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Full Length Research Article

GUM EXUDATES OF *ACACIA SENEGAL* LINN IS AN ALTERNATIVE BINDING AGENT IN *DROSOPHILA MELANOGASTER* CULTURE FOR LABORATORY MAINTENANCE OF STOCKS

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ABSTRACT
The gum exudates of *Acacia senegal* Linn was utilized as a single agent or in combination with agar-agar in the formulation of *Drosophila* diet. Eight (8) corn-meal diets were formulated in two sets consisting of 15 – 40 % (w/w) *A. senegal* as a single binding agent or a mixture of *A. senegal* in the ratios of 1:5, 1:2, 1:1 and 2:1 to agar-agar per 100 g corn-meal diet. Biochemical markers of toxicity were analyzed spectrophotometrically. Standard methods of AOAC were employed to determine the physicochemical and proximate compositions of the formulated corn-meal diets. The results from this study showed high level of safety of the gum on adult *Drosophila melanogaster* (Harwich strain) of both sexes and of the same lineage. LC_{50} > 100 mg/g with insignificant mortality in all groups at varying concentration (1 – 100 mg/g) of the gum exudate was observed after 7 days of treatment. Significant increases in eclosion in the *A. senegal* – exposed flies at concentrations of 2, 4 and 5 mg/g diet was also observed after the treatment. A normal trend in locomotor activity was observed in all groups when flies were subjected to negative geotaxic assay, however, at concentrations of 50 mg/g there was an impairment in locomotion. The formulated *A. Senegal* containing diets have shown varying differences in physicochemical properties, even though no significant changes in the biochemical parameters including SOD1, Catalase and GST in all groups were seen. The collective findings of the present study revealed that the gum exudates of *A. senegal* L. may be a cost-effective alternative of agar-agar in corn-meal diet for laboratory maintenance of *Drosophila melanogaster* stocks.

Keywords: Acacia, Alternative, Corn-meal, Diet, *Drosophila melanogaster*, Exudate, Gum, Toxicity.

INTRODUCTION

Acacia senegal L. is a plant of the Leguminosae family originating from Sahel Sudan region of Africa (Kew Gardens, 2016). The plant is a producer of a natural gum called acacia gum, gum Arabic or locally '*Karo*' in Hausa. One of the largest producers of acacia gum is Nigeria (UNCTAD, 2018) with trees producing gums of export quality found growing in the North east (Borno and Yobe states) and North west (Jigawa, Sokoto, Zamfara, Katsina states) regions. Although, Nigeria is producer of acacia gum, it still remains an importer of processed gums which usually costs more than the exported unprocessed and semi-processed gums. Acacia gum is an edible biopolymer obtained as plant exudates from Acacia species (Williams & Phillips, 2001).

Drosophila melanogaster is a small fly, belonging to the family Drosophilae, whose members are often called "fruit flies. *Drosophila melanogaster* is an indispensable model for researches in biosciences and the use of this model dates back to over 100 years (Abolaji *et al.,* 2015). Nutrition is an important component of life and in order to maintain Drosophila cultures, a meal usually made up of all the necessary ingredients – carbohydrate, proteins, vitamins, minerals and water must be steadily supplied such that it permits the efficiency in mating, egg production, larval growth, emergence and sustenance of life in adulthood (Piper, 2017). Different laboratories have their optimized culture mixes for routine maintenance of Drosophila cultures which may constitute yeast as well as a variety of other ingredients that can include banana, powdered potatoes, rolled oats, molasses, cornmeal, malt extract, various sources of fat and purified sugars (Piper and Partridge, 2007). Different recipes are available for varying experimental purposes (BDSC, 2021) however, one ingredient that remains similar in most culture media is agar. Agar is a water soluble, non-digestible polysaccharide obtained from plant sources which act as a stabilizer, gelling and binding agent in foods and pharmaceuticals (Armisen & Galatas, 2009). In Drosophila culture, agar is used as a binder to achieve setting of the media in the most appropriate consistency that is suitable for burrowing by the growing larva and non-sticky for the flying adults. Dry food is a bad food unsuitable for Drosophila due to its hard consistency as result of very low moisture content often arising from evaporation during preparation.

The life cycle of drosophila involves complete metamorphosis with the fertile female laying hundreds of eggs that grow through the larval and pupa before emergence of a new adult. These stages require nutrients and energy for the growth and developmental stages of the fly life. During embryogenesis, the entire larval body plan is established though the expression of a number of genes and proteins which diffuse across to form the anterior- posterior axis and the dorsal-ventral axis. The diffusion of these proteins across the embryo forms gradients of each, and the varying levels of each protein will activate the transcription of specific cascades of genes that divides the embryo into segments, regions and structures (Gebelein *et al.,* 2004). Upon completing embryonic development, a first instar larva hatches from the egg and begins to eat. At this stage it is necessary for the larva to consume food not just for growth, but also to convert into storage as fats and sugars in the fat body, from where it will be used to sustain the larva though metamorphosis. At the end of the third instar stage, larvae begin to wander to find a place to pupate and are appropriately referred to as "wandering larvae." As adults do not grow, their final body size is primary regulated by the growth occurring after the critical size is reached (Nijhout *et al.,* 2014).

Acacia gum is an exudate formed by the bark of acacia trees as a result of wounding. It is rich in phytochemicals hence its antioxidative and anti-inflammatory potentials as reported by El – Garawani *et al*., (2021). Owing to its water solubility, emulsifying, thickening and gelling properties, acacia gum finds applications in food, pharmaceuticals and many other industrial processes. Agar is a natural product obtained from seaweeds. It is an effective thickener and binding agent in Drosophila culture media. However, acacia gum from *Acacia senegal* L. grown in Nigeria may be a cost-effective alternative to agar in Drosophila culture media. Innovation, problem solving and value addition while maintaining standard is key in every scientific research. This work aimed at the utilization of gum exudates of *Acacia senegal* L. in formulation of corn-meal diet cultures for routine laboratory maintenance of *Drosophila melanogaster*.

MATERIALS AND METHODS

Source of Acacia Gum: Crude, unprocessed acacia gum was purchased from the gum Arabic market in Nguru town Yobe state. It was authenticated at the department of biological sciences, Kaduna State University and given a voucher No KASU/BCH/881.

Preparation of Acacia Gum: The purchased acacia gum was cleaned by hand picking barks and other dirt. About 200g of the cleaned acacia gum was first ground by mortar and pestle to obtain powder which was further pulverized to finer particles by electric grinding with laboratory grinder (Binatone BLG 450). The resultant fine powder was stored in moisture-free, airtight bottle until needed.

Acute toxicity study of gum exudates of *Acacia senegal* in *Drosophila melanogaster*: Experiment to assess the toxicity of oral administration of *Acacia senegal* gum was done in two phases as previously described by Maduagwuna *et al*., (2020) and Abolaji *et al*., (2017) with some modifications. Adult flies (Harwich strain) of both sexes and 3 – 5 days old were grouped into six (6) each consisting of thirty (30) flies per treatment vial in 3 replications for 3 independent experiments in both phases I and II. Flies were treated with 10 mg, 20 mg, 30 mg, 40 mg and 50 mg gum exudates of *A. senegal* per 10 g diets in phase I. Phase II consisted of 100 mg, 250 mg, 500 mg, 750 mg and 1000 mg gum exudates of *A. senegal* per 10 g diets. The control group received acacia-free diet. Flies were allowed to feed on the treated diets in the various groups for 7 days while changing flies into a new treatment vial after the 3rd day. The number of dead flies were recorded after every 24 hours throughout the study. The lethal concentration (LC_{50}) was determined by plotting percentage survival versus log concentrations in a dose-response simulation using Graph pad prism 8.0.2. Effect of the gum exudates on fecundity and locomotion was also noted. At the end of the 7 days period, flies were harvested, homogenized and the hemolymph collected for further assays.

Drosophila corn-meal diet preparation: The *Drosophila* corn-meal diet is made up of yellow cornmeal, baker's yeast, agar-agar and nipagin (methyl paraben). About 1700 mL was measured, out of which 50 mL was used to dissolve one hundred grams (100 g) of finely ground yellow corn (local variety) to make a smooth paste of fluid consistency. Two-gram (2 g) nipagin was dissolved in 1 mL dilute ethanol (50 % v/v). The remainder of the water was poured into a stainless-steel pot and brought to boil. An aliquot (10 mL) of boiled water was used to dissolve two grams (2 g) baker's yeast. Two grams (15 g) agar-agar was carefully stirred into the remaining boiling water to complete dissolution. This was allowed to continue boiling for about 10 minutes before adding the cornmeal paste by stirring. The cornmeal mixture is allowed to further cook for another 10 minutes before adding dissolved baker's yeast while continuously stirring to avoid clumping. The meal was allowed to briefly cool for 2-3 minutes before adding nipagin. The meal was thoroughly mixed before storing in a vacuum flask until needed for the experiment. Experimental corn-meal diets were formulated by total or partial substitution of agar-agar in the standard diet for Drosophila.

Behavioral Assay

Negative geotaxis assay: The climbing performance of flies was carried out with the aid of a negative geotaxis assay, in which the percentage of flies that reached the 6 cm mark of the 15 cm treatment vial in 6 seconds was calculated. The experiment was repeated three times at each time point and recorded (Abolaji *et al*., 2017).

Bioassay

Homogenization of flies: The flies were manually homogenized in 2 mL of phosphate buffer saline (0.1 M pH 7.4) using a mini pestle in a 1.5 mL tube. This was centrifuged at 3000 rpm for 15 minutes and the supernatant collected as the hemolymph, stored at 4 ^{o}C for biochemical assessment.

Determination of catalase activity: As reported by Aebi (1984), about 250 µL of 50 mM potassium buffer (PH 7.0) and 250 µL of 300 mM H_2O_2 were measured and transferred into 1.5 mL centrifuge tube, 10 µL of the sample lysate was added to the tube and mixed thoroughly. A maximum of 200 µL was dispensed into 96 well plates in triplicate and the loss in absorbance was monitored on BIOTEK microplate reader at the interval of 30 seconds for 2 minutes and 240 nm. Catalase activity was then calculated and expressed as µmol of utilize by the enzyme per minutes per unit milligram of protein.

Determination of glutathione-s-transferase activity: As previously described by Habig and Jakoby (1981) 20 µL of 0.25 M potassium buffer pH 7.0 was weighed and transferred into 1.5 mL centrifuge tube. About 50 µL of 2.5 mM EDTA was added to the tube. Also, 10.5 µL of distilled water and 500 µL of 0.1 M GSH were added to and incubated at 25 ^{o}C. 10 µL of 25 mM CDNB and 20 µL of sample diluted at 1:5 ratio was equally added. The mixture was monitored for 5 min (10 s intervals) at 340 nm on BIOTEK microplate reader.

Determination of superoxide dismutase activity: As reported by Misra and Fridovich (1972) a volume of 200 µL of the sample was diluted with 800 µL of distilled water (1:5). Then 200 µL of the diluted sample was added to 2500 µL of 0.05 M carbonate buffer (pH 10.2) in the cuvette and allowed to equilibrate in a spectrophotometer. Then, 300 µL freshly prepared 0.3 M adrenaline was added to start the reaction. The cuvette used as standard contained 2500 µL buffer, 300 µL adrenaline, and 200 µL of water. Absorbance was monitored every 30 seconds for 2.5 minutes at 480 nm.

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Physicochemical Assay

pH Measurement: The acidity of the formulated diets and the control were analysed by a digital pH meter.

Viscosity: The viscosity of formulated diets was measured by a digital rotational viscometer using spindle 4 of viscometer NDJ-5S as described by the attached manual.

S**pecific gravity**: The specific gravity of formulated diets was determined using a density bottle fitted with a stopper having a hole. The density bottle is cleaned and dried at a temperature of 105 ^{o}C to 110 ^{o}C and cooled. The mass of the bottle, including that of stopper was taken. About 5 g of each formulated diet was taken in the bottle and weighed. Distilled water was added to cover the sample. The formulated diet was allowed to soak water for about 2 h and water added until the bottle was half full. More water was added to the bottle to make it full. The stopper was then inserted in the bottle and its mass taken. The bottle was emptied, washed and then refilled with distilled water. The bottle was filled to the same mark as in the previous case and the process repeated several times to collect readings for the analysis.
M_1= mass of empty bottle
M_2 = mass of bottle and diet
M_3= mass of bottle, diet and water
M_4= mass of bottle filled with water
Specific Gravity = Mass of Diet/(Mass of Diet+(M4-M3)).

Proximate Analysis and Evaluation

Proximate Composition of the Formulated Corn-meal Diet: The methods of AOAC 2010 were used to evaluate the proximate compositions of the formulated diets and the control.

Modified Sensory Evaluation of the Formulated Corn-meal Diet: A modification of the 5-point hedonic scale was used to analyze some sensory parameters in the formulated diets. Five (5) panelists were guided on the requirements of Drosophila diet in respect of texture/consistency, aroma/odor, colour and overall/general acceptability based on the control (standard diet). The panelists rated the prepared formular to meet the acceptance of *Drosophila melanogaster* using an ordered but arbitrary scale of 5-point hedonic scale (1 = fail, 2 = fair, 3 = good, 4 = very good and 5 = excellent) for odor, texture and overall acceptability. For color of the formulated diets, a descriptive judgement based on 3 colour shades (1 = deep cream, 2 = cream and 3 = pale cream) was used.

Statistical analysis: The results of the present study are reported as means ± SD (or SEM where applicable) and the data were collected and analyzed in triplicates. The data were analyzed by GraphPad Prism (v. 6) using one-way analysis of variance (ANOVA). Tukey test was used to compare differences among the formulated diets and the control at 95% confidence limit.

RESULTS

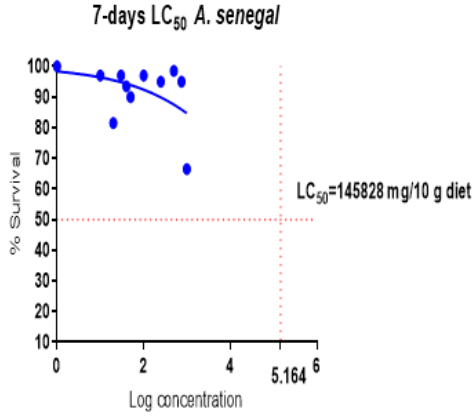

Figure 1: Acute toxicity assessment of gum exudate of *A. senegal* in *D. melanogaster* (LC_{50}): The log concentration of the extracts corresponding to 50 % death of Drosophila in the study is determined by joining the scattered dots representing the expected percentage of fly survival to form a trend line representative of the LC_{50}. Data is presented as Mean ± SEM of 3 replications for 3 independent experiments using all the various experimental concentrations

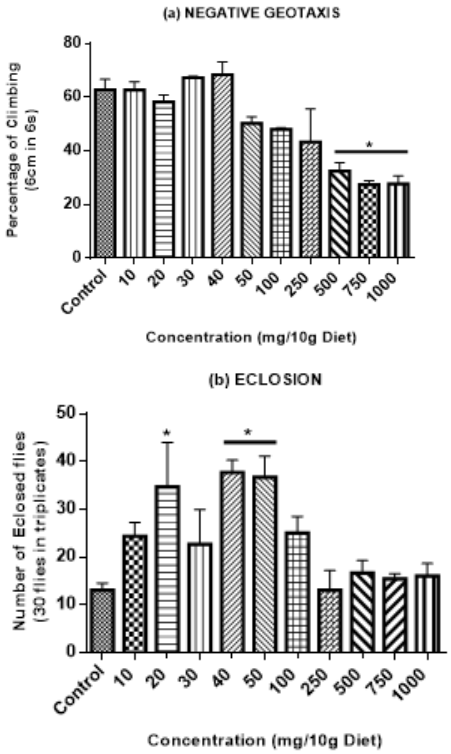

Figure 2: (a) Incidence of locomotor impairment (expressed as percentage of climbing 6 cm in 6 s) determined in a negative geotaxis assay in control and treated flies during a 7-day exposure period to *A. senegal* gum. $p < 0.05$ at concentrations of *A. senegal* gum 500 – 1000 mg/10g diet against control. (b) Effect of exposure of flies to *A. senegal* on eclosion, $p < 0.05$ in eclosion of *D. melanogaster* fed with acacia gum at concentrations of 20, 40 and 50 mg/10g diet. Values are mean ± SEM and n = 30 flies/three replicates

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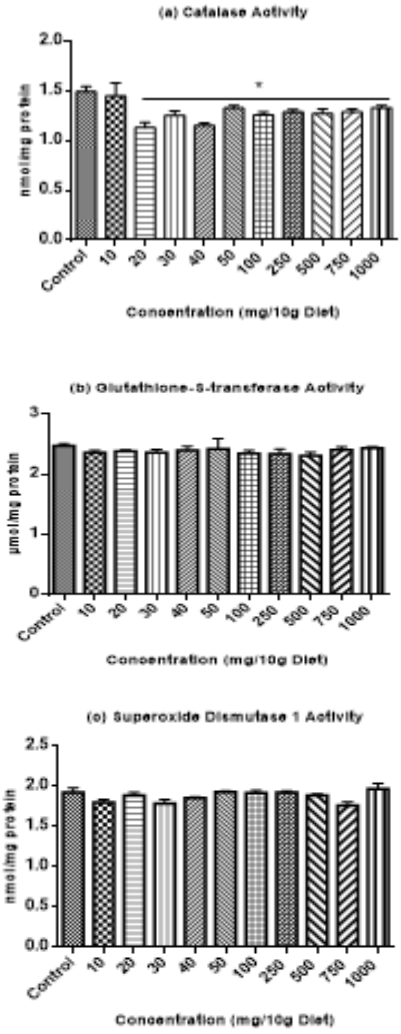

Figure 3: Catalase, glutathione-S-transferase and superoxide dismutase activities in control and treated flies after administration of acacia gum – substituted diet for a period of 7 days. Values are mean ± SEM and n = 30 flies/three replicates. (a) $P < 0.05$ against control. (b) $P > 0.05$ against control. (c) $P > 0.05$ against control

DISCUSSION

Acacia senegal is a tropical tree producing gummy exudates from its stem bark upon wounding by physical or mechanical process. This gum exudates find applications as a binder, stabilizer and thickener in food, pharmaceuticals and cosmetics. *Drosophila melanogaster* as it is called vinegar fly thrives on left over fermented foods in kitchen garbage. Maintenance of Drosophila in the laboratory involves supply of nutritious meal made up of all the components of a balanced diet – carbohydrate, proteins, lipids, vitamins, minerals and water. Agar – agar is a component of Drosophila culture which serves as a carbohydrate source and act as a stabilizer and binding agent to keep the food in a consistency suitable for livelihood. This is the first report that attempted to use gum exudates from *A. senegal* as a binding agent in Drosophila corn-meal culture. The study showed the potential applicability of Acacia gum as an alternative binding agent in preparation of drosophila culture. We reported here the safety of inclusion of Acacia gum as a component of Drosophila corn-meal diet for laboratory maintenance of flies. Acacia Senegal is rich in ascorbic acid and phenolic compounds and exhibited antioxidant potentials *invitro* (Authors' unpublished work). The toxicity assessment after 7 days exposure to *Acacia senegal* (Figure 1) yielded an LC_{50} value of 1.46e3 mg/g diet. This high value of LC_{50} is suggestive of non-lethality of the *A. senegal* gum in exposed flies. There are no similar reports on toxicity of *A. senegal* in *Drosophila melanogaster*, however a number of reports have shown safety of *A. senegal* in rodents. In a study of leaves extract of *A. senegal*, an LD_{50} value of 5000 mg/kg body of rodents was reported by Magnini *et al*., (2021). Doi and co-workers (2006) also reported the safety of a processed gum from *A. Senegal* in F344 rats. European Food Safety Authority (EFSA) reported no biologically relevant adverse effects on short-term and sub-chronic administration of oral doses up to 5,000 mg acacia gum/kg bw per day to rats and 20,000 mg acacia gum/kg bw per day to mice (Mortensen *et al*., 2017). Therefore, FDA (2014) generally regards Acacia gum as a safe excipient.

Eclosion is one of the end-point assays of toxicity in drosophila. In this study, *D. melanogaster* fed with acacia gum at concentrations of 20, 40 and 50 mg/10g diet demonstrated significant increases in eclosion when compared to the acacia-free control diet. This means that more flies eclosed from these concentrations during the first phase as compared to the second phase of toxicity assessment where less flies emerged. This finding could be attributable to ageing of the flies at the second phase of the toxicity assessment. Although the emergence of flies in the eclosion assay in this study was not in a concentration dependent pattern, it may likely be that the slightly acidic Acacia gum has mixed with yeast to come up with fermentation odor suitable for oviposition as reported by Baumberger, (1917). There is no reported study on the effect of *Acacia senegal* gum on eclosion or fertility in drosophila, however, Nasir *et al*., (2020) reported dose-dependent increases in spermatogenesis upon oral administration of *A. senegal* gum to adult male Balb/c mice. Deshpande *et al*. (2015) in another study reported increased gustatory responses and food intake with prolonged life span in Drosophila upon exposure to acidic diet.

The profile of antioxidant enzymes SOD1, catalase and glutathione-s-transferase activity of flies that received oral administration of *A. senegal* gum (Figure 3). The toxicity or safety of a substance is determined by the release of antioxidant and oxidative stress marker enzymes. Antioxidant enzymes are released to protect the cells against free radical toxicity (He *et al*., 2017). SOD, Catalase and Glutathione peroxidase are considered the first line of defense in response to toxicity or free radicals (Ighodaro & Akinyele, 2018). Superoxide anion (*O2) is a highly reactive anion which is constantly generated through endogenous and exogenous processes. It is neutralized to a less reactive H_2O_2 by dismutation reaction catalyzed by superoxide dismutase. Three types of SOD exist in humans – SOD1 localized in the cytosol with Cu/Zn active site, SOD2 localized in the mitochondria with Mn active site and SOD3 located extracellularly with a Cu/Zn active site (Fukai & Ushio-Fukai, 2011). In Drosophila, the Cu/Zn SOD1 is important in linking diet to lifespan whereas Mn SOD2 is involved in mitochondrial activation proteins (Tower, 2015). Increases in SOD levels is expected when the defense system is switched on to neutralize superoxide radicals. However, findings from this study did not show any activation in SOD1 in all treatment groups.

Though a significant decrease of 15 – 25 % in catalase activity at concentrations ≥20 mg/10g was observed in this study, a possible explanation to this drop experienced may be the elemental composition of *A. senegal.* Since enzyme activities are generally affected positively or negatively by the presence of metals, *A. senegal* gum used in this study was found to be composed of metals such as magnesium and iron (unpublished) which may have affected the catalase activity. Zhang et al., (2021) in their study showed significant decreases in catalase activity in gills of crayfish arising from heavy metal composition.

Catalase and GST are antioxidant enzymes which protect cellular macromolecules by neutralizing intracellular peroxides and electrophilic oxidants respectively (Adedara *et al*., 2015). As part of the phase II conjugation reactions of xenobiotics, Glutathione-S-transferases are activated in order to conjugate a foreign compound or toxicant to glutathione during the detoxification process to protect cellular macromolecules from reactive species (Townsend and Tew, 2003). GST activity was significantly decreased at 20 mg/10g diet compared to the control group. However, in all other treatment concentrations, GST activity was not significantly different from the control group. This drop observed at 20 mg/10g diet could be due to possibility of stress during fleeting in the course of the assay. Acacia gum is reported to have no carcinogenic or teratogenic properties, and showed the absence of histopathological toxicities when administered to rats at doses > 5 g/kg/day for 3months (Ahmed, 2018). The enzyme profile of the *in-vivo* antioxidant defense system of exposed flies in this study was not reflective of toxicity, stress or damages. Taken together, this data further shows the safety of oral administration of *A. senegal* in Drosophila.

Different culture media and recipes exists for various experimental purposes using Drosophila as a model. The composition of the media usually covers all the nutritional requirements of living organisms – from water to all the macromolecules and vitamins needed for maintenance of life. Agar or agar-agar have been reported as an important ingredient of standard drosophila culture which helps to solidify the media (Baumberger, 1917; Spencer, 1947; Priyadarsini et al., 2020). In the absence of agar or agar-agar, the culture may be prepared by making a thick corn meal (Spencer, 1947) which may be too hard and difficult to pour and also inaccessible to the burrowing larva**.** In this study, acacia gum has been used to substitute agar-agar completely or partly to come up with a diet or culture media which is not too hard or difficult for drosophila larva or adults to burrow. Acacia gum is made up of non-digestible fibers which is full of amino acids. The presence of these amino acids offers acacia gums their antioxidant and anti-inflammatory potentials (Musa *et al*., 2019). Structurally, *A. senegal* gum consists of a core of (1,3)-β-d-galactose units with extensive branching at the C6 position. It is a mixture of a major component of lower molecular weight polysaccharides (M.Wt 0.25 × 10^6) and a minor component of higher molecular weight hydroxyproline-rich glycoprotein (M.Wt 2.5 × 10^6) (Ocheri *et al*., 2017). Owing to the highly branched structure, *A. senegal* gum forms viscous solutions only at high concentrations of approximately 50 % w/w (Williams, 2016; Abdalbasit, 2018).

The proximate composition of the formulated corn-meal diets in this study (Table 2) shows some variations. Crude protein composition of all formulated diets (diets 1-8) was significantly different from the control diet. The higher percentage was observed in diet1 (1.40±0.10 %), diet 3 (4.37±0.06 %) and diet 5 (4.53±0.06 %). Crude fat composition of the formulated diet 3 was significantly different from the control diet. However, there was no significant differences in the percentage crude fibre contents of all groups in this study. All the formulated diets and the control are the same in respect to the fibre content.

The percentage ash contents of the formulated diets 1,2,3,7 and 8 were significantly different from the control diet. However, diets 4,5 and 6 were not significantly different from the control. Significantly higher crude ash content was observed in Diet 3 compared to all the studied formulated diets. The percentage carbohydrate contents of the formulated diet 7 and the control diet were not significantly different; both recorded the highest carbohydrate contents in this study.

In terms of moisture contents, there was significant difference in all the formulated diets 1-8 and the control. Diet 1 recorded the highest percentage moisture content (17.47±0.06 %). While the lowest moisture content was seen in the control (9.47±0.06 %). Diet 6 and 8 share approximately same percentage moisture contents (10.97±0.06) with no significant difference between the two. Water is an important component of drosophila culture, the high moisture content observed in diet 1 is reflective of the inability of 40% acacia gum only to absorb water to form colloidal suspension in the formula. Mixture of *A. senegal* gum with a little percentage of agar-agar enhanced the water absorption capacity of the formula. Moisture content of the formulated diets and control showed some effect on specific gravity. Significant differences were observed in the diets with higher *A. senegal* contents than the agar-agar mixes. Diets 7, diet 8 and the control were almost same in proximate composition, for there were no significant differences in all the 3.

The physicochemical properties of the formulated diets in this study are presented in table 3. The acidity of the formulated diets 1 – 7 were significantly higher than the control. The pH of diet 8 and the control are not significantly different. All pH values in the formulated diets studied are within the mildly acidic range of 5.8- 6.8. This acidity is reflecting the composition of *A. senegal* gum in all the formulated diets. *Drosophila melanogaster* like humans respond to a repertoire of taste, they show preference to low levels of acidity which stimulate feeding and reproduction (Charlu *et al*., 2013; Chen & Amrein, 2017; Mi *et al*., 2021).

The texture/consistency of formulated diets 1-5 show significant differences with the control. However, diets 6,7 and 8 are not significantly different with the control. The texture/consistency of the formulated diets are affected by the moisture content of the diets. Less water content may be required to achieve the desired consistency for effective feeding of adult drosophila. Formulas with some amount of agar-agar had consistency similar to the control diet. Ocheri *et al*., (2017) reported acacia gum to be readily soluble in aqueous solution to give relatively low viscosity Newtonian solutions, even at higher concentration (20 – 30 % w/w). These macromolecules are weak polyelectrolytes and therefore, it is expected that pH, ionic strength and type of ions must have a significant effect on the viscosity of the colloidal solution (Sanchez, 2018). Overall, only diets 1 and 4 were significantly different from the control diet in term of an aided sensory evaluation.

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Table 1: Composition of formulated corn-meal diet for Drosophila

	Diet 1	Diet2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Control
Cornflour (g)	100	100	100	100	100	100	100	100	100
Agar-agar (g)	—	—	—	—	2.5	5	7.5	10	15
Acacia gum (g)	40	30	20	15	12.5	10	7.5	5	—
Baker's Yeast (g)	20	20	20	20	20	20	20	20	20
Nipagin (g)	2	2	2	2	2	2	2	2	2
Water (mL)	1700	1700	1700	1700	1700	1700	1700	1700	1700

Table 2: Proximate composition of the formulated corn-meal diet for Drosophila

	DIET 1	DIET 2	DIET 3	DIET 4	DIET 5	DIET 6	DIET 7	DIET 8	CONTROL
Protein (%)	1.40±0.10*	3.60±0.10	4.37±0.06*	1.58±0.03	4.53±0.06*	4.37±0.06	1.77±0.06	2.73±0.06	3.37±0.06
Carbohydrates (%)	78.43±0.32*	80.53±0.15*	78.56±0.21*	81.05±0.30*	76.03±0.21*	82.47±0.12*	84.73±0.15	83.80±17*	85.10±0.26
Crude fiber (%)	0.57±0.06	0.37±0.06	0.37±0.06	0.33±0.12	0.33±0.12	0.57±0.06	0.37±0.06	0.37±0.06	0.37±0.06
Fat (%)	1.17±0.06	1.37±0.06	1.77±0.06*	1.37±0.06	1.17±0.06	1.17±0.06	1.17±0.06	1.17±0.06	1.17±0.06
Moisture (%)	17.47±0.06*	13.17±0.25	13.47±0.06	15.00±0.20	15.47±0.06	10.97±0.06	11.00±0.10	10.97±0.06	9.47±0.06
Ash (%)	0.97±0.57*	0.97±0.57*	1.5±0.57*	0.47±0.57	0.47±0.57	0.47±0.57	0.97±0.57*	0.97±0.57*	0.47±0.57

Values are mean ± SD (n = 3).
Values with asterisks are significantly ($p < 0.05$) different from the control.

Table 3: Physicochemical properties of the formulated corn-meal diet for Drosophila

	Diet 1	Diet2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Control
pH	5.80±0.10	6.13±0.06	6.23±0.21	6.33±0.31	6.13±0.1	6.37±0.06	6.40±0.10	6.76±0.10	6.83±0.12
Specific gravity	1.03±0.10*	1.03±0.1	1.02±0.1	1.02±0.21	1.02±0.06	1.02±0.31	1.01±0.10	1.01±0.11	1.01±0.12
Temperature (°C)	26.1	26.9	28.1	26.8	27.1	27.7	27.4	27.9	27.7
Speed (rpm)	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Torque (%)	32.4	25.2	50.9	28.4	22.1	38.4	58.4	66.5	86.3
SpL4 mpa*s	16284	12622	30456	14266	17050	16692	29094	33359	86282
Viscosity (Pa*s)	16.3	12.6	30.5	14.3	17.05	16.7	29.1	33.4	86.3

Values are mean ± SD (n = 3).
Values with asterisks are significantly ($p < 0.05$) different from the control

Conclusion

Acacia gum from *Acacia senegal* trees is safe, easily available and cheap source of binding agent which finds application in many processes. The formulated diets in this study exhibited some degree of consistency in physicochemical, proximate composition and considerably less expensive. This gum may be suitable as an alternative to agar-agar in routine maintenance of *Drosophila melanogaster* cultures in the laboratories.

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REFERENCES

Abdalbasit, A. Mariod (2018). Chemical Properties of Gum Arabic, Editor(s): Abdalbasit Adam Mariod, Gum Arabic, Academic Press 2018, Pages 67-73. ISBN 9780128120026, https://doi.org/10.1016/B978-0-12-812002-6.00006-3. (https://www.sciencedirect.com/science/article/pii/B9780128120026000063)

Abolaji, A. O., Olaiya C. O., Oluwadahunsi, O. J. and Farombi, E. O. (2017). Dietary consumption of monosodium L-glutamate induces adaptive response and reduction in the life span of *Drosophila melanogaster*. *Cell Biochem Funct. 2017 Apr;35(3):164-170. doi: 10.1002/cbf.3259. Epub 2017 Mar 16. PMID: 28303588*.

Abolaji, A.O., Kamdem, J.P., Lugokenski, T.H., Farombi, E.O., Souza, D.O., Da Silva Loreto, E.L., Rocha, J.B.T. (2015). Ovotoxicants 4- vinylcyclohexene 1,2- monoepoxide and 4-vinylcyclohexene di-epoxide disrupt redox status and modify diff erent electrophile sensitive target enzymes and genes in *Drosophila melanogaster*, *Redox Biol. 5:328–339*.

Adedara Isaac A., Abolaji Amos, O., Rocha Joao, B. T. and Farombi Ebenezer, O. (2015). Diphenyl Diselenide Protects against Mortality, Locomotor Deficits and Oxidative Stress in *Drosophila melanogaster* Model of Manganese-Induced Neurotoxicity. *Neurochem Res DOI 10.1007/s11064-016-1852-x*

Aebi, H. (1984): Catalase activity. *Methods in Enzymology*, 105, 121–126.

Ahmed, A. A., Fedail, J. S., Musa, H. H., Musa, T. H., Sifaldin, A. Z. (2016). Gum Arabic supplementation improved antioxidant status and alters expression of oxidative stress gene in ovary of mice fed high fat diet. *Middle East Fertil. Soc. J. 21, pp 101–108.*

AOAC, (2000). Official methods of analysis of the Association of Official Analytical Chemists.,15th ed. Washington DC, USA. Pp. 56-60.

Armisén, R. and Gaiatas, F. (2009). 4 - Agar, Handbook of Hydrocolloids (Second Edition).

Baumberger, J. P. (1917). The Food of *Drosophila melanogaster* Meigen. *Proceedings of the National Academy of Sciences of the United States of America*, *3*(2), 122–126. https://doi.org/10.1073/pnas.3.2.122

Bloomington Drosophila Stock Centre (BDSC) (2021). In: Fly food Recipes accessed at URL: https://bdsc.indiana.edu/ Dept Biology, Indiana University 1001 E.Third St. Bloomington, IN 47405-7005 USA

Charlu, S., Wisotsky, Z., Medina, A. and Dahanukar, A. (2013). Acid sensing by sweet and bitter taste neurons in *Drosophila melanogaster*. *Nature communications*, *4*(1), pp.1-10.

Chen, Y., Amrein, H. (2017). Ionotropic Receptors Mediate Drosophila Oviposition Preference through Sour Gustatory Receptor Neurons. *Curr Biol. 2017 Sep 25;27(18):2741-2750.e4. doi: 10.1016/j.cub.2017.08.003. Epub 2017 Sep 7. PMID: 28889974; PMCID: PMC5680077.*

Doi, Y., Ichihara, T., Hagiwara, A., Imai, N., Tamano, S., Orikoshi, H., Ogasawara, K., Sasaki, Y., Nakamura, M. and Shirai, T. (2006). A ninety-day oral toxicity study of a new type of processed gum arabic, from Acacia tree (*Acacia senegal*) exudates, in F344 rats. *Food Chem Toxicol. 2006 Apr;44(4):560-6. doi: 10.1016/j.fct.2005.09.002. Epub 2005 Oct 25*. PMID: 16256256.

Editor(s): G.O. Phillips, P.A. Williams. Woodhead Publishing Series in Food Science, Technology and Nutrition Woodhead Publishing. Pages 82-107. ISBN 9781845694142 https://doi.org/10.1533/9781845695873.82.

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen, A, Aguilar, F, Crebelli, R, Di Domenico, A, Frutos, MJ, Galtier, P, Gott, D, Gundert-Remy, U, Lambré, C, Leblanc, J-C, Lindtner, O, Moldeus, P, Mosesso, P, Oskarsson, A, Parent-Massin, D, Stankovic, I, Waalkens-Berendsen, I, Woutersen, RA, Wright, M, Younes, M, Brimer, L, Christodoulidou, A, Lodi, F, Tard, A and Dusemund, B. (2017). Scientific Opinion on the re-evaluation of acacia gum (E 414) as a food additive. *EFSA Journal* 2017; 15 (4): 4741-51 pp. doi:10.2903/j.efsa.2017.4741

El-Garawani, I., Hassab El-Nabi, S., El Kattan, A., Sallam, A., Elballat, S., Abou-Ghanima, S., El Azab, I. H. (2021). The Ameliorative Role of *Acacia Senegal* Gum against the Oxidative Stress and Genotoxicity Induced by the Radiographic Contrast Medium (Ioxitalamate) in Albino Rats. *Antioxidants*, *10*(2), 221. MDPI AG. Retrieved from http://dx.doi.org/10.3390/antiox10020221

Gebelein, B., McKay, D. J. and Mann, R. S. (2004). Direct integration of Hox and segmentation gene inputs during Drosophila development. *Nature. 2004;431(7009):653-659*

Habig, W. H. and Jakoby, W. B. (1981) Assays for differentiation of glutathione S- transferases. *Methods Enzymol 77:398–405*

He, L., He, T., Farrar, S., Ji, L., Liu, T., Ma, X. (2017). Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. *Cell Physiol Biochem 2017;44:532-553. doi: 10.1159/000485089.*

Kew, G. (2016). *Acacia senegal* (gum arabic). Board of Trustees of the Royal Botanic Gardens, Kew accessed at http://www.kew.org/science-conservation/plants-fungi/acacia-senegal-gum-arabic

Maduagwuna, C. A., Omale, S., Etuh, M. A., Gyang, S. S. (2020). nHexane Extract of *Caryota no* Seeds Modulates Survival and Longevity in *Drosophila Melanogaster. J Gerontol Geriatr Med 6: 069.*

Magnini, R. D., Nitiéma, M., Ouédraogo, G.G., *et al.* (2021). Toxicity and bacterial anti-motility activities of the hydroethanolic extract of *Acacia senegal* (L.) Willd (Fabaceae) leaves. *BMC Complement Med Ther* **21,** 178 https://doi.org/10.1186/s12906-021-03348-5

Mi, T., Mack, J. O., Lee, C. M., Zhang, Y. V. (2021). Molecular and cellular basis of acid taste sensation in Drosophila. *Nat Commun. 2021 Jun 17;12(1):3730. doi: 10.1038/s41467-021-23490-5. PMID: 34140480; PMCID: PMC8211824*.

Misra, H. P. and Fridovich, I. (1972). The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. *Journal of Biological Chemistry, 247, 3170-3175.*

Mortensen, A., Aguilar, F., Crebelli, R., Di Domenico, A., Frutos, M. J., Galtier, P., Gott, D., Gundert-Remy, U., Lambré, C., Leblanc, J. C., Lindtner, O., Moldeus, P., Mosesso, P., Oskarsson, A., Parent-Massin, D., Stankovic, I., Waalkens-Berendsen, I., Woutersen, R. A., Wright, M., … Dusemund, B. (2017). Re- evaluation of acacia gum (E 414) as a food additive. *EFSA journal. European Food Safety Authority, 15(4), e04741. https://doi.org/10.2903/j.efsa.2017.4741*

Musa, H. H., Ahmed, A. A. and Musa, T. H. (2019) Chemistry, Biological, and Pharmacological Properties of Gum Arabic. In: Mérillon JM., Ramawat K. (eds) Bioactive Molecules in Food. Reference Series in Phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-319-78030-6_11

Nasir, O., Alqadri, N., Elsayed, S., Ahmed, O., Alotaibi, S. H., Baty, R., Omer, H., Abushal, S. A., and Umbach, A. T. (2020). Comparative efficacy of Gum Arabic (*Acacia senegal*) and *Tribulus terrestris* on male fertility. *Saudi pharmaceutical journal: SPJ: the official publication of the Saudi Pharmaceutical Society, 28(12), 1791–1796. https://doi.org/10.1016/j.jsps.2020.11.005*

Nijhout, H. F., Riddiford, L. M., Mirth, C., Shingleton, A. W., Suzuki, Y., Callier, V. (2014). The developmental control of size in insects. *Wiley Interdisciplinary Reviews Developmental Biology. 2014;3(1):113-134*

Ocheri, C., Agboola, J. B. and Moses, O. M. (2017). Investigation of Gum Arabic and Its Suitability as a Composite Binder for Core Production. *J Powder Metall Min 6147. doi:10.4172/2168-9806.1000147*

Phillips, G. O. and Williams, P. A. (2001). Tree exudate gums: natural and versatile food additives and ingredients. Food Ingredients and Analysis International, Vol.23, (nd) pp. 26-28, ISSN: 0968-574X.

Piper, M. D. and Partridge, L. (2007). Dietary restriction in Drosophila: delayed aging or experimental artefact? *PLoS Genet.2007;3:e57*.

Piper, M. (2017). Using artificial diets to understand the nutritional physiology of *Drosophila melanogaster*. *Current Opinion in Insect Science. 23. 10.1016/j.cois.2017.07.014.*

Priyadarsini, S., Mukherjee, S., and Mishra, M. (2020). Formulation of Drosophila food for various feeding studies In: Fundamental approaches to screen abnormalities in Drosophila, Springer Protocols Handbooks. New York, USA. ISSN 1949-2456. https://doi.org/10.1007/978-1-4939-9756-5_1

Sanchez, C., Michael, N., Véronica, M. T., Thierry D. and Pascale, W. (2018). Acacia gum: History of the future. *Food Hydrocolloids*, Elsevier, 2018, 78, pp.140-160. ⟨10.1016/j.foodhyd.2017.04.008⟩. ⟨hal-01602791⟩

Spencer, W. P. (1947). Genetic Drift in a Population of Drosophila immigrant. *Evolution*, *1*(1/2), 103–110. https://doi.org/10.2307/2405408

Townsend, D. and Tew, K. (2003). The role of glutathione-S-transferase in anti-cancer drug resistance. *Oncogene 22, 7369 – 7375.* https://doi.org/10.1038/sj.onc.1206940

UNCTAD (2018). Round Table: The Economics of gum Arabic in Africa accessed at URL: https://unctad.org/meeting/round-table-economics-gum-arabic-africa

Williams, P. A. (2016). Gums: Properties and Uses in: Encyclopaedia of Food and Health. Editor(s): Benjamin Caballero, Paul M. Finglas, Fidel Toldrá. Academic Press. Pages 283-289. ISBN 9780123849533. https://doi.org/10.1016/B978-0-12-384947-2.00364-0.

Zhang, C., Yingxiao, L., Linfeng H., Fengjun S., Weitang, Y. and Xuegang, L. (2022). Effects of Uranium on the Antioxidant Responses of Chinese Oak Silkworm, *Antheraea pernyi. Pakistan J. Zool., 54: 339-346. DOI: https://dx.doi.org/10.17582/journal.pjz/20190715000752*