginger's active ingredients have a variety of physiologic effects. For example, the gingerols have analgesic, sedative, antipyretic and antibacterial effects *in vitro* and in animals **(Mascolo *et al.*, 1989).** Although an intravenous bolus of gingerol had a half-life of 7.23 min in rats **(Ding *et al.*, 1991)**, it is not clear how this relates to pharmacokinetics after oral administration in humans. In human aortic endothelial cells, zingerone demonstrated significant antioxidant effects on low density lipoproteins **(Pearson *et al.*, 1997; Zhou and Xu, 1992).** In human erythrocyte membranes, ginger extracts inhibited lipid peroxidation by 72% (Sujatha and Srinivas, 1995). In addition, in rats fed with a high fat diet, supplementation with ginger provided significant antioxidant effects, raising tissue concentrations of superoxide dismutase and catalase and reduced gluatathione **(Jeyakumar *et al.*, 1999**). So, this study is concerned with the role of water extracts of thyme and ginger to detoxify the injuries of alcohol abuse on liver and brain.

Z. officinale has been shown to have antimicrobial activity **(Habsah *et al.*, 2000 and Srinivasan *et al.*, 2001).** Ethanolic extract of the rhizomes of *Z. officinale* showed significant inhibition of growth of both certain Gram-positive and Gram-negative bacteria. It also displayed antiinflammatory, analgesic, antipyretic and antimicrobial activities. The essential oils of *Z. officinale* showed antimicrobial activity against Gram-positive and Gram negative bacteria using the agar diffusion method **(Martins *et al.*, 2001).** Toxicity studies conducted on *Z. officinale*, used as aphrodisiacs in Arab Medicine showed no toxicity during acute toxicity test. The percent lethality was insignificant as compared to the control **(Qureshi, 1999).** The safety and efficacy of herbal remedies is a concern for many people.

Methanolic extract of the dried powdered ginger rhizome and the isolated constituents, 6-, 8-, 10-gingerol and 6-shogoal were tested against 19 strains of *H. pylori*. It inhibited growth of all 19 strains *in vitro* with a minimum inhibitory concentration range of 6.25-50 µg/ml. The crude extract, containing gingerols, inhibited the growth of all strains of *H. pylori* with an MIC range of 0.78 to 12.5 µg /ml and with significant activity against the CagA+ strains **(Mahady *et al.*, 2003).** The extracts of ginger exhibited antibacterial activity against the pathogens *S. aureus*, *S. pyogenes*, *S. pneumoniae*and *H. influenzae*. The MIC of extracts ranged from 0.0003 µg/ml to 0.7 µg/ml for ginger, while MBC ranged from 0.135 µg/ml to 2.04 µg/ml for ginger. Results indicated that extracts of ginger and *Garcinia kola* roots may contain compounds with therapeutic activity **(Akoachere*et al.*, 2002).**

Ginger has been widely studied for its pharmacological activities and has been reported to exhibit anti-inflammatory, antipyretic, hypoglycaemic, antimigraine, antischistosomal, antioxidant, hepatoprotective, diuretic, hypocholesterolaemic **(Mascolo *et al.*, 1989; Langner *et al.*, 1998**), hypotensive **(Ghayur and Gilani, 2005)** and gastrointestinal prokinetic activities **(Ghayur *et al.*, 2006).**

It has been shown that ginger extracts no affect blood lipids, blood sugar and fibrinogen in patients with coronary artery disease **(Bordia, 1997)**. Other study brought out a new property of ginger, whereby it neutralizes the altered fibrinolytic state induced by fatty meal in healthy individuals **(Verma and Bordia, 2001)**

It has been also reported that ginger inhibits platelet aggregation in healthy individuals **(Verma *et al.*, 1993)** and patients with coronary artery disease **(Bordia, 1997).** The concurrent use of ginger and anticoagulants may result in increased risk of bleeding **(Kruth *et al.*, 2004).**

Materials and Methods

1- Materials:

1.1: Plant material: Tow plants were used in this study including ginger rhizomes (***Zingiber officinale***), Thyme leaves (***Thymus vulagaris***) were kindly provided from medicinal and aromatic plants department-Pharmacy and Pharmaceutical industries division-National Research Centre, Dokki, Giza

1.2: Microorganisms and media:

Eight well identified test microorganisms including (*Aspergillus niger, Aspergillus Flavus, Penicillium chrysogenum, Escherichia coli, Klebsiella pneumonia, Bacillus subtilis, Staphylococcus aureus* and *Candida Albicans)* , were kindly provided from the National Research Centre, Dokki, Cairo, Egypt.

1.2.1: Preparation of standard bacterial suspensions: according to (**Miles and Misra, 1938**).

1.2.2: Preparation of standard fungal suspensions: The fungal cultures used were maintained on potato dextrose agar. **(Brantner *et al.*, 1994)**

1.3: Chemicals and reagents:

(Barium chloride-Tween 20) reagent: This was prepared according to (**Larsen *et. al.*, 1986**)

Lowery reagents: **Lowry A**: 2% sodium carbonate in 0.IN sodium hydroxide solution.

Lowry B: **(**1% cupric sulphate solution) – (2% sodium potassium tartrate solution).

Folin-Ciocalteu's Phenol Reagent: One volume of Folin- Ciocalteu's phenol reagent was diluted with two volumes of water before use to determine soluble protein.

Standard antibiotic discs: Streptomycin (10µg/disc) and erythromycin (15µg/disc) were purchased from Bioanalyse company, Ltd., Ankara, Turkey and Griseofulvin (20 mg/ml) from local pharmacy (125 mg/tablet).

Heparin: (Heparin sodium) this was purchased from SIGMA chemicals Co., U.S.A.

Hemoclar: (Pentosan sulfuric polyester) it was the commercial product prepared by Clin- Midy Paris and supplied by the Nile Co. pharmaceuticals. Cairo, Egypt.

Plasma: Human plasma was purchased from The Holding Company for Biological Products and Vaccines (EGYVAC-VACSERA), El-batal Ahmed Abdel Aziz Street, Dokki, Egypt.

Calcium chloride solution 2 % (w/v) & **Saline solution 0.9 % (w/v)** and **Other Chemicals:** All chemicals were analytical grade – products purchased from Sigma, Mark and BDH companies.

2: Methods:

2.1: Biochemical analysis of the mushroom

2.1.1: Determination of moisture content and ash : was attained according to **(A. O. A. C., 1970**).

2.1.2: Determination of total lipids: according to the Method of **(A. O. A. C. 1970).**

2.1.3: Analysis of the polymeric carbohydrates:

2.1.3.1: Acid hydrolysis: Complete acid hydrolysis of the ground samples was carried out according to the modified method of (**Fischer and Dorfel 1955**).

2.1.3.2: Qualitative examination of the hydrolysis products: This was performed by chromatography of the hydrolysates on thin-layer chromatography (TLC) according to **(Adachi, 1965) and** detection of spots was achieved by spraying with an aniline-phthalate reagent (**Partridge, 1949**).

2.1.3.3: Quantitative Determination of the Hydrolysis Products: according to the method of (**Wilson 1959**).

2.1.5: Determination of total carbohydrates: was determined, after hydrolysis according to **(Haug and Larsen, 1962)**. The color density was measured at 490 according to (**Dubois *et al.*, 1956**).

2.1.6: Determination of total nitrogen and crude protein: was determined according to the adopting the usual micro-Kjeldahl method of **(A. O. A. C. 1970**).

2.2: Preparation of organic, methanolic and aqueous extracts:

2.2.1: Grinding of the selected plant materials.

2.2.2: Extraction with Methanol according to **(Menaga D, 2012)**

2.2.3: Aqueous extraction of defatted plant material:

2.2.3.1: Extraction with deferent pH [Acidic with HCl at (pH 3), Neutral at (pH 6.6) and Alkaline with (1N) NaOH at (pH 12)]: 5 gm of the ground mushroom sample were extracted successively 3 times at 85°C under reflux for 3 hr (1 hr x 3 times) adopting the method of (**Jindal and Mukherjee 1970**).

2.3: Analysis of aqueous extracts: This was done using the same methods previously adopted for analysis of the plant sample except the protein assay which performed according to the method of (**Lowry *et al*., 1951**) .

2.4: Sulphation of aqueous extracts: achieved adopting the method of (**Hussein, 1994**)

2.4.1: Determination of Sulfate Ester Groups: was done in two following steps:

2.4.1.1: Cleavage of the Sulfate Ester Groups: by using the method of (**Larsen *et. al*., 1986**)

2.4.1.2: Turbidimetric Assay of the Liberated Sulfate: Sulfate content of the aforementioned hydrolyzate was determined adopting the turbidimetric procedure of (**Garrido, 1964)** with some modifications

2.5: Biological activities of aqueous extracts:

2.5.1:-Anti-coagulation activity: by using the method of (**U.S.A., Pharmacopoeia 1960**) for the assay of sodium heparin .

2.5.2: Evaluation of Fibrinolytic activity: This was performed by exposing a plasma clot to the effect of an aqueous solution according to (**U. S. A., Pharmacopeia, 1960**).

2.5.3: Antimicrobial activity *in vitro*:

2.5.3.1: Testing for antibacterial activity: The disc agar diffusion method was adopted according to (**Kavanagh, 1972**) with some modification to assess the antibacterial activity of the prepared extracts.

2.5.3.2: Anti-fungal activity: The cup-plate agar diffusion method was adopted according to **(Brantner**, ***et. al*., 1994)** to assess the antifungal activity of the prepared extracts.

2.5.3.2: Anti-viral activity : by two steps**:**

- **cytotoxicity assay (TC^{50}):** of the extracts were tested by method (**Mossman, 1983**)
- **Plaque reduction assay:** was carried out according to the method of (**Hayden *et al*., 1980**)

2.5.4: Antioxidant test by DPPH Radical Scavenging Assay according to the method of **(Gamez EJ, *et al* 1998) and (Liu L, et al, 2009).**

2.5.5: Antitumor activity by using SRB Cell survival assay: Cell survival will be determined using SulphoRhodamine-B (SRB) method as previously described by (**Skehan *et al*., 1990**)

Results and Discussion:

Chemical composition of ginger and thyme.

The present work was initiated to evaluate the anticoagulation, fibrinolytic, antibacterial and antifungal activities of crude aqueous, methanolic extracts and their sulfated extracts of Ginger rhizomes (*Zingiber officinale*) and Thyme leaves (*Thymus vulagaris*). The aforementioned plants collected from local market were subjected to the following analysis:

Biochemical evaluation of the investigated medicinal plants

Table (1): Ash, lipid, total carbohydrates and their monosaccharide constituents of the two investigated plant (mg/100mg)

Plants	Ash	Lipid	Protein	Total Carbohydrates	Monosaccharide Composition (w/w %)					
					U.A	Gal	Glu	Man	Ara	Xyl
T. vulgaris	7.38	8.5	9.56	14.01	0.7	17.2	43.9	8.6	14.4	15.2
Z. officinale	9.15	5.7	18.32	45.52	1.9	20.9	61.7	6.9	5.6	2.6

U.A (Uronic acid), Gal (Galactose), Glu (Glucose), Man (Mannose), Arab (Arabinose) and Xyl (Xylose)

The contents of ash, lipids, carbohydrates and protein were measured in gram per 100 gram of dry weight (g/100 g dry weight) in ginger and thyme samples. As can be seen from Table 1 chemical composition of the powdered ginger and thyme samples were characterized by the value of ash (9.15%) which was higher than the 7.64% reported by **Altman and Marcussen (2001)**. In addition total lipid of the investigated ginger samples constitute of the dry weight of lipids 5.7%.

In addition to, total carbohydrates of the investigated of ginger samples 45.52% of the dry weight (Table 1). The results also indicated that protein represents another constituent of most of the ginger samples were recorded 18.32% which was higher than the 12.6% according to **Bhat *et al.* (2010).** Also of thyme the results of the value of ash (7.38%) reported by **(Lee *et al*., 2004).** In addition total lipid of the investigated thyme plant constitute of the dry weight of lipids 8.5%.

In addition to, total carbohydrates of the investigated of thyme plant 14.01% of the dry weight (Table 1). The results also indicated that protein represents another constituent of most of thyme plant was recorded 9.56% which was higher than the 12.6% according **(Lee *et al*., 2004)**.

Figure (1): The Chemical composition of investigated plants.

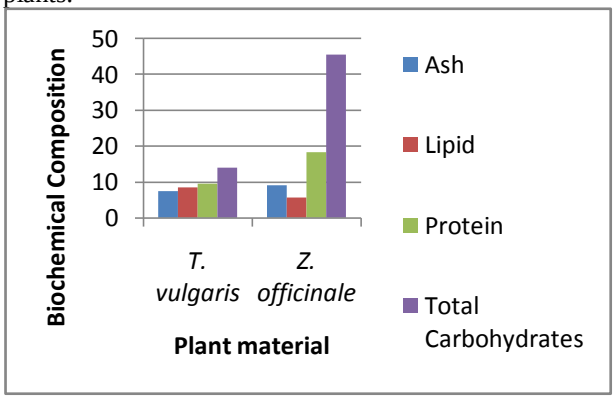
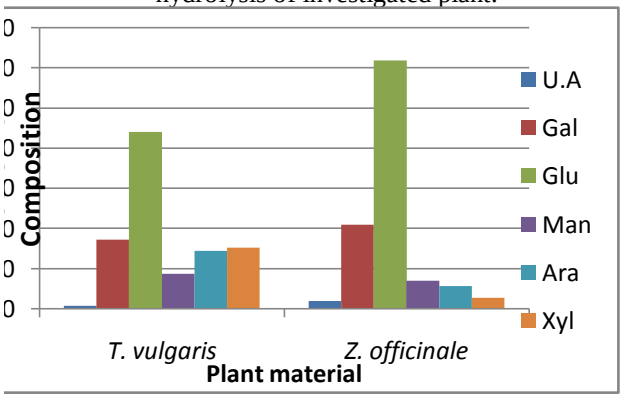


Figure (2): Monosaccharide constituents of acid hydrolysis of investigated plant.



It has been observed from chromatographic analysis of the acid hydrolysates of investigated plant that presence of glucuronic acid, galactose, glucose, mannose, arabinose and xylose in different value.

1.2. Biochemical composition of aqueous and methanolic extracts of thyme "*Thymus vulgaris*".

It can be seen that Table (2) and Figure (3) showed a wide range of variation in the yield of the investigated aqueous extracts. The highest value in aqueous extract was recorded in neutral extract of (42.2%) and lowest in acidic extract (10.1%). In addition, determination of highest sulphate content in aqueous extract was recorded in acidic extract (12.7%) and lowest in alkaline extract (8.6%). Another variation was also observed in the soluble-protein content the studied thyme extract. Thus, the percentages of protein varied in aqueous extract from (47.5%) in acidic extract to (35.7%) in neutral extract. Also the in aqueous extract the highest value of total carbohydrate contents of (38.2%) in alkaline extract and lowest in neutral extract (31.5%).

Table (2): Yield, total carbohydrates, protein, sulphate content and monosaccharide constituents of aqueous and methanolic extracts of thyme "*Thymus vulgaris.*

Extract	Yield	Sulfate content	Protein	Total Carbohydrates	Monosaccharide Composition (w/w %)					
					U.A	Gal	Glu	Man	Ara.	Xyl
T-1	10.1	12.7	47.5	34.7	1.2	16.2	45.4	7.7	24.9	4.6
T-2	42.2	10.2	35.7	31.5	1.3	15.4	49.2	6.6	23.2	4.3
T-3	19.5	8.6	42.1	38.2	1.5	14.6	49.9	5.7	21.8	6.5
T-4	29.3	9.8	40.7	32.9	1.6	17.1	47.3	6.4	22.7	4.9

T-1 (Acidic), T-2 (Neutral), T-3 (Alkaline), T-4 (Methanolic) extract of Thymus vulgaris, U.A (Uronic acid), Gal (Galactose), Glu (Glucose), Man (Mannose), Ara (Arabinose) and Xyl (xylose).

It has been observed from Table (2) and Figure (4) showed chromatographic analysis of the acid hydrolysates of various thyme aqueous extracts that presence of glucuronic acid, galactose, glucose, mannose, arabinose and xylose in different value.

Figure (3): Yield, total carbohydrates, protein and sulphate content of Thyme (*Thymus vulgaris*) in aqueous and methanolic extracts.

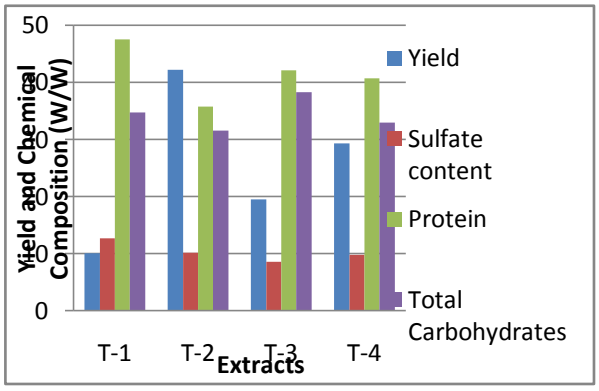


Figure (4): Monosaccharide constituents of acid hydrolysis of (*Thymus vulgaris*) aqueous and methanolic extracts.

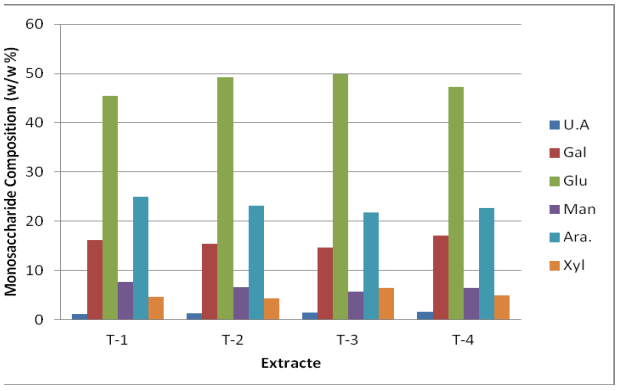


Table (3) and Figure (5) showed a wide range of variation in the yield of the investigated aqueous extracts. The highest value in sulfated extract highest yield obtained from alkaline extract of (63.6%) and lowest of (18.8%) in methanolic extract. In addition, determination of highest sulphate content in in acidic extract of (45.9%) and lowest in neutral extract of (35.5%). Another variation was also observed varied from (45.9%) in acidic to (35.5%) in neutral. And the highest value of total carbohydrate contents of (39.4%) in alkaline extract and lowest value recorded in neutral extract of (30.3%).

Table (3): Yield, total carbohydrates, protein, sulphate content and monosaccharide constituents of chemically modified (sulfated) aqueous and methanolic extracts of *Thymus vulgaris* in (mg/100mg)

Extract	Yield	Sulfate content	Protein	Total Carbohydrates	Monosaccharide Composition (w/w %)					
					U.A	Gal	Glu	Man	Ara.	Xyl
ST-1	25.2	37.2	45.9	33.5	1.3	16.6	45.9	7.4	24.7	4.1
ST-2	35.5	41.4	35.5	30.3	1.4	15.8	49.7	6.5	21.8	4.8
ST-3	63.6	33.6	41.9	39.4	1.1	14.8	50.4	5.6	21.6	6.5
ST-4	18.8	35.9	39.8	35.8	1.5	18.7	47.8	8.5	19.8	3.7

ST-1 (Sulphated acidic), ST-2 (Sulphated neutral), ST-3 (Sulphated alkaline), ST-4 (Sulphated methanolic) extract of Thymus vulgaris, U.A (Uronic acid), Gal (Galactose), Glu (Glucose), Man (Mannose), Ara (Arabinose) and Xyl (xylose).

Figure (5): Yield, total carbohydrates, protein and sulphate content of Thyme (*Thymus vulgaris*) in aqueous extracts.

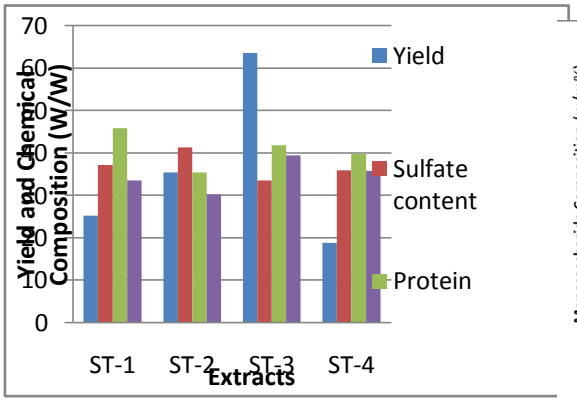
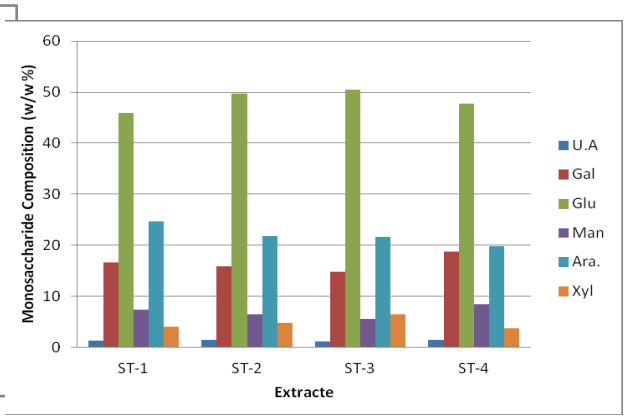


Figure (6): Monosaccharide constituents of acid hydrolysis of (*Thymus vulgaris*) aqueous extracts.



On the other hand in sulfated extract as seen in the Table (3) and Figure (6) Chromatographic analysis of the acid hydrolysates of various thyme sulfated aqueous extracts that presence of glucuronic acid, galactose, glucose, mannose, arabinose and xylose in different value.

Table (4): Yield, total carbohydrates, protein, sulphate content and monosaccharide constituents aqueous and methanolic extracts of ginger (*Zingiber officinale*) (mg/100mg).

Extract	Yield	Sulfate content	Protein	Total Carbohydrates	Monosaccharide Composition (w/w %)					
					U.A	Gal	Glu	Man	Ara.	Xyl
Z-1	17.1	3.7	22.9	32.8	1.5	11.0	51.1	12.6	20.3	3.5
Z-2	31.8	4.3	38.5	34.4	1.6	14.4	48.5	10.1	19.4	6.0
Z-3	24.5	3.6	23.7	43.5	1.3	20.7	47.0	8.2	18.7	4.1
Z-4	38.4	3.9	39.8	42.8	0.8	17.5	48.4	13.6	16.3	3.4

Z-1 (Acidic), Z-2 (Neutral), Z-3 (Alkaline), Z-4 (Methanolic) extract of *Zingiber officinale*, U.A (Uronic acid), Gal (Galactose), Glu (Glucose), Man (Mannose), Ara (Arabinose) and Xyl (xylose

In Table (4) and Figure (7) results showed a variation in the yield of the investigated extract *Z. officinale* in the aqueous extract highest value was recorded in methanolic extract of (38.4%) and lowest in acidic extract (17.1%). On the other hand, the highest sulfate content was recorded in neutral extract (4.3%) and lowest in alkaline extract (3.6%). Noteworthy, highest soluble-protein values were recorded of (39.8%) in methanolic extract and lowest in acidic extract of (22.9%). And also determination of total carbohydrate contents showed a variation in the proportions of this constituent highest value was recorded (43.5%) in alkaline extract and lowest in acidic extract (32.8%).

Figure (7): Yield, total carbohydrates, protein and sulphat content of *Z. officinale* in crude aqueous and methanolic extracts.

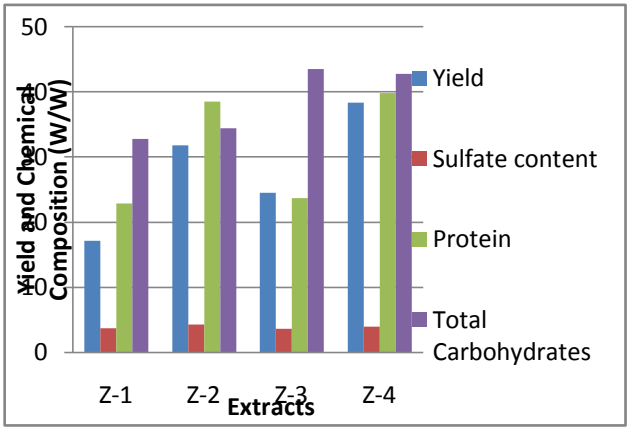
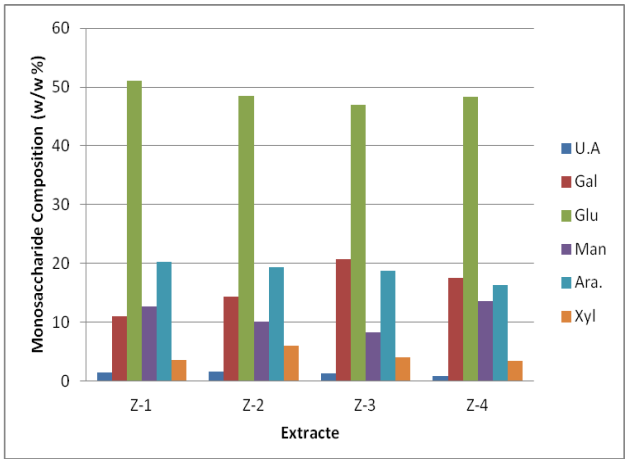


Figure (8): Monosaccharide constituents of acid hydrolysis of *Z. officinale* in crude aqueous and methanolic extracts.



As seen in the Table (4) and Figure (8) chromatographic analysis of the acid hydrolysates of the acid hydrolysates of various thyme aqueous extracts that presence of glucuronic acid, galactose, glucose, mannose, arabinose and xylose in different value.

Table (5): Yield, total carbohydrates, protein, sulphate content and monosaccharide constituents of chemically modified (sulfated) aqueous and methanolic extracts ginger (*Zingiber officinale*) (mg/100mg).

Extract	Yield	Sulfate content	Protein	Total Carbohydrates	Monosaccharide Composition (w/w %)					
					U.A	Gal	Glu	Man	Ara.	Xyl
SZ-1	20.8	44.2	21.1	29.4	1.2	11.5	51.4	12.3	19.9	3.7
SZ-2	17.4	41.9	35.4	32.8	1.4	15.8	49.0	9.8	19.2	4.8
SZ-3	58.3	49.6	21.6	41.2	1.0	19.1	47.5	8.0	18.5	5.9
SZ-4	16.8	42.8	26.9	35.8	1.5	11.0	51.1	12.6	20.3	3.5

SZ-1 (Sulphated acidic), SZ-2 (Sulphated neutral), SZ-3 (Sulphated alkaline), SZ-4 (Sulphated methanolic) extract of Zingiber officinale, U.A (Uronic acid), Gal (Galactose), Glu (Glucose), Man (Mannose), Ara (Arabinose) and Xyl (xylose

Figure (9): Yield, total carbohydrates, protein and sulphat content of *Z. officinale* in aqueous sulfated and methanolic extracts.

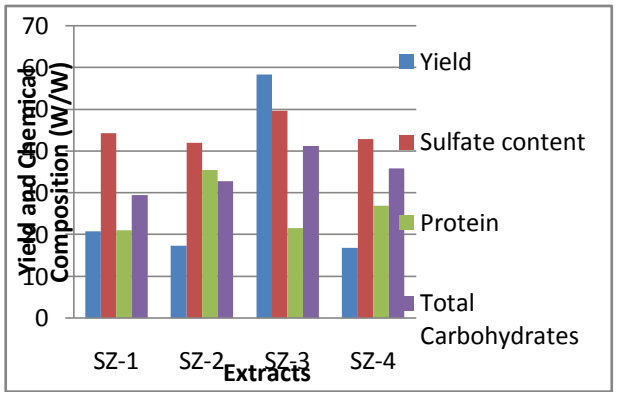
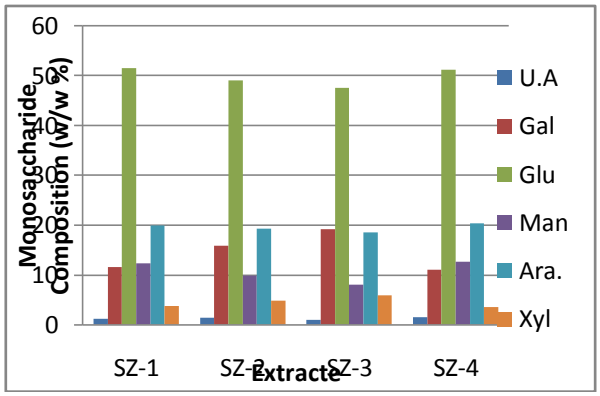


Figure (10): Monosaccharide constituents of acid hydrolysis of *Z. officinale* in aqueous and methanolic extracts.



Chromatographic analysis of the acid hydrolysates of chemically modified ginger extracts as show in Table (5) and Figure (10) revealed the presence of glucuronic acid, galactose, glucose, mannose, arabinose and xylose.

Biological activities:

A part of the present work has been devoted to evaluate the (anticoagulant, fibrinolytic, antimicrobial, antioxidant, antitumor and antiviral activities) of the aqueous and methanolic extracts from thyme leaves "*Thymus vulagaris*" and ginger rhizomes "*Zingiber officinale*"

1. **Testing aqueous extracts for its anticoagulation activity *in vitro:***

Table (6): The anticoagulation activity of aqueous extracts of tested plants and their sulphated aqueous extracts against human plasma (min)

Extracts	Clotting Time (min)							
	Concentrations of extracts (µg /ml)							
	cont.	2000	1000	500	200	100	50	25
T-1	5	90	25	---	---	---	---	---
T-2	5	70	15	---	---	---	---	---
T-3	5	65	10	---	---	---	---	---
T-4	5	70	12	---	---	---	---	---
ST-1	5	> 600	> 600	> 600	> 600	70	25	---
ST-2	5	> 600	> 600	> 600	> 600	60	20	---
ST-3	5	> 600	> 600	> 600	> 600	55	15	---
ST-4	5	120	45	13	---	---	---	---
Z-1	5	30	---	---	---	---	---	---
Z-2	5	20	---	---	---	---	---	---
Z-3	5	45	8	---	---	---	---	---
Z-4	5	60	15	---	---	---	---	---
SZ-1	5	> 600	> 600	> 600	> 600	110	35	10
SZ-2	5	> 600	> 600	> 600	> 600	> 600	85	20
ZS-3	5	> 600	> 600	> 600	> 600	> 600	180	40
ZS-4	5	110	35	8	---	---	---	---

(T-1, T-2, T-3, T-4, TA-1, ST-2, ST-3 and ST-4) acidic, neutral, alkaline, methanolic and its sulfated extract of *Thymus vulagaris* respectively and (Z-1, Z-2, Z-3, Z-4, SZ-1, SZ-2, SZ-3 and SZ-4) acidic, neutral, alkaline, methanolic and its sulfated extract of *Zingiber officinale* respectively

Briefly, the obtained results in Table (6) could be revealed that most of the studied all aqueous extracts (T-1, T-2, T-3, T-4, Z-1, Z-2, Z-3 and Z-4) have no anticoagulation activities or weak anticoagulation activities comparable to that of standard preparation of heparin sodium. But on other hand results indicated that sulphated aqueous extracts have activities. The highest clotting times were found by using sulphated acidic extract (SZ-1), sulphated neutral extract (SZ-2) and sulphated alkaline extract (ZS-3) of *Zingiber officinale* at concentration of 25µg/ml and the lowest clotting time were reported by alkaline aqueous extract of *Zingiber officinale* at concentration of (1000 µg/ml). It has been also reported that ginger inhibits platelet aggregation in healthy individuals **(Verma, 1993).**

From these data, It was concluded that addition of sulphate group into the extracts enhance anticoagulation activities. However the obtained results are in parallel to that obtained by **Akoachere *et al*., (2002**). Any way we can concluded from these data, that addition of sulphate group into the extracts enhances anticoagulation activities however the obtained results are in parallel to that obtained by **(Akoachere *et al*., 2002).**

2. Testing of aqueous extracts for its fibrinolytic activity *in vitro*.

As part of our interest in this study was to evaluate various aqueous and methanolic extracts and their corresponding sulphates against fibrinolytic activity compared with standard fibrinolytic, Hemoclar drug (Pentosan sulfuric polyester, product of Clin Midy. Paris).

From the results in Table (7) that the sulfated acidic, sulfated neutral and sulfated alkaline extract of *T. vulagaris* and sulfated neutral, alkaline extract , sulfated alkaline and methanolic extract of *Z. officinale* exhibited fibrinolytic activities equivalent to double of the same amount of standard "Hemoclar" preparation at concentration of (2000 µg/ml). On the other hand, the acidic, sulfated acidic extract of *Z. officinale* show fibrinolytic activities about of the same amount of standard "Hemoclar" preparation. In addition, the natural and methanolic extract of *Z. officinale* exhibited fibrinolytic activities equivalent to about half that of standard "Hemoclar" preparation at same concentration (2000 µg/ml). The data recorded in Table (11) show that original aqueous extracts have no fibrinolytic activity while the chemically modified extracts have high fibrinolytic activities. Fortunately the obtained results are in agreed with many authors as **Mascolo *et al*., (1998) and Langner *et al*., (1998**).

Finally, we can conclude that fibrinolytic activities of the modified (Sulphated) products revealed high fibrinolytic activity but, there is no activity of the original extracts. Also these results considered as initial steps in the way of using medicinal plants as a sources of cheap natural sources for the production of substances that have anti-microbial activity , anti-coagulation and fibrinolytic drugs to our knowledge, this is the first report investigating the fibrinolytic activities of isolated ginger rhizome extracts.

Table (7): Fibrinolytic activities of aqueous extracts of tested plants and their corresponding chemically modified extracts of the selected plants *(2*000 µg/ml)

Extracts		Fibrinolytic activity *(2*000 µg/ml)	
		aqueous extract	Modified extract
T. vulagaris	Acidic	+2	+8
	Neutral	+1	+7
	Alkaline	+3	+7
	Methanolic	--	+1
Z. officinale	Acidic	+4	+6
	Neutral	+3	+7
	Alkaline	+8	+9
	Methanolic	+3	+7
Standard (Hemoclar)		**+4**	

Lysis of plasma clot using standard Hemoclar ((2000µg/ml): 4(+)., (+9): Lysis of more than 90 % of plasma clot, (+8): Lysis of more than 80 % of plasma clot (+7): Lysis of more than 70 % of plasma clot, (+6): Lysis of more than 60 % of plasma clot, (+5): Lysis of more than 50 % of plasma clot, (+4): Lysis of more than 40 % of plasma clot, (+3): Lysis of more than 30 % of plasma clot, (+2): Lysis of more than 20 % of plasma clot, (+1): Lysis of more than 10 % of plasma clot, (---): not Lysis of plasma clot

3. Antimicrobial activity *In vitro*.

3.1. Testing of aqueous extracts for Antibacterial activity:

Results obtained in the present study relieved the tested four medicinal plants aqueous extracts and their corresponding sulphate derivatives posses potential antibacterial activity against Gram-positive (*B. subtilis* and *S. aureus*) and the Gram-negative (*E. coli* and *K. pneumonia*) organisms

Results in Table (8) are variable in its antimicrobial activity the high activity show in acidic extract of *ginger* (23 mm) against *B.subtilis* and against *K.pneumonia* (26mm). Any way the obtained results was parallel with that obtained by **Martins *et al.* (2001).**

Table (8): The Antibacterial activity of aqueous, methanolic extracts and corresponding chemically modified extracts (mm):

Extracts (20 mg/ml	Diameter of inhibition zone (mm					
	Control		Gram positive		Gram Negative	
	solvent	*Streptom*	*B.subtilis*	*S.aureus*	*E.coli*	*K.pneumonia*
T-1	--	22	13	14	12	14
T-2	--	20	19	16	--	22
T-3	--	19	9	19	7	8
T-4	--	18	10	10	8	--
ST-1	--	20	--	13	15	--
ST-2	--	21	15	11	9	--
ST-3	--	18	10	16	--	9
ST-4	--	15	10	13	14	7
Z-1	--	17	23	--	--	26
Z-2	--	18	--	--	--	11
Z-3	--	20	--	8	--	--
Z-4	--	19	14	--	15	9
SZ-1	--	21	--	--	10	--
SZ-2	--	20	--	11	--	--
ZS-3	--	17	--	11	--	15
ZS-4	--	15	16	11	--	--

(T-1, T-2, T-3, T-4, TA-1, ST-2, ST-3 and ST-4) acidic, neutral, alkaline, methanolic and its sulfated extract of *Thymus vulagaris* respectively and (Z-1, Z-2, Z-3, Z-4, SZ-1, SZ-2, SZ-3 and SZ-4) acidic, neutral, alkaline, methanolic and its sulfated extract of *Zingiber officinale* respectively and (*Streptom)* Streptomycin.

3.2. Testing of aqueous extracts for its Antifungal activity:

Table (9): The Antifungal activity of aqueous,methanolic extracts and corresponding chemically modified extracts (mm).

Extracts (20 mg/ml	Diameter of inhibition zone (mm					
	Control		Organisms			
	solvent	Griseo	*C.albicans*	*A.niger*	*A.flavus*	*p.chrysogenum*
T-1	--	18	23	18	13	--
T-2	--	17	11	14	9	--
T-3	--	17	--	--	--	7
T-4	--	18	--	17	--	--
ST-1	--	16	--	--	--	7
ST-2	--	18	10	--	9	--
ST-3	--	17	--	--	--	6
ST-4	--	16	--	13	--	--
Z-1	--	17	12	18	--	7
Z-2	--	18	16	--	--	10
Z-3	--	17	--	8	--	--
Z-4	--	18	--	--	8	--
SZ-1	--	18	--	15	--	--
SZ-2	--	17	8	--	11	--
ZS-3	--	17	--	10	--	--
ZS-4	--	16	10	--	8	--

(T-1, T-2, T-3, T-4, TA-1, ST-2, ST-3 and ST-4) acidic, neutral, alkaline, methanolic and its sulfated extract of Thymus vulagaris respectively and (Z-1, Z-2, Z-3, Z-4, SZ-1, SZ-2, SZ-3 and SZ-4) acidic, neutral, alkaline, methanolic and its sulfated extract of Zingiber officinale respectively and (Griseo) Griseofulvin

Antifungal activity of aqueous and methanolic extracts their corresponding chemically modified extracts showed significant activity when compared with Griseofulvin as standard antifungal agent (Table9) Highly activity recognized in acidic aqueous extracts of *T. vulagaris* (23 mm) against *C. albicans* and lowly activity recognized in modified alkaline aqueous extracts of *T. vulagaris* in the same concentration of extracts in 20 mg /ml. However, the obtained results was also in agree with many authors as they indicated that extracts of ginger plant may contain compounds with therapeutic activity **(Akoachere *et al*., 2002).**

that the sulphation process improve the antifungal activity of acidic and neutral extracts of ginger rhizomes, since both extracts (SZ-1 and SZ-2) have high antifungal activity against *A. niger* compared with their corresponding native extracts.

3.3. Testing of extracts and its sulfated extracts for its Antiviral activity Results:

Table (10): Antiviral activity measured using Plaque reduction assay of H5N1 influenza virus

Extracts	Conc.µg/µl	Initial viral count	Viral count (PFU/ml)	% of Inhibition
T-1	50	0.71×10^{10}	0.63×10^{10}	11
	100		0.60×10^{10}	15
T-2	50	0.71×10^{10}	0.62×10^{10}	13
	100		0.39×10^{10}	45
T-3	50	0.58×10^{10}	0.58×10^{10}	0
	100		0.39×10^{10}	33
T-4	50	0.58×10^{10}	0.46×10^{10}	21
	100		0.29×10^{10}	50
ST-1	50	0.83×10^{10}	0.71×10^{10}	14
	100		0.58×10^{10}	30
ST-2	50	0.83×10^{10}	0.78×10^{10}	6
	100		0.70×10^{10}	16
ST-3	50	0.70×10^{10}	0.71×10^{10}	0
	100		0.71×10^{10}	0
ST-4	50	0.70×10^{10}	0.68×10^{10}	3
	100		0.64×10^{10}	8.5
Z-1	50	0.74×10^{10}	0.74×10^{10}	0
	100		0.74×10^{10}	0
Z-2	50	0.74×10^{10}	0.74×10^{10}	0
	100		0.74×10^{10}	0
Z-3	50	0.55×10^{10}	0.55×10^{10}	0
	100		0.55×10^{10}	0
Z-4	50	0.50×10^{10}	0.40×10^{10}	20
	100		0.40×10^{10}	20
SZ-1	50	0.91×10^{10}	0.53×10^{10}	42
	100		0.51×10^{10}	44
SZ-2	50	0.91×10^{10}	0.55×10^{10}	39.5
	100		0.54×10^{10}	40.6
SZ-3	50	0.70×10^{10}	0.70×10^{10}	0
	100		0.40×10^{10}	43
SZ-4	50	0.70×10^{10}	0.70×10^{10}	0
	100		0.70×10^{10}	0

The samples show from very weak to moderate activity against M7217B H5N1 influenza virus and result varied and ranged from (0%) to the highest value at inhibition (50%) in (T-4) Methanolic aqueous extract of *T. vulagaris.*

4. Testing of aqueous and methanolic extracts and its sulfated extracts for its Antioxidant activity

An antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness.Although. Antioxidants also have many industrial uses, such as preservatives in food and cosmetics and to prevent the degradation of rubber and gasoline.

Table(11) Antioxidant activities of aqueous and methanolic extracts and its modified (sulfated) extracts.

Extracts		Antioxidant activities	
		aqueous extract	Modified extract
T. vulagaris	Acidic	38.91	39.36
	Neutral	32.83	38.94
	Alkaline	35.16	36.58
	Methanolic	29.92	30.94
Z. officinale	Acidic	38.95	39.97
	Neutral	37.56	39.78
	Alkaline	32.78	36.88
	Methanolic	38.95	42.56
Standard	**Rutin**	**62.27**	
	Vitamin C	**61.33**	

Antioxidant activities of samples compared with Rutin and Vitamin C.

Table (11) shows that antioxidant materials in comparison with Rutin and vitamin C as references. According to the results obtained the antioxidant activity of sulfated cellulose depends upon the degree of substitution of sulfated groups. In the future, maybe we can use sulfated investigated plants as antioxidant materials in drug production or in human food matching with **(Grigore e*t al.,* (2010).**

5: Testing of aqueous and methanolic extracts for its Antitumor test:

(Table 12): Testing of extracts and its sulfated extracts for its Antitumor test to HEPG-2 Cells Table (12) (liver Cancer)

Extracts		IC_{50} (µg/ml)	
		aqueous extract	Modified extract
T. vulagaris	Acidic	55.0267	3.5997
	Neutral	26.6479	13.076
	Alkaline	19.5085	99.9364
	Methanolic	3.0738	26.6604
Z. officinale	Acidic	20.9316	22.4621
	Neutral	11.1237	13.8522
	Alkaline	14.8476	0.0942
	Methanolic	22.4621	120.1218
Standard (Doxorubicin)		**3.0738**	

IC_{50} meaning (concentration inhibiting 50% of the growth) the results in Table (12) are variable in its antimicrobial activity the lowest IC_{50} show in sulfated alkaline aqueous extracts of *Z. officinale* (0.0942µg/ml) and the highest IC_{50} show in sulfated methanolic aqueous extracts of *Z. officinale* (120.1218µg/ml).

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