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Antioxidant and Insecticidal Effect of Some Plant Extracts against *Callosbruchus maculates* (coleoptera: Bruchidae)

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Abstract The aim of the study was to test the effect of methanolic guava leaf and pomegranate peel extracts as antioxidant and insecticide. In the Gas Chromatography and Mass spectroscopy analysis, 30 bioactive phytochemical compounds were identified in the methanolic extract of guava leaf and 23 bioactive phytochemical compounds were identified, in the methanolic extract of pomegranate peel. The fraction for guava leaf methanol extract was characterized by large amounts of caryophyllene , β- humulene and longifolene, which constituted 16.42% , 15.74% and 12.13% respectively , while the fraction of pomegranate peel methanol extract was characterized by large amounts of longifolene, alloaomadendrene and β- humulene which constituted 23.34% , 13.57% and 12.53% respectively .The phenolic contents of guava leaves and pomegranate peel extracts were found to be 154.16 and 280 (mg/g extract), respectively, while total flavonoids content were 55.45 and 69.24 (mg/g extract) respectively. Studies have confirmed that the antioxidant activity of plants extracts depends on the concentration of phenolic compounds, the methanolic extracts were assayed by DPPH scavenging activity and reducing power. Both methanolic guava leaf and pomegranate peel extracts appeared antioxidant activity in reducing power and DPPH scavenging activity assay, while the methanolic pomegranate peel extract was the more effective one compared with guava leaf extract. Insecticidal effects of these extracts on insect beetle cowpea were studied by determination LC50 which were 1.22 and 2.3 % for pomegranate peel and Guava leaves methanol extracts, respectively. The biochemical effects of these extracts were studied. Total protein, total carbohydrate and lipids increased but decreased acetylcholine esterase, GOT and GPT .The results showed that peels of pomegranate greatest influential extract and attributed this to the large content of phenols and Flavonoids.

Keywords Antioxidant, Insecticidal Effect, *Callosbruchus maculates*

1. Introduction

Psidium guajava L. (Myrtaceae),guava is tree small, with less than 3m, rustic,native of tropical America, specially of Brazil and Antilhas [1]. Guava leaf extract is used in the treatment of many diseases. Pomegranate (*Punica granatum* L.) have been variously placed in the *Lythraceae*or *Punicacea* family, Pomegranate grows as a shrubor small tree reaching 4-10 m. The fruit size can vary from 6-12 cm in diameter and has a tough, leathery skin2. Pomegranate and its derivates such as juice, peel and seeds are rich source of several high-value compounds with beneficial physiological activities. Its high has led to applications in functional food formulation, mainly for heart and prostate health. Pomegranate the main source of many vitamins, such as vitamin C containing also vitamin E,

pectin, and carotenoids. Studies have proved that extracts of these plants have an anti-oxidant activity as a result because it contains large amounts of phenols tannin, essential oils, flavonoids, triterpenoids and sesquiterpenoids [2-3]. This result demonstrates that these bioactive compounds may beresponsible for the antioxidant activity of the extracts of guava leaves [4], obtained the highest antioxidant activity as well as the largest concentration of flavanols. These compounds have the ability to donate hydrogen atoms, and therefore inhibit the chain reactions caused by free radicals.

Protection of pulses stored against insect pests is one of the major problems in all parts of the world. Conventional methods have been used for including a long period of use of chemicals to control insects. Plants having insecticidal effects and repellent properties have traditionally been used by people all over the world, the focus today turns back into this option in light of the dangers of chemical pesticides. The harmful effects of phytochemcials or crude plant extracts against insects in many ways, including the suppression of calling behavior [5], growth retardation [6], toxicity [7], oviposition deterrence [8], feeding inhibition [9]**,** and decreasing in fecundity and fertility [10]. *Callosobruchus maculates* Linn. (Coleoptera: Bruchidae) is one of the major pests infesting stored pulses. The eggs are laid on the host grains; the larvae bore inside and after feeding and pupating emerge out as adults leaving behind damaged hollow seed-grains.

Some studies have shown that some plant extracts may be helpful in reducing the infestation of pests stored grain and can be a useful alternative to pesticides and so inexpensive and available for the farmers because they are not harmful to humans or the surrounding environment, as well as natural enemies. Researches indicate that these extracts contain some compounds such as phenols and flavoniods which have an effect on insects. The present study was conducted to investigate antioxidant and insecticidal activity of methanol extracts of guava leaves and Pomegranate peel against the cowpea beetles

2. Materials and Methods

2.1. Plant extracts preparation

Plant materials (Guava leaves and Pomegranate peel) were collected and dried. The dried plant materials were powdered using a grinder. The extraction was done at room temperature. About 100 g of dried, ground plant materials were soaked in methanol (1 L) for 5-7 days separately. The soaked material was stirred every 24 h using a sterilized glass rod. The final extracts were passed through Whatman filter paper No.1. The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40^{o}C and stored at 4^{o}C for further use.

2.2. GC / MS analysis of plant extracts

The analysis was carried out using a GC (Agilent Technologies 7890 A) interfaced with a mass – selective detector (MSD, Agilent 7000) equipped with an apolar Agilent HP-5ms (5%-phenyl methyl poly siloxane)capillary column (30 m×0.25 mm i.d. and 0.25 um film thickness) the carrier gas was helium with the linear velocity of ml/min . The identification of components was based on comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature

2.3. Determination of total phenolic compounds:

The amounts of phenolic compounds in different extracts were determined with Folin-Ciocalteu reagent using the reported method [11]. 2.5 ml of 10% Folin Ciocalteu reagent and 2 ml of Na_2CO_3 (2% w/v) was added to 0.5 ml of each sample of plant extract solution (1 mg/ml). The resulting mixture was incubated at 45°C with shaking for 15 min. The absorbance of the samples was measured at 765 nm using UV/visible spectrophotometer against blank. The content of phenolic in extracts was expressed in terms of gallic acid equivalent (mg of GA / g of extract).

2.4. Estimation of total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination [12]. One millilitre (1 ml) of sample (1 mg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 min. The absorbance of the reaction mixture

was measured at 420 nm with UV/ visible spectrophotometer against blank. The content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of RU / g of extract).

2.5. *In vitro* antioxidant activity

2.5.1. 2, 2'-diphenly-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method [13] . The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard. 0.004% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 20 min and optical density was measured at 517 nm using Spectrophotometer. Methanol (1 ml) with DPPH solution (0.004%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below:

Percent (%) inhibition of DPPH activity = A – B /A *100

Where A = optical density of the blank and B = optical density of the sample.

2.5.2. Reducing power assay

The reducing power of different extracts was determined according to the reported method [14]. 2.5 ml of extract (25-400 µg/ml) in water were mixed with a phosphate buffer (2.5 ml, 0.2M, pH6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50^oC for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm against blank. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as a positive control

2.6. Biochemical studies of adult insects treated with LC_{50}

2.6.1 Determination of LC_{50}

-Mass rearing of insects

It has been breeding the insect for several generations in the Plant Protection Research Institute pests of stored grain and then were taken to the cultured samples in the lab Economic Entomology Department and the agricultural animal College of Agriculture, University of Menoufiya for several also generations at a temperature of 33±2 ° C and relative humidity of 65± 5%. Full insects lay eggs individually cowpea seeds. Eggs are grouped and placed in glass cages with 200 grams of seeds of cowpea and covered in cloth Mosselln until hatching. After emergence of adult insects collect to reproduction other generations or to be used in experiments.

Five concentrations were prepared of the extracts under study and were treated adult of insects. And was the work of three replicates for each concentration and each replicate contain 20 insect The assessment of mortality rate was corrected for control mortality [15], and been using propane program to determine LC_{50}. After that, the adult treatment with LC_{50} to be used in chemical experiments to see the biochemical effects of extracts under study on these insects.

- Chemicals

Bovine albumin standard was purchased from Stanbio laboratory (Texas , USA) . Commasie brilliant blue G-250 was from sigma (sigma chemical co.).P- nitroanisole (purity 97%) was obtained from Ubichem Ltd.(Ham pshire),while nicotinamide ademine dinucleotide phosphate (reduced form , NADPH)was from BDH chemicals Ltd. (Poole , England). The rest of chemicals were of high quality and purchased from commercial local companies .

2.6.2. Apparatus

Insects treated with LC50 were homogenized for biochemical analysis in a chilled glass Teflon tissue homogenizer (ST – 2 Mechanic-Preczyina , Poland). After homogenation , supernatants were kept in a deep freezer at -20ºC till use for biochemical assays . Double beam ultraviolet / visible spectrophotometer (spectronic 1201 , Milton Roy Co. ,USA) was used to measure absorbance of colored substances or metabolic compounds .

2.6.3. Preparation of insects for analysis

The insects were prepared as described [16]. They were homogenized in distilled water (50 mg /1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2 ºC in a refrigerated centrifuge. The deposits were discarded and the

supernatants ,which is referred as enzyme extract ,can be stored at least one week without appreciable loss of activity when stored at 5 °C .

2.6.4. Total proteins

Total proteins were determined by the reported method [17]. Protein reagent was prepared by dissolving 100mg of Coomassie Brilliant blue G-250 in 50ml 95% ethanol. To this solution 100 ml 85% (W/V) phosphoric acid were added . The resulting solution was diluted to a final volume of 1 liter.

Sample solution (50µl) or for preparation of standard curve 50µl of serial concentrations containing 10 to 100µg bovine serum albumin were pipetted into test tubes . The volume in the test tube was adjusted to 1 ml with phosphate buffer(0.1M,pH 6.6). Five millimeters of protein reagent were added to test tube and the contents were mixed either by inversion or vortexing. The absorbance at 595 nm was measured after 2 min and before 1 hr against blank prepared from 1 ml of phosphate buffer and 5 ml protein reagent.

2.6.5. Determination of total carbohydrates

Total carbohydrates were estimated in acid extract of sample by the phenol-sulphuric acid reaction [19].

Total carbohydrates were extracted and prepared for assay as per reported method [19].

Sample (1 g) was homogenized in 0.3N $HClO_4$ (5 ml) at 0˚C for 1 min. The homogenate was kept in ice for further 10 min. Insoluble matter was removed by centrifugation for 3 min at 2000 rpm and washed twice in ice –cold $HClO_4$ (5 ml) by redispertion and centrifugation. The three supernatant combined into acid extract.

Hundred microliters of the acid extract were added into a colorimetric tube to 0.5 ml of phenol (20 percent w/v). Then 5 ml of concentrated sulfuric acid were added rapidly with shaking. The tubes were allowed to stand 10 min, then they were shaken and placed for 10-20 min in water bath at 25 to 30 °C before readings.

Blanks were prepared by substituting distilled water for the sugar solution. The absorbance of characteristic yellow – orange color is measured at 490 nm against blank. Total carbohydrate is expressed as :µg glucose / gm fresh weight.

2.6.6. Determination of total lipids

Total lipids were estimated by the method of [20] using phosphovanillin reagent prepared by dissolving of 0.6 gm pure vanillin in 10 ml ethanol and completed to 100 ml with distilled water. Then 400 ml conc. Phosphoric acid wAs added.

250 ul of sample were added to conc sulphuric acid (5 ml) in a test tube and heated in aboiling water bath for 10 min. After cooling to room temperature, the digest was added to phosphovanillin reagent (6 ml). After 45 min , the developed color was measured at 525 nm against reagent blank .Optical density was compared to that of a reference standard and results expressed as mg lipids/ ml heamolymph .

2.6.7. Acetylcholinesterase determination

AchE (acetylcholinesterase) activity was measured according to the method described by [21], using acetylcholine bromide (AchBr) as substrate.

The reaction mixture contained 200 µl enzyme solution, 0.5 ml 0.067 M phosphate buffer (pH 7) and 0.5 ml AchBr (3 mM). The test tubes were incubated at 37 ºC for exactly 30 min, 1 ml of alkaline hydroxylamine (equal volume of 2 M hydroxylamine chloride and 3.5 M NaOH) was added to the test tubes. Then 0.5 ml of HCl (1 part of conc. HCl and 2 parts of H_2O) was added. The mixture shaken vigorously and allowed to stand for 2 min, 0.5 ml of ferric chloride solution (0.9 M $FeCl_3$ in 0.1M HCl) was added and mixed well. The decrease in AchBr resulting from hydrolysis by AchE was read at 515 nm.

2.6.8. Transaminases determination

Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase

(GOT) were determined colorimetrically according to the method [22]. GOT transfer amino group from L-aspartate to α-keto acid (α-ketoglutaric acid),producing a new amino acid (L-glutamate) and a new keto acid acid (oxaloacetic acid).GPT transfer the amino group from D,l alalnine to α-keto acid (α-ketoglutaric acid), resulting in a new amino acid (L-glutamate) and a new Keto acid (pyruvic acid) .

Pyruvate or oxaloacetate reacts with 2,4-dinitrophynylhydrazine, formig pyruvate or oxaloacetate hydrazone which in alkaline medium form a brown color , which can be measured spectrophotometrically . The reaction mixture

consisted of 100 sample , and 1 ml of mixture of phosphate buffer (PH 7.2) ,0.2 mM α-ketoglutaric and 200 mM D,L alanine or L-aspartate . Incubate for exactly 30 minutes . Add 1 ml of 0.001M 2.4-dinitrophenylhydrazine. Wait for at least 30 minutes. Then after, 10 ml of 0.4 N NaOH were added. The optical density of the produced brown colour is measured after 5 minutes , using spectrophotometer at 520 nm. The enzyme activity is expressed as U /gm body weight.

3 Statistics

All experiments contained 3 replicates (insects homogenates), and the results of biochemical determinations were pooled from triplicate determinations. The results were analyzed by one – way analysis of variance (ANOVA) using cost at statistical software (cohort software , Berkeley). When the ANOVA statistics were significant ($P < 0.01$), means were compared by the Duncan's multiple range test .

4. Results and Discussions

4.1. Chemical Composition of Plant Extracts

Gas Chromatography and Mass spectroscopy analysis of compounds was carried out in methanolic leaf extract of guava and methanolic peel extract of pomegranate shown in Table 1 and 2. In the GC- MS analysis, 30 bioactive phytochemical compounds were identified in the methanolic extract of guava leaf and 23 bioactive phytochemical compounds were identified in the methanolic extract of pomegranate peel . The identification of phytochemical compounds is based on the peak area, retention time molecular weight and molecular formula.

The fraction for guava leaf methanol extract was characterized by large amounts of caryophyllene , β- humulene and longifolene, which constituted 16.42% , 15.74% and 12.13% respectively , while the fraction of pomegranate peel methanol extract was characterized by large amounts of longifolene, alloaomadendrene and β- humulene which constituted 23.34% , 13.57% and 12.53% respectively .

Table1: Phytocomponents identified in the methanolic leaf Extract of guava by GC-MS

No	Compound	Area sum%	No	Compound	Area sum%
1	Amino-6-tert-butyl-4-methylphenol	0.24	16	Longifolene	12.13
2	Geranyl isovalerate	0.74	17	α- Elemene	1.95
3	β – Guaiene	1.08	18	Trans- Calamenene	3.5
4	α- Ylangene	2.64	19	Cedrene	1.71
5	β – Copaene	2.03	20	Zearalenone	1.26
6	Cubedol	1.25	21	(+)-α-Tocopherol	0.95
7	Patchoulene	1.1	22	Cis- Jasmone	4.95
8	Caryophyllene	16.42	23	Limonene-6-ol, pivalate	0.95
9	γ- Selinene	0.51	24	Sinapic acid	7.92
10	β- Humulene	15.74	25	Butylated hydroxytoluene	2.06
11	Cis-α- Bisabolene	2.65	26	Cholecalciferol	3.04
12	Ledene	1.45	27	Retinol , acetate	0.75
13	α- Himachalene	1.57	28	Cis-13,16-Docasadienoic acid	1.29
14	γ- Muurolene	3.28	29	Retinoic acid	0.93
15	Alloaromadendrene	5.45	30	Phytol	0.48

Table2: Phytocomponents identified in the methanolic leaf Extract of pomegranate by GC-MS

No	Compound	Area sum%	No	Compound	Area sum%
1	Amino-6-tert-butyl-4-methylphenol	0.26	16	δ- Selinene	2.85
2	α- Ylangene	2.94	17	Zearalenone	3.48
3	β – Copaene	4.3	18	(+)-α-Tocopherol	1.43
4	Cubedol	1.83	19	Cis-Jasmone	1.88
5	Patchoulene	0.54	20	Globulol	4.81
6	γ- Selinene	0.92	21	Retinol , acetate	1.6

7	β- Humulene	12.53	22	Cis-13,16-Docasadienoic acid	0.54
8	Clovene	5.67	23	Retinoic acid	0.33
9	Ledene	5.75			
10	γ- Muurolene	5.36			
11	Alloaromadendrene	13.57			
12	Longifolene	23.34			
13	α- Elemene	1.65			
14	Trans- Calamenene	3.13			
15	Cedrene	1.27			

4.2. Total phenolic, total flavonoids contents in plant extracts

In extracted antioxidants from pomegranate peel and seed with the use of methanol, acetone and water and found that methanol gave maximum antioxidant yield. So we used methanol to extract active compounds in our tested plants [23].

The antioxidant activity of plants is mainly contributed by the active compounds present in them. The phenolic contents of guava leaves and pomegranate peel extracts were found to be 154.16 and 280 (mg/g extract), respectively, while total flavonoids content were 55.45 and 69.24 (mg/g extract) respectively (Table 3). These results agree with [24] who determined total phenols and total flavonoids of Pomegranate peel and pulp and [25] who studied total phenols in different guava leaves extracts.

From our data we can reported that methanolic extract of Pomegranate peel have high concentration of total phenolic compounds and total flavonoids content compared with the methanolic extract of guava leaves.

Table 3: Total phenolic and total flavonoids contents of guava leaves and pomegranate peel extracts.

Plant extract	**Total phenolic content (mg/g extract)**	**Total flavonoids content (mg/g extract)**
Methanol extract of guava leaves	154.16	55.45
Methanol extract of Pomegranate peel	280	69.24

4.3. Antioxidant Activity of Plant Extracts

Recently, DPPH scavenging method has been widely used in antioxidant activity studies of herb extracts. DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen –or electron– donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [26]. In fact, free radical scavenging method (DPPH) show the reduction of alcoholic DPPH solutions in the presence of an hydrogen donating antioxidant [27] and phenolic compound have been reported and provided to be potent hydrogen donators to the DPPH radical [28] because of their excellence structural chemistry [29]. The values of absorbance for guava leaf extract and pomegranate peel extract ranged from 54.54 to 65.37 and 59.6 to 94.66 respectively compared with the values of absorbance for ascorbic acid ranged from 94.66 to 98.31 and followed the order of effectiveness as: ascorbic acid > pomegranate peel extract > guava leaf extract . In general, the methanol extract of the pomegranate peel, exhibiting greater total phenolic and total flavonids content in the present analysis.

Fe (III) reduction is often used as an indicator of electron- donating activity, which is an important mechanism in phenolic antioxidant action [30]. In this assay, the presence of reductants (antioxidants) in the samples would result in the reduction of Fe^{+3} to Fe^{+2} by donating an electron. The amount of Fe^{+2} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Fig. 2 shows the dose– response curves for the reducing powers of the plant extracts. It was found that the reducing powers of extracts also increased with an increase in their concentrations. At the highest concentration (100 µg/ml) pomegranate peel extract showed highest activity (1.594) while guava leaves extract was (1.009), at the same concentration.

The free radical scavenging power of antioxidant components is very much associated with their total phenolic and total flavonids content [31-32]. The plant extract with higher levels of total phenolics and flavonoids also exhibit greater free radical scavenging [33-34]. Pomegranate peel extract evaluated by Ferric reducing antioxidant power (FRAP) assay, was found to be the richest source of antioxidants among peel extracts of the most commonly consumed fruits. Similarly, pomegranate peel extract demonstrated 2.8-fold higher antioxidant activity compared to pomegranate seed and leaf extracts [35].

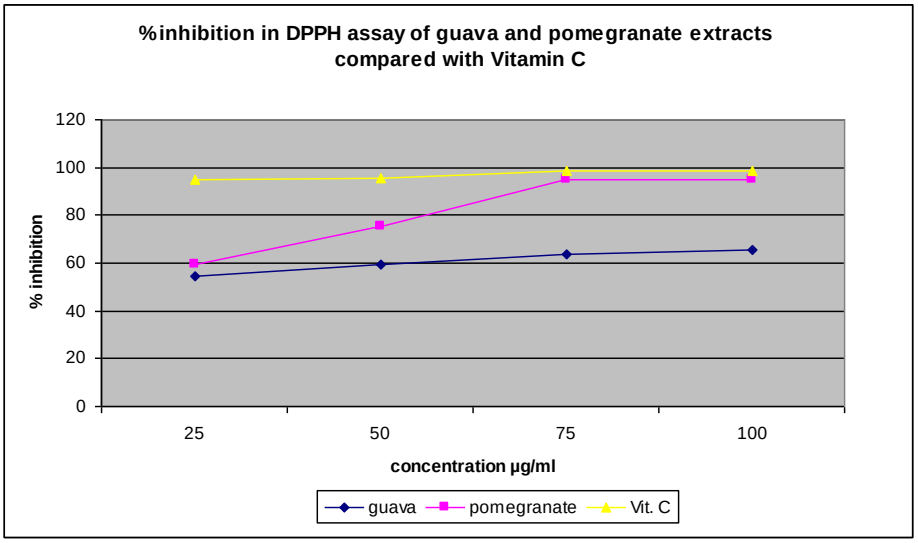

Figure 1: % inhibition in DPPH assay of guava and pomegranate extracts compared with Vitamin C

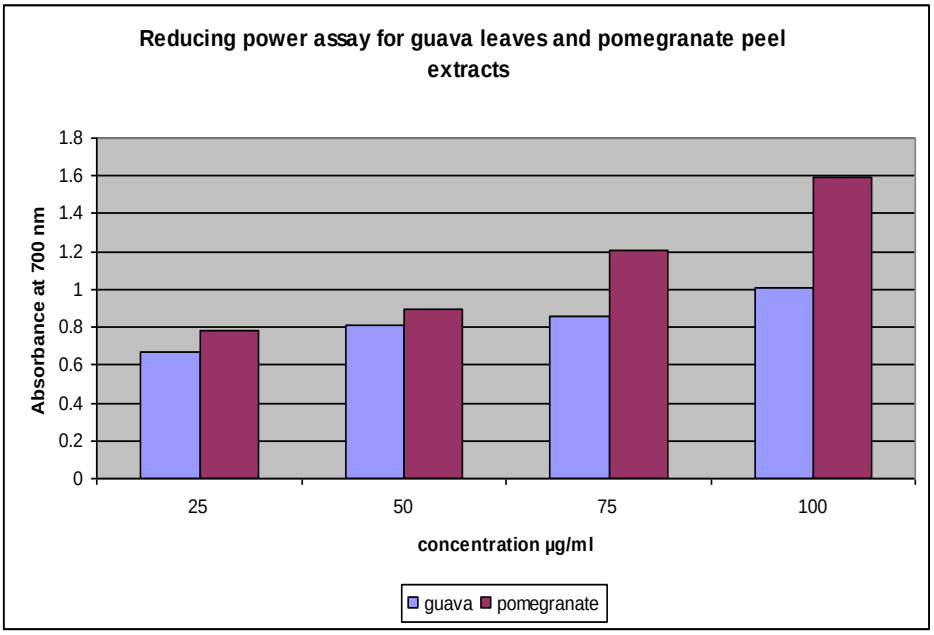

Figure 2: Reducing power assay for guava leaves and pomegranate peel methanol extracts

The free radical scavenging activity of plant phenolics involves electron donation to free radicals that converts them to relatively more stable compounds [36-37]. Studies have confirmed that the antioxidant activity of plants extracts depends on the concentration of phenolic compounds, and the antioxidant power of pomegranate peel extract has been found to linearly increase with the concentration of peel phenolics up to the level of 100 mg/g [38].

Guava leaf extracts from all conditions possess higher antioxidant capacities with higher total phenolic contents [39]. It could be considered that the attributed phenolic compounds in guava leaf extracts were the major group that

contributed strongly to the antioxidant activities of the extracts [25]. And also [24, 40] reported that the high antioxidant activity of the pomegranate peel extract appeared to be attributed to its high phenolics content. So we can explained our results by the correlation between both total phenolic and total flavonoids content and *in vitro* antioxidant activity of tested plant extracts.

4.4. Effect of plant extracts on biochemical aspects

Table (4) shows LC_{50} of methanol extracts of pomegranate peel and guava leaves. LC_{50} % were 1.2 % and 2.3 %. for pomegranate peel and guava leaves , respectively. The results showed that the toxicity of methanol extract of pomegranate peel was higher than methanol extract of guava leaves, so high content of total phenolic and total flavonoids may explain these results.

Table 5 shows the effect of the treatment with LC_{50} concentrate of methanol extracts of guava leaves and Pomegranate peel on biochemistry of beetle cowpea adults such as total proteins , total carbohydrates and total lipids, as well as the enzyme of acetylcholine esterase and transaminase (GOT and GPT). The results showed a significant increase in both protein , carbohydrates and lipids, respectively, and a significant decrease in the enzyme acetylcholine esterase and GOT and GPT respectively. Results indicated that the effect of treatment of these extracts may provide potential alternatives to currently used insect control tools because they constitute a rich source of bioactive chemicals [41] such as Pomegranate peel which contain a large amount of phenol and flavinoid compounds [42-46] showed that the methanol extract of Pomegranate peel exhibited anti-feeding effects activity against *T. castaneum* larvae whereas exhibited a low anti-feeding activity against adults. The results showed that Pomegranate peel extract the strongest, followed by guava leaf extract [47] showed that the oil and extracts of guava leaf significantly performed better than the control in terms of reduced population and emergence of *T. granarium* larvae and adults. Percentage mortality of *T. granarium* in groundnut seeds treated with the oil increased with rate of treatment. The presence of flavonoids, alkaloids, steroids, triterpenoids, cynogenic glycosides, in various amounts is an indication of insecticidal properties of the oil. Lipids and carbohydrates offered reliable nutritional components of the guava oil. Oxalates, tannins, saponins and phenols were absent in the studied leaf oil [48-50].

Table 4: LC_{50} of methanol extracts of pomegranate peel and guava leaves

Plant Extract	**LC_{50} %**	**Slope**	**95%fiducial limits**	
			lower	**upper**
Methanol extract of pomegranate peel	1.2	1.29±0.33	0.31	2.04
Methanol extract of guava leaves	2.3	1.27±0.31	1.09	3.28

Table 5: Effect of methanol extract of pomegranate peel and guava leaves on biochemical aspects of *Callosbruchus maculatus* adults which treated with LC_{50} of methanol extracts of pomegranate peel and guava leaves

	Total proteins ***(mg/g.b.wt)***	**Carbohydrates** ***(mg/g.b.wt)***	**Total lipids** ***(mg/g.b.wt)***	**AchE** ***(ug AchBr/min/g.b.wt)***	**Transaminases** (U 10^3/g.b.wt)	
					GOT	**GPT**
Control	9.18 c	4.8 c	2.80 c	163.6 a	3863a	1020b
Treatment 1	13.8 a	7.2 a	4.32 a	142 b	1740b	189.6a
Treatment 2	11.6 b	5.4 b	3.91 b	135.5 c	1650c	150c

Treatment (1) methanol extract of pomegranate peel

Treatment (2) methanol extract of guava leaves.

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