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# Antioxidant Activities and Related Phytochemical Constituents of Ethanolic Extract of Some Fruits in Vitro

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## ABSTRACT

This work was achieved to study the antioxidant activities of ethanolic extracts of four fruits, *physalis peruviana* L.( pp ), *Ziziphus spina-christi* (z c ), *Syzygium cumini* (s c) and *Eriobotrya japonica* (E j) using in vitro antioxidant methods and determine the total phenolic compounds (TP) , flavonoids (F), total tannins( TT) and flavonols( FL) contents in fruits ethanolic extracts. *Syzygium cumini* (Sc) extract gave the highest contents of TP,F, TT , and FL contents. Also, *Syzygium cumini* (Sc) extract gave the highest total antioxidant activity, reducing power, scavenging activity of $H_2O_2$ radical, superoxide radical, DPPH radical, and the highest metal chelating activity followed by *Eriobotrya japonica* (E j) extract, *Ziziphus spina-christi* (z c) extract and *physalis peruviana* L.( pp) extract respectively .

**Keywords**: *Physalis peruviana* L., *Zziziphus spina-christi*, *Syzygium cumini*, *Eriobotrya japonica*, ethanolic extract, phytochemical analysis, antioxidant activity.

## INTRODUCTION

Free radicals have unpaired electrons. The oxygen radicals, for instance ($O_2$-), ($^{\cdot}OH$) and non-free radical species, for instance $H_2O_2$ and ($^1O_2$) are generated in many redox processes (Gulcin *et al*,2002) and cause oxidative damage to biological compounds for instance lipids , DNA , proteins and carbohydrates (Wiseman and Halliwell,1996),thus cause many diseases such as cancer, cardiovascular diseases, , inflammation , aging, and Alzheimer (Valko *et al* , 2007)

Green nature full of several biologically active compounds such as secondary metabolites especially Polyphenols which are effective antioxidants( Salah *et al*., 1995;Saskia *et al*.,1996) and can scavenge many kinds of radicals (Etoh *et al* 2004). In addition many polyphenols can chelate metal ion catalysts such as Cu and Fe to prevent free radicals formation. Also, polyphenols can reduce oxidative damage to lipids, proteins and DNA in cells (Fedeli *et al* 2004). Food rich in antioxidants had antiatherosclerotic , anticancer activities , reduce cardiovascular risk (Gerber *et al*.,2002; Kris-Etherton etal.,2002 , Ren *et al* 2003 and Seeram *et al* 2006), prevent osteoporosis , reduce inflammation (Nijveldt *et al,* 2001) prevent aging(Ames *et al* 1993) and prevent Alzheimer's diseases (DiMatteo and Esposito,2003)).

*Physalis peruviana Linnaeus*, are most commonly known as Cape gooseberry belonging to the family *Solanaceae* .fruits are eaten and have antispasmodic ,diuretic, antiseptic, sedative, analgesic properties eliminate intestinal parasites, Antidiabetic properties and anticancer (Puente et at , 2011 ). *Ziziphus spina-christi (L.)* Willd. (ZSC) commonly known as Christ's thorn (in English) and Sidr (in Arabic )belongs to family *Rhamnaceae.* (ZSC) fruits are usually eaten fresh and have antinflammatory effects (Waggas and Al-Hasni ,2009 and Asgarpanah *et al* ,2012), antibacterial , antifungal activity and treat tuberculosis, cough, fever, healing fresh wounds ( Abalaka *et al* , 2010). *Syzygium cumini (L.)* fruits are eaten and has medicinal properties, treat diabetes, pharyngitis, spleenopathy, urethrorrhea and ringworm infection(Warrier *et al* , 1996) . Loquat (*Eriobotrya japonica Lindil,* ) belongs to family Rosaceae. Fruits are eaten and has antidiabetic properties, antinflammatory effects ,anti tumor and treat diuretic ( Singh *et al* ,2010)

Limited information is available about phytochemical constituents and antioxidant activities of four fruits in Egypt i.e.. *Physalis peruviana* L *Ziziphus spina-christi (L.)* , *Syzygium cumini (L.)* and *Eriobotrya japonica* and which fruits had the higher antioxidants activity.

The present study has been under taken to determine total phytochemical constituents of ethanolic extracts of *Physalis peruviana* L*., Ziziphus spina-christi, Syzygium cumini and Eribotrya japonica* fruits, To evaluate their antioxidant activities.

## MATERIALS AND METHODS

**Plant materials**

*Physalis peruviana* L ( pp )., *Ziziphus spina-christi* ( z s ) and *Eriobotrya japonica* (E j) fruits were collected in March 2016, and the *Syzygium cumini* (s c )collected in November 2016. from experimental farm, Faculty of Agriculture, Ain Shams University. Then washed with distalled water and stored in deep freezer-80°c.

**Extraction**

Fruits were homogenated with absolute ethanol (1: 3 w/v) and then macerated for 24h. The ethanolic extracts were filtered and evaporated to dryness under vacuum.

**Methods**

**Phytochemical analysis:-**

Total phenolic compounds % was determined in ethanolic extracts using Folin-ciocalteus reagent at 725nm by the colorimetric method of Shahidi and Naczk (1995) and Gallic acid was used as standard. Favonoids % was determined by the aluminum chloride colorimetric assay at 510nm according to Marinova *et al.* (2005) using quercetin as standard. The total tannins % was evaluated by the method reported by Price and Butler (1977) using tannic acid as standard. Flavonols % was estimated using the method of Kumaran and Karunakaran( 2007)using quarcetin as standard. All previous % were expressed as mg/100 g.DW. of extract

**Antioxidant activities:-**

The total antioxidant activity (TAA) % of the extracts was evaluated by green phosphomolybdenum complex according to Prieto *et al* (1999)using ascorbic acid as reference. The TAA % was expressed as mg ascorbic acid /100g. DW. ). The reducing power was determined according to the method of (Oyaizu, 1986) using from 0.4 to 2 mg /ml of each extract for

determination and the formed colour was measured at 700 nm. A higher absorbance indicates a higher reducing power. Ascorbic acid, butylated hydroxy anisole (BHA) and butylated hydroxyl toluene (BHT) were used as controls.

The DPPH radical scavenging activity% of extracts was determined according to Gulluce *et al.* (2004)using concentration from 4 to 20mg/ml of extracts. The hydrogen peroxide scavenging activity% was determined according to the method of Ruch *et al*. (1989) using extract concentration(8-40 mg/ml). The superoxide anion scavenging activity% was measured as described by Dasgupta and De (2007) using riboflavin-light-NBT system and extracts concentrations from 4 to 20mg/ml.

The ferrous ion chelating ability % of the extracts was evaluated by Dinis *et al*. (1994) method. The reaction mixture contained 1.0 ml of various concentrations of the extracts (8-40 mg/ml).

Ascorbic acid, BHA and BHT were used as standard for comparison in all scavenging activity or chelating ability methods and the scavenging activity% or chelating ability% was calculated from the following :-

**scavenging activity% or chelating ability% = [(Control- Test) / control] ×100**

**Statistical analysis:**

The data were statistically analyzed by (ANOVA) using the (SAS Institute, inc, 1996). Means were separation by (L.S.D.) Test at $P < 0.05$ level

## RESULTS AND DISCUSSION

Data presented in table (1) revealed the total phenolic compounds% ( TP) , flavonoids (F) %, total tannins (TT), flavonols( FL) % and total antioxidant activity( TAA) % of the tested fruits ethanolic extracts %( mg/100g.DW) ( *physalis peruviana* L.( pp ), *Ziziphus spina-christi*( z c ), *Syzygium cumini* ( s c) and *Eriobotrya japonica* (E j)) . There were significant differences between all extracts in TP, F, TT, FL% and TAA %( mg/100g.DW). *Syzygium cumini* (sc ) ethanolic extracts had the highest contents of TP (2871.8 mg/100g DW), F (926.1 mg/100g DW), TT(4611 mg/100DW) and FL(28.55 mg/100g DW) and had the highest TAA (1323 mg/100g DW), followed by *Eriobotrya japonica*( Ej)extract , *ziziphus spina-christi* (zc)extract and *physalis pubescens L*(pp) extract respectively. The highest TAA% of Sc extract correlated with the highest contents of TP , F , TT and FL % . TT% was the highest component in all extracts followed by TP , F and FL % respectively.

**Table 1. Total phenolic compounds%(TP), flavonoids%(F) ,flavonols%( FL), total tannins%(TT) and total antioxidant activity( TAA)% of fruits ethanolic extracts mg/100g. DW**

| Extract | Total phenolic compounds% ( TP) | Flavonoid% (F) | Total tannins% (TT) | Flavonols% (F) | total antioxidant activity% (TAA) |
|---|---|---|---|---|---|
| pp extract | $262.7^{d}$±1.08 | $35.3^{d}$±0.46 | $483.3^{d}$±2.08 | $2.74^{d}$±0.09 | $954^{d}$±4.58 |
| z s extract | $812.9^{c}$±2.5 | $222.4^{c}$±0.44 | $1512^{c}$±2 | $8.71^{c}$±0.2 | $1008^{c}$±1.52 |
| sc extract | $2871.8^{a}$± 1.51 | $926.1^{a}$±0.71 | $4611^{a}$±2 | $28.55^{a}$±1.02 | $1323^{a}$±3.51 |
| Ej extract | $910.5^{b}$±1.39 | $678.8^{b}$±0.62 | $3952.7^{b}$±1.52 | $16.5^{b}$±0.49 | $1146^{b}$±2.64 |
| L.S.D | 3.21 | 1.07 | 3.6 | 1.09 | 6.14 |

**Values are expressed as means ±SD of three replicates .In the same colon the values affected with defferent letters(a-e) are significantly differented at p<0.05**

Generally there was significant increase in reducing power with increasing the concentration of extracts from 4mg/ml to 20mg/ml (Fig. (1). 20mg/ml concentration gave the highest reducing power in all extracts and standard antioxidants. Sc extract had the highest reducing power in all concentrations followed by E j extract, Z s extract and P p extract respectively. Ascorbic acid had the higher value between other standard antioxidants (BHA and BHT) .20 mg / ml Sc extract at concentration score the highest reducing power (1.632) followed by Ascorbic (1.586), E j extract (1.389), BHA acid (1.231), BHT acid (1.174), Zs extract (0.696) and Pp extract (0.267) respectively.

DPPH is a stable free radical. Antioxidants interact with DPPH, transfer electrons or hydrogen atoms to DPPH, thus neutralizing free radical character (Naik *et al*., 2003).

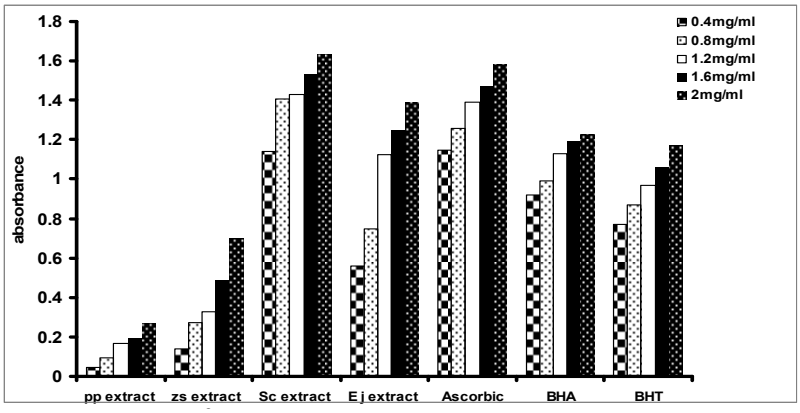


**Fig. 1. Reducing power of different concentrations of fruits ethanolic extracts and standared antioxidants (Ascorbic ,BHA and BHT) (LSD,0.009).**

There were positive relation between DPPH radical scavenging ability and concentrations of fruit ethanolic extracts or standard antioxidants (Fig. 2).20mg/ml concentration gave the highest ability to scavenge radical . Ascorbic acid score the highest scavenging activity (99.95%) followed by S c extract (97.4%), E j extract (95.27), BHA (90.85), BHT (88.31), z c extract (88.01) and P p extract gave the lowest value (51.68) respectively at (20mg/ml) concentration.

Low concentrations of hydrogen peroxide were found in air, water, human body, plants, microorganisms and food. Hydrogen peroxide can enter the human body across inhalation or through eye or skin and it decompose rapidly to $O_2$ and $H_2O$ and this may form ($^{\cdot}OH$) which can cause lipid peroxidation and DNA damage.

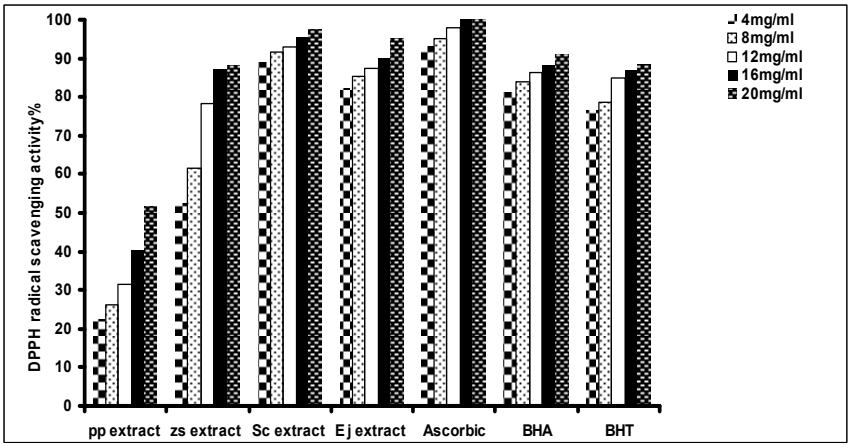


**Fig. 2. DPPH radical scavenging activities of different concentrations of fruits ethanolic extracts and standared antioxidant ( Ascorbic ,BHA and BHT) (LSD,0.141).**

The results revealed that there were positive relation between $H_2O_2$ scavenging ability and the concentrations of extracts or standard antioxidant (Fig. 3).Scavenging activity of $H_2O_2$ radical score the highest value in Ascorbic acid (99.95%) followed by E j ( 99.85%) extract, Sc (99.11%) extract, BHT (80.73%), BHA (55.54), Z s extract (43.14%) and Pp extract (26.31) at 40 mg/ml concentration.

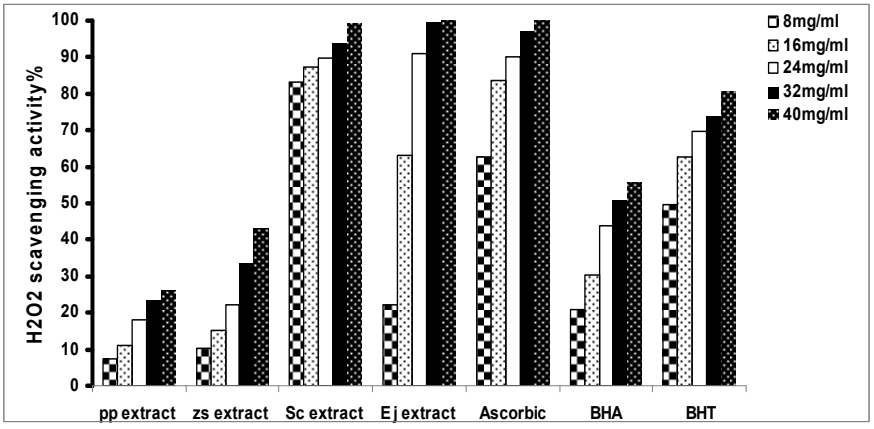


**Fig. 3. Hydrogen peroxide radical scavenging activities of different concentrations of fruits ethanolic extracts and standared antioxidant ( Ascorbic ,BHA and BHT) (LSD,0.382).**

The superoxide radical($O^{-}_{2}$) is formed in biological systems with photochemical reactions and cause oxidative stress (Meyer-Isaksen,1995) so it is very dangerous.

The results showed that superoxide radical scavenging activity had the higher value with standard antioxidant comparing with extracts (Fig, 4). Increasing concentration from 4mg/ml to 40mg/ml increased superoxide radical scavenging activity in extracts or standard antioxidant. Sc extract score higher reduction in superoxide radical (70.33), followed by E j extract (56.86), Zs extract (51.84) and Pp extract which gave the lowest value (40.82%).

The results concluded that there were positive correlation between concentrations of fruits extracts and scavenging ability against DPPH , $H_2O_2$ and $O_2^{-}$ radicals. The high scavenging ability % correlated with high content of TP, F, TT and FL. So Sc extract has the highest scavenging activity in comparison with others extracts. These results may be due to aromatic hydroxyl (OH) group( presented in phenolic compounds , flavonoids , tannins and flavonols) which can be give hydrogen atom to a undesired free radical which become inactive and the aromatic compounds become more stable radicals by delocalization around the π- electron system (Duthie *et al* 2000 and Nijveldt *et al* , 2001).

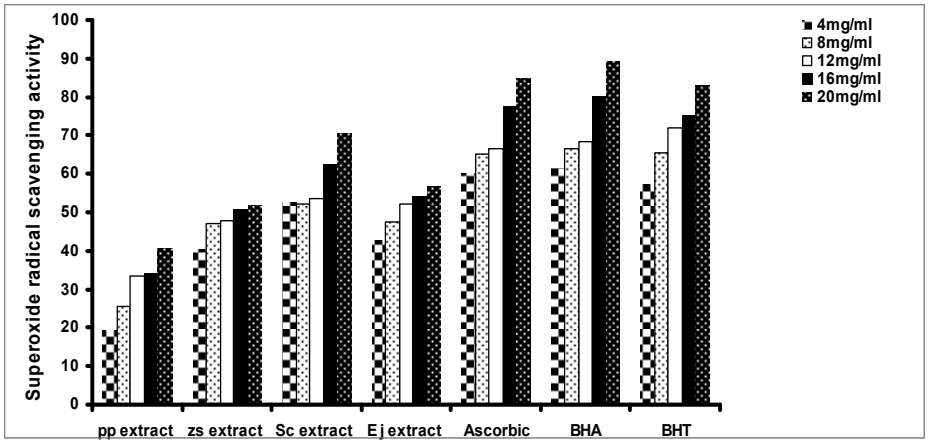


**Fig. 4. Superoxide radical scavenging activities of different concentrations of fruits ethanolic extracts and standared antioxidant ( Ascorbic , BHA and BHT) (LSD,0.234).**

Fruit extract can chelate $Fe^{++}$ and decrease the red colour of ferrozine-$Fe^{++}$ complexes. The decrease in absorbance in comparing with control indicate metal chelating ability of fruit extract. The result indicated that metal chelating ability of fruits extracts had the highest value in Sc extract, followed by standard antioxidants, E j extract, P p extract and the Z s extract gave the lowest value (Fig. 5).High ability of fruits extracts to chelate metal correlated with the high content of TP, F, TT and FL% .TP which contain one OH group on the aromatic ring can not chelate Cu and Fe ions . The presence of two hydroxyl groups or three hydroxyl groups on aromatic ring is essential to the chelation ability to metal ions (Andjelkovic *et al* ,2006). The metals chelation can prevent radical generation. There are three sites in flavonoids can chelate metal ion:- (Between $OH^-$ group on ring A and C=O group on ring C) , (Between $OH^-$group on ring C and C=O group on ring C ) and ( Between two $OH^-$groups in B ring) and can form different complexes. The complexes between Metal and flavonoid was stronger than the free flavonoids in chelation ability ( Marzena and Mateusz , 2012).

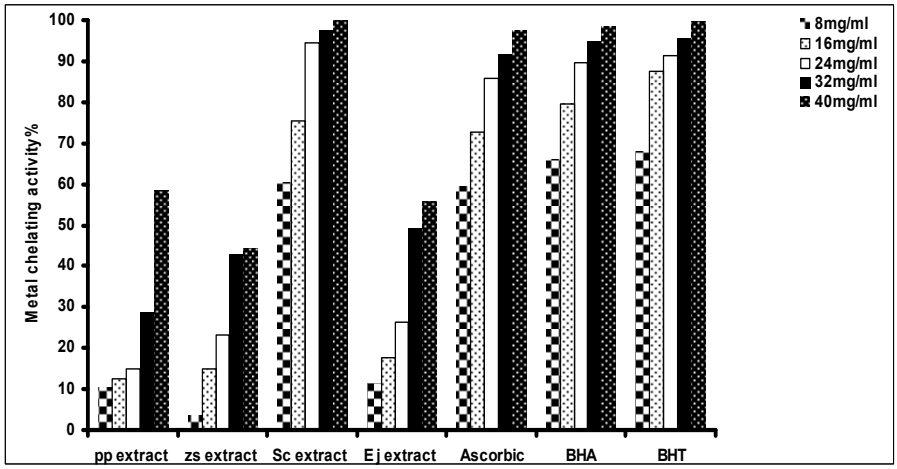


**Fig. 5. Metal chelating activities of different concentrations of fruits ethanolic extracts and standared antioxidant ( Ascorbic ,BHA and BHT) (LSD,0.173).**

The results were in harmony with Banerjee *et al* (2005) they reported that there were linear correlation between concentration of *Syzygium cumini* fruit water extract and superoxide and DPPH radicals scavenging ability % .This ability was correlated with fruit contents of vitamins , TP or TT and anthocyanins .. Also (Alhakamani *et al*, 2014 )found that alcoholic extract of zsc was rich in TP (24.64mg/ml ) and the extract exhibited 54.1% inhibition of DPPH radical at 200µg/ml. and consider good antioxidant.On the other hand Rajinder *et al* (2015) observed that *Ziziphus maurtiana* and *Eriobotrya japonica* were rich in phenol and flavonoids and had hydrogen donating activity . *Ziziphus maurtiana* had higher DPPH scavenging activity than *Eriobotrya japonica.* Medicinal properties of *Physalis peruviava Linnaeus* fruit are associated with the antioxidant capacity of polyphenol (Puente *et al* ,2011). Also *Physalis angulata L.*fruit methanolic extract possesses scavenging activities against DPPH ,superoxide and $H_2O_2$ radical. So it can play important role in biomolecules protection(Murali *et al* ,2013).

Singh *et al* (2012) investigated that phenolic compound and flavonoid contents of *Ziziphus spina-christi* fruit methanolic extract was 1644mg/100g .DW and 47 mg/100g.DW. respectively .the scavenging activity of extract were 51%against DPPH radical (at

140mg/ml) , 47% against superoxide radical (at 20µg/ml ) . Metal chelating ability of extract was 94% (at 100µg/ml).This antioxidant activity may be due to TP content which increase the electron donating ability.

## CONCLUSION

Sc ethanolic extracts consider strong antioxidant followed by Ej extract, which gave activity near of standard antioxidant. Zs and Pp extracts had lower antioxidant activity in all experiments, that correlated with extracts content of phenolic compounds, flavonoid, tannins and flavonols.

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## الانشطة المضادة للاكسدة والمكونات الكيميائية للمستخلص الايثانولى لبعض الثمار معمليا

**همت عبد الفتاح ابراهيم سعيد و محمد سيد صلاح هيكل**

**قسم الكيمياء الحيوية الزراعية – كلية الزراعة – جامعة عين شمس**

اجريت هذة الدراسة لتقييم الانشطة المضادة للاكسدة للمستخلصات الايثانولية لأربعة من الفواكة :- الحرنكش(*physalis peruviana* L. )، النبق ( *Ziziphus spina-christi* ) ، البامبوزيا (*Syzygium cumini* ) والبشملة ( *Eriobotrya japonica* ) باستخدام طرق معملية لتقييم النشاط المضاد للاكسدة باستخدام خمسة تركيزات مختلفة من المستخلصات ، كذلك تم تقدير محتوى المستخلصات من المركبات الفينولية الكلية ، الفلافونويد ، التانينات الكلية و الفلافونول. اظهرت النتائج ان المستخلص الايثانولى للبامبوزيا يحتوى على اعلى محتوى من المركبات الفينولية الكلية ، الفلافونويد ، التانينات الكلية و الفلافونول يليه مستخلص البشملة ، النبق و الحرنكش على الترتيب. اعطت كل المستخلصات تأثير مضاد للاكسدة واظهر مستخلص البامبوزيا اعلى نشاط مضاد للاكسدة يليه مستخلص البشملة ، النبق و الحرنكش على الترتيب.وبمقارنة النشاط المضاد للاكسدة للمستخلصات ( تحت الدراسة) بالنشاط المضاد للاكسدة لمضادات الاكسدة القياسية ( Ascorbic , BHA and BHT) اعطى مستخلص الايثانولى للبامبوزيا نشاط مماثل لنشاط مضادات الاكسدة القياسية فى معظم التجارب المستخدمة ( فى جميع التركيزات المختلفة من المستخلصات).