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Chemistry and Medicinal Potentials of the Seed Essential Oil of *Eucalyptus Toreliana* F. Muell Grown in Nigeria

By Ololade Z.S. & Olawore N.O.
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Abstract - E. toreliana is a distinct aromatic plant with several medicinal applications to cure many ailments. Chemistry and medicinal potentials of the seed essential oil of Eucalyptus toreliana F. Muell grown in Nigeria were examined in this study. The phytochemical composition of the seed essential oil was evaluated using multidimensional GCxGC-MS, MS and FT-IR. The seed oil was also investigated for its total phenolic content, antioxidant and acute toxicity, anti-inflammatory and antinociceptive potentials. Analyses of the seed essential oil extract resulted in the identification of 70 compounds representing 98.53 % of the oil. α-Pinene (16.0%), Copaene (10.0%), 1R-α-Pinene (8.0%), DL-Pinene (8.0%), β-trans-Ocimene (5.0%), α-Bisabolol oxide B (5.0%), Oleamide (5.0%) and Globulol (4.0%) were detected as the major components accounted for 65% of the oil. The total phenolics content of the seed oil of E. toreliana was estimated as 191.68±0.0006 μgmg^{-1} GAE. The results of DPPH and FRAP antioxidant showed that the oil possessed strong free radical scavenging and reducing potentials with IC_{50} 9.0 μgml^{-1} each in both methods. No sign of toxicity was noticed in the rats which indicate that the seed oil was relatively non-toxic and safe.

Keywords : *eucalyptus toreliana, seed essential oil, phytochemicals, phytotherapeutic.*

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Ololade Z.S. [α] & Olawore N.O. [σ]

Abstract - *E. toreliana is* a distinct aromatic plant with several medicinal applications to cure many ailments. Chemistry and medicinal potentials of the seed essential oil of *Eucalyptus toreliana* F. Muell grown in Nigeria were examined in this study. The phytochemical composition of the seed essential oil was evaluated using multidimensional GCxGC-MS, MS and FT-IR. The seed oil was also investigated for its total phenolic content, antioxidant and acute toxicity, anti-inflammatory and antinociceptive potentials. Analyses of the seed essential oil extract resulted in the identification of 70 compounds representing 98.53 % of the oil. *α*-Pinene (16.0%), Copaene (10.0%), 1R-*α*-Pinene (8.0%), DL-Pinene (8.0%), *β-trans*-Ocimene (5.0%), *α*-Bisabolol oxide B (5.0%), Oleamide (5.0%) and Globulol (4.0%) were detected as the major components accounted for 65% of the oil. The total phenolics content of the seed oil of *E. toreliana* was estimated as 191.68±0.0006 μgmg^{-1} GAE. The results of DPPH and FRAP antioxidant showed that the oil possessed strong free radical scavenging and reducing potentials with IC_{50} 9.0 μgml^{-1} each in both methods. No sign of toxicity was noticed in the rats which indicate that the seed oil was relatively non-toxic and safe. The oil at 1000 μgkg^{-1} (*p.o.*) gave 99.61% significant inhibition of paw edema. In the antinociceptive assay the oil inhibited the licking time by 88.69% and 75.90% in first phase (neurogenic pain) and second phase (inflammatory pain) respectively. These results showed that the seed essential oil of *E. toreliana* possesses antioxidant, anti-inflammatory and antinociceptive potentials, which provided an initial scientific validation of the seed essential oil as a phytotherapeutic agent against reactive oxidative, nociceptive and inflammatory processes.

Keywords : eucalyptus toreliana, seed essential oil, phytochemicals, phytotherapeutic.

I. Introduction

Eucalyptus toreliana (*Myrtaceae*) is a tall evergreen and a dense shade plant with an irregular crown, a very hard tree with smooth, tight and grey-green bark with persistent scaly, sub-fibrous base and tessellated. The leaves has a simple, leathery, variable but usually ovate, wavy margin, green above or with a pink tint, generally pubescent when young and with a wider leaf than other *Eucalyptus.* It processes attractive flowers with large creamy white clusters and numerous stamens, the creamy fruit is large and ovoid shape and with valves well below rim of the fruit.

Globally, there is a rapid increase in screening of plants that can lead to the discovery and development of novel therapeutics. Plants from different continents have shown considerable pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, antiviral, anti-allergic and vasodilatory properties (Rustaiyan *et al.,* 2011; Newman and Cragg, 2007).

The leaf essential oil of *E. toreliana* has been used in the treatment of lung diseases and was shown to have anti-tubercular properties (Alain *et al.*, 2012). The extracts of the leaf and stem of the plant were reported to have antibacterial and gastroprotective properties, it inhibits the growth of *Helicobacter pylori* (Adeniyi *et al.,* 2006). The leaves extracts of the plant is applied over wounds and ulcers, also used to treat gastrointestinal disorders, they decrease gastric acid production and used for the treatment of gastric ulcers, cough associated with most pulmonary diseases and medically importance for the treatment of infections caused by the non-tuberculous mycobacteria (Lawal *et al.*, 2011). The plant is also used locally in the treatment bacterial infections of the urinary tracts, respiratory tracts, inflammation of the mucous membranes and sore throat (Farah *et al.,* 2002).

Moreover, *Eucalyptus* essential oils has long history of safe use in food preservation, pharmaceuticals, phytotherapies, pesticides and have attracted extra attentions for more intensive studies (Tepe *et al.*, 2004). It was also reported that *Eucalyptus* leaf essential oil had a direct effect on the coxsakievirus B3 and ethno-pharmacologically been used to treat respiratory tract disorders such as pharyngitis, bronchitis, and sinusitis (Elaissi *et al.,* 2012).

To best our knowledge, no literature on the chemistry, phenolic content, antioxidant, anti-inflammatory and antinociceptive potentials of the seed essential oil of *E. toreliana* have been reported so far. The present research was therefore undertaken for the first time with the main objective to isolate and characterize the seed essential oils of *E. toreliana* cultivated in Nigeria for their detailed chemical constituents, pharmacological properties.

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II. Materials and Methods

a) Plant Material

Seed of *E. toreliana* were collected from Ogbomoso, Nigeria. The plant was authenticated at the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria.

b) Extraction of the Essential Oil

Seed of *E. toreliana* was air-dried in a well ventilation place till when the moisture content reduced to a minimum suitable for grinding; the plant material was pulverized and used immediately. The crushed plant material (100 g) was subjected to hydrodistillation for 2.5 hours using a Clevenger-type apparatus (European Pharmacopoeia, 2004). The oil collected was stored in vial at low temperature.

c) Instrumentation and Analytical Conditions

i. *Multi-Dimensional GCxGC-MS Analysis*

Analysis of the seed essential oils of *E. toreliana* was performed using multi-dimensional gas chromatograph coupled with Gas Chromatography-Mass Spectrophotometer (Shimadzu, Japan) equipped with double capillary columns (25.0 m x 0.25 μm i.d., 0.25 μm df) that have different characteristics (non-polar and polar). High purity helium was used as the carrier gas at a constant flow rate of 0.99 ml/min. A total of 1 μl sample was injected (split ratio 100:1) into GCxGCMS using AOC20i auto injector for analysis. The initial temperature was set at 60 °C, heated at a rate of 3℃/minutes to 280℃ and held isothermally for 6 minutes. Ion source temperature for these analyses was set at 200℃, while the interface temperature was set at 250℃, solvent cut time was 3.0 minutes and the mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70 eV as acquisition mass range from 40-700 a.m.u. at 0.50 scan/s.

ii. *Mass Spectra Data Analysis*

MS parameters were as follows: EI mode, with ionization voltage 70 eV, ion source temperature, 180 °C. The mass spectra were generally recorded over 40-700 amu that revealed the total ion current (TIC) chromatograms. The MS fragmentation pattern was compared with those of pure compound, by matching the MS fragmentation patterns with NIST mass spectra libraries and with those given in literature.

iii. *Identification through Comparison with Reference Standards*

Identification of phytochemical organic constituents in the seed essential oil was confirmed using published electron impact-mass spectra (EI-MS) in the NIST and Shimadzu's Flavours and Fragrance of Natural and Synthetic Compounds (FFNSC) and published spectral data. The retention indices were determined based on a homologous series of *n*-alkanes of *n*-alkanes internal standard analyzed under the same operating conditions and calibrated based on the Automatic Adjustment of Compound Retention Time (AART) function of the GC-MS. Relative concentrations of the essential oil components were calculated based on GC peak area.

iv. *Fourier-Transform Infra-Red (FT-IR) Analysis*

The IR spectra in KBr pellets were recorded using a spectrophotometer. 0.25 μl of the seed essential oil was deposited in the middle of a KBr pellet and the IR spectrum was recorded at different times. The FT-IR conditions were: 4 cm^{-1} spectral resolution, 20 kHz scan speed, 128 scan co-additions and scanning range 400-4000 cm^{-1}.

d) Total Phenolic Content (TP) and Antioxidant Capacity

Total phenolic content of the seed oil of *E. toreliana* was analysed by the Folin-Ciocalteu method (Wua and Ng, 2008). A solution of the seed oil (0.2 ml) containing 1000 μgml^{-1} of the oil in methanol was pipetted into a 50 ml volumetric flask, 46 ml distilled water and 1ml Folin-Ciocalteu's phenol reagent were added, and the opaque flask was thoroughly shaken. After 3 minutes, 3 ml of (2% w/v) Na_2CO_3 solution was added and the mixture was allowed to stand for 2 hours for incubation in dark with intermittent shaking. Absorbance values of the clear supernatants were measured at 760 nm against a blank (0.5 ml Folin-Ciocalteu's reagent + 1 ml Na_2CO_3) on UV-Visible spectrophotometer. The same procedure was repeated for all the standard gallic acid solutions (0-1000 μg/0.1 ml) and a standard curve obtained with the following equation:

$$\text{Absorbance} = 0.0008 \times \text{gallic acid } (\mu g) + 0.0068$$

Calculation of percentage total phenols content was based on Gallic Acid Equivalents (GAE).

e) Pharmacological Assays

i. *In vitro DPPH Free Radical Scavenging Assay*

The antioxidant activity of the seed oil extract was measured using the stable radical 2,2´-diphenyl-1-picrylhydrazyl (DPPH). 1.0 ml of the seed *E. toreliana* essential oils (10, 100 and 1000 μgml^{-1}) in methanol was added to 1.0 ml of a 0.004% w/v methanol solution of DPPH. The mixture was shaken vigorously and the absorbance was monitored at 517 nm after 30 minutes of incubation, when the reaction reached a steady state. Ascorbic acid was used as reference compound. Assays were carried out in triplicate. The inhibition percentage (%) of radical scavenging activity was calculated by using following formula (Ololade *et al.*, 2012).

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance values of the test compounds.

ii. *In vitro Ferrous Reducing Power Assay (FRAP)*

The reducing power of the seed oil was determined by the method of (Saeed *et al*., 2012). An aliquot of the sample (1.0 ml) at various concentrations (10, 100 and 1000 μgml^{-1}) were mixed with phosphate buffer (0.2 M, pH 6.6, 2.5 ml) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50℃ for 20 minutes. After adding 10% Trichloroacetic acid (2.5 ml), the mixture was centrifuged at 1000 rpm for 10 minutes. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.1% $FeCl_3$ (0.5 ml) and the absorbance was measured at 700 nm using an appropriate blank. Assays were carried out in triplicate. Ascorbic acid was used as a reference. The average values were plotted to obtain the half maximum effective concentration (EC_{50}) of Fe^{3+} reduction.

iii. *In vivo Anti-inflammatory Assay*

Healthy rats (200 ±30 g) acclimatized to laboratory hygienic conditions were housed in polycarbonated clean cages under standard conditions of temperature (25 ± 2^0C) and RH was % 55-60, 12 hours light/dark cycle were maintained in the quarantine and were fed with standard pellet diet and water *ad libitum*. The handling and uses of animals were in accordance to the institutional guidelines. The *in vivo* toxicity of seed essential oil *E. toreliana* was also observed during and after the experiment (Santin *et al*., 2011).

In vivo antiinflammatory assay of the seed oil of *E. toreliana* was studied in rat paw edema. The rats of were divided into three groups of five animals each and the rats were fasted for 12 hours in order to avoid food interference with substance absorption, ensure uniform hydration and minimize variability in edematous response. 1% carrageenan (0.1 ml) was injected into the plantar surface of the rat hind paw 30 minutes after oral administration of the test compounds or vehicle. Indomethacin (25 $mgkg^{-1}$) was used as reference drug. Paw volume was determined immediately after the injection of the phlogistic agent and again 2 and 4 hours later by means of a digital vernier calliper. The anti-inflammatory activity of the seed oil was expressed as the percentage of inhibition calculated from the difference between the responses of the treated and the control groups. The inhibition percentage of the inflammatory reaction which was calculated by the formula given in equation below was determined for each rat by the comparison of each group with controls (Sousa *et al.,* 2010).

$$I\ \% = 1-(dt/dc) \times 100$$

Where:

I % = Percentage inhibition

'd*t*' is the difference in paw volume in the drug-treated group and 'd*c*' is the difference in paw volume in control group.

iv. *In vivo Antinociceptive Assay*

In vivo antinociceptive activity of the seed oil of *E. toreliana* was studied in rats according to (Ouedraogo *et al*., 2011). The rats of were divided into three groups of five animals each and the rats were fasted for 12 hours in order to avoid food interference with substance absorption, ensure uniform hydration and minimize variability in response. The rats were treated respectively with 1000 μgkg^{-1} of *E. toreliana* seed essential oil or indomethacin. Thirty minutes later, the pain was induced by injecting 0.05ml of 2.5%v/v formalin (formaldehyde) in distilled water into the sub-plantar right hind paw of rat, immediately placed in a transparent plastic cage separately; the amount of time spent in licking the injected paw was monitored and was considered as an indicative of pain and frequency of the injected paw were recorded for 30 minutes. The number of lickings from 0-5 minutes (first phase) and 15-30 minutes (second phase) were counted after injection of formalin.

The percentage inhibition (I) was calculated accordingly.

III. Results and Discussion

a) Identification and Quantification of the Essential Oil

Exhaustive hydrodistillation of the seed of *E. toreliana* of afforded light-cream coloured oil with 1.30 % v/w yield per 100g of dried seed sample and possessed a distinct sharp aromatic scent. The analyses of the seed essential oil were carried out using GCxGC-MS, MS and FT-IR systems. The percentage composition and retention index are given in Table 1. Seventy compounds were identified from the seed essential oil of *E. toreliana* amounting to 98.53 % of the oil. The seed oil was dominated by *α*-Pinene (16.0%), Copaene (10.0%), 1R-*α*-Pinene (8.0%), DL-Pinene (8.0%), *β-trans*-Ocimene (5.0%), *α*-Bisabolol oxide B (5.0%), Oleamide (5.0%), 2-fluoro-*β*-3,4-trihydroxy-N-isopropyl-Benzeneethanamine (4.0%) and Globulol (4.0%). None of these principal compounds has ever been detected in the leaves extracts of *E. toreliana* that had been investigated before except *α*-Pinene. Monoterpenes (43.40%) dominated the seed essential oil, because of the remarkable proportions of Pinene derivatives. The percentage composition of monoterpenoid was very low (3.50%), while the level of sesquiterpene was relatively high (20.70%), sesquiterpenoids constituted (10.50%), but few diterpenoids (2.30%) were also available in the seed oil of *E. toreliana*. We also identified some new phytocompounds which are not reported in the previous study on the leaf essential oil of this plant. Most of the principal components present in the seed oil were not available in the leaf oils and many components identified in the current study have not been previously identified in the leaf oil of the same species. Apart from the main compounds, other newly identified

phytochemicals in the seed oil of *E. toreliana* are: compound 1, 3, 4, 12, 13, 14, 15, 16, 21, 25, 26, 27, 29, 30, 31, 40, 49, 59, 60, 61, 62, 63, 64 and 65.

The result shows that the percentage of eucalyptol is lower in the seed oil *E. toreliana* compared to the leaf oil, but more components were present in the seed oil investigated than in the leaf oil results presented in the literatures. 45 and 41 components were detected in the leaf oils by Ogunwande *et al.*, 2011; Coffi *et al.*, 2012 respectively.

As shown in Table 1, the main constituents of *E. toreliana* seed oil were different from that of the same species investigated in the leaves from Nigeria (30%, 4.2%) (Ogunwande *et al.*, 2011), Ethiopia (44%, 7%) (Dagne *et al.*, 2000), Congo-Brazzaville (78%, 1%) (Loumouamou *et al.*, 2009), Brazil (40%, 55%; 24%, 7%), Morocco (14%, 64%) (Coffi *et al.*, 2012), Republic of Benin (38, 18 and 14%) (Alain *et al.,* 2012; Sohounhloue *et al.*, 1996), Mali (Chalchat *et al.*, 2000).

From the mass spectrometry analysis, Compound **5, 7** and **8** are pinene derivatives, they are bicyclic monoterpenes with molecular ion peak 136, the relatively low abundance of the molecular ion peak is consistent with the view that the molecular structure of the compound is crowded; the base peak m/z 93 corresponds to the loss of 43 mass units and relatively abundance of the ion m/z 41 is about one quarter of the base peak. A point of distinction between the isomers arises from the abundance of the ion m/z 29 and 39 in 1S-α-pinene which is not feature in α-pinene and 1R-α-pinene. The failure to detect the isopropyl ion strengthens that the loss of 43 mass units is not an entity. Therefore, the groups elided may be obtained by the breaking of two tertiary bonds with the removal of or concomitant hydrogen migration. The occurrence of gem dimethyl group as a part of ring system is common feature of many monoterpenes. Compound **10** a monocyclic monoterpene, with molecular formula of $C_{10}H_{16}$ (m/z 136) is β-*trans*-Ocimene, m/z 43 ($C_3H_7^+$) which was due to the detachment of isopropyl [$(H_3C)_2$ C-] group attached to the quaternary carbon of the compound and weak bond is broken to give a fragment at m/z 93 as the base peak. Compound **24** was obtained as Copaene a bicyclic sesquiterpene with molecular formula of $C_{15}H_{24}$ (m/z 204) and base peak at m/z 161 due to loss of m/z 43 ($C_3H_7^+$) which occurred as a result of propyl fragment from compound, other prominent peaks observed in Copaene occurred at m/z 93, 104 and 120. Compound 34 is Globulol, a tricyclic oxygenated sesquiterpenoid of alkanol family, the prominent peaks observed in this compound are m/z 43 ($C_3H_7^+$) which occurred due to detachment of propyl fragment from the compound and m/z 41 ($C_3H_5^+$) while molecular ion peak is m/z 222. Compound **54** also a bicyclic oxygenated sesquiterpenoid with molecular formula $C_{15}H_{26}O_2$ is α-Bisabolol oxide B, m/z 238 as molecular ion peak, the molecular ion peak of the mass spectrum indicates that there are no heteroatoms in the molecule other than oxygen, the even m/z indicates absence of nitrogen atom and that the compound has a molecular weight of 238amu. Base peak was observed at m/z 43 ($C_3H_7^+$) in the compound, which is due to the formation of $(H_3C)_2C^+H$ or $CH_3C{=}O$, this is due to α-cleavage at C_3 of the compound. Other prominent peaks are m/z 41due to ($C_3H_5^+$) or $((H_3C)_2{=}C^+$, m/z 59 which is due to the formation of $(H_3C)_2{}^+COH$, fragmentation of weak bond at tertiary carbon atom and cleavage of C-C bond next to hetero atom with the elimination of largest group of the molecule confirms that the compound is tertiary alkanol, m/z 70 calculated for C_5H_{11} (m/z 71) due to formation of pentenyl fragment from compound. m/z 161 which is due to the loss of propyl [$(H_3C)_2$C-] group attached to the quaternary carbon of the compound and weak bond is broken to give a fragment at m/z 43.

The FT-IR spectra of the seed oil of *E. toreliana* revealed some prominent peaks especially in the regions around 3700-2933 cm^{-1}, 2300-1447 $cm^{-1}$and 1440-1090 cm^{-1} the oil showed a peak. The band at 3500-3400 cm^{-1} was due OH stretching vibration, 3600 (sharp) was due to unassociated OH, while 3400 cm^{-1} (broad) was due to associated (hydrogen bonded) OH; both bands frequency present alkanol spectra; bands at 3400-3200 are due to N-H stretching vibrations, 3400 (sharp) was due to free N-H, while 3200 cm^{-1} (broad) was due to associated N-H; Peaks at 1750 and 1447 cm^{-1} were attributed to >C=O stretch and -C=C- stretch and can be used as an indicative for the presence of unsaturated bonds in the oil. These functional groups detected by FT-IR are futures of the compounds found in the seed essential oil.

Table 1 : Chemical Compositions of the Seed Essential Oil of *Eucalyptus toreliana*

Compounds	Percentage Composition	Retention Index
3-Methylene-1,7-octadiene	0.3	863
D-Sabinene	0.4	897
9-Oxabicyclo[6.1.0]non-6-en-2-one	2.0	908
1R-α-pinene	8.0	937
1S-α-pinene	0.4	941

1R-α-pinene	8.0	937
1S-α-pinene	0.4	941
DL-pinene	8.0	943
α-pinene	16.0	948
β-pinene	2.0	970
trans-β-Ocimene	8.0	976
L-β-pinene	0.5	978
Ethyl-2,2,3,3-tetramethylcyclopropanecarboxylate	0.3	1002
2-fluoro-β-3,4-trihydroxy-N-isopropylbenzeneethanamine	4.0	1100
5-Methylsulfanyl-2H-[1,2,4]-triazol-3-ylthiophen-2-ylmethyla- mine	0.3	1102
1,5-Dimethyl-1,5-cyclooctadiene	0.3	1103
3,7-dimethyl-(*Z*)-1,3,6-Octatriene	0.1	1029
Limonene epoxide	0.3	1031
D-*trans*-Limonene oxide	0.4	1039
cis-Sabinenhydrate	0.6	1041
Eucalyptol	1.0	1059
DL-Lavandulol, trifluoroacetate	0.2	1124
4-Caranol	0.5	1125
p-Menth-8-en-2-ol	0.5	1196
Cycloisolongifolene	0.5	1197
Copaene	10.0	1221
2*E*-1-Methoxy-3,7-dimethylocta-2,6-diene	0.3	1222
2-Methylene-4,8,8-trimethyl-4-vinylbicyclo[5.2.0]nonane	0.5	1301
4,8-dimethyl-3,7-Nonadien-2-ol	1.0	1329
α-Cubebene	1.0	1344
4,4-Dimethyl-1-[2*E*]-2,7-octadienyl]-1-cyclobutene	0.2	1380
2-Bromotetradecane	0.2	1401
5*E*,9*E*-12-Methyl-1,5,9,11-tridecatetraene	0.2	1404
β-*trans*-Caryophyllene	0.2	1418
Caryophyllene	1.0	1428
5-Phenylnonane	0.4	1437
allo-aromadendrene	1.0	1445
α-Selinene	0.2	1474
γ-Gurjunene	0.5	1476
β-*cis*-Caryophyllene	0.2	1494
α-Farnesene	0.4	1496
1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-3-methylbut-2-enylcyclohexane	0.3	1501
α-Bulnesene	1.0	1508
[s-*E*,*E*]-Germacrene D	0.5	1515
(+)-δ-Cadinene	2.0	1517
Ledane	0.4	1530
α-Muurolene	0.5	1546
L-Globulol	0.5	1578
(+)-Viridiflorol	0.4	1587
Globulol	4.0	1591
2-Ethyl-2-methyl-tridecanol	0.3	1601
D-Dihydrocarveol	0.6	1611
5-phenyldecane	0.3	1633
trans-Longipinocarveol	0.3	1634
3-Phenylundecane	0.3	1646
α-Bisabolol oxide B	5.0	1655
n-Heptadecane	0.2	1700
6-Phenyldodecane	0.1	1718
7-Phenyltridecane	0.03	1818
1-pentyloctylbenzene	0.2	1829
2*E*,6*E*-Farnesyl acetate	0.4	1843
4,6-Diisopropylidene-8,8-dimethylbicyclo[5.1.0]octan-2-one	0.4	1883

Z-5,17-Octadecadien-1-ol acetate	1.0	2082
Sulfurous acid, cyclohexylmethyltetradecylester	0.3	2100
Farnesyl-β-D-Mannofuranoside	0.2	2102
Geranylgeraniol	0.3	2201
Oleamide	5.0	2397
n-Heptacosane	0.2	2700
n-Octacosane	0.2	2800
n-Hentriacontane	0.2	3100
Percentage Total	98.53	

b) Phenolic Content and Antioxidant Property

The seed essential oil of *E. toreliana* is highly rich in phenoloids. Based on the absorbance value of the seed oils solution reacting with Folin-Ciocalteu phenol reagent and compared with the absorbance values of standard solutions of Gallic acid, total phenolics content of the seed oil of *E. toreliana* was estimated as 191.68±0.0006 μgmg^{-1} of GAE. This might be due to the presence of low molecular mass phenolic compound like 2-fluoro-β-3,4-trihydroxy-N-isopropyl-Benzeneethanamine (4.0%) as revealed by GCxGC-MS and others which might be available among other unidentified compounds in the essential oil. The Folin-Ciocalteu assay relies on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic or phosphotungstic acid complexes to form blue complexes.

Folin-Ciocalteu measured both total phenolics and antioxidation strength base on the nature of its chemistry, to prevent inhibitory effects due to the oxidants competing with Folin-Ciocalteu reagent and/or air oxidation after the sample is made alkaline, the Folin-Ciocalteu reagent is added before the alkali (Singleton *et al.*, 1999).

c) Pharmacological Activities

i. *In vitro Antioxidant Activity by DPPH Radical Scavenging Method*

The free radicals scavenging activity of the seed oil of *E. toreliana* was estimated by DPPH and FRAP´S assays. DPPH involves single electron transfer and hydrogen atom transfer reactions (Prior *et al.*, 2005). The oil was able to inhibit the formation of DPPH radicals in a concentration dependent manner. The percentage inhibitions of the essential oil at various concentrations (10, 100 and 1000 μgml^{-1}) are 50.07±0.0006, 62.48±0.0006 and 81.14±0.0006 % respectively; while the IC_{50} values was found to be 9.0 μg/ml in comparison to ascorbic acid which gave 54.37±0.00, 84.51±0.001 and 95.50±0.00 as the percentage inhibitions with IC_{50} value of 9.0 μgml^{-1}. The DPPH radical scavenging capacity of the seed oil of *E. toreliana* is at same concentration as observed for ascorbic acid as shown in Table 2.

The result of reducing power (FRAP) of the seed oil of *E. toreliana* in comparison with ascorbic acid as a reference antioxidant is also shown in Table 2. FRAP involves single electron transfer (Prior *et al.*, 2005). The reducing power of ascorbic acid used as standard in this study was EC_{50}: 20.00. The seed oil exhibited the high of reducing power value at concentrations of 10, 100 and 1000 μg/ml with effective dose value at (EC_{50}: 9.00 μgml^{-1}). Reducing power of *E. toreliana* oil increases from 0.641±0.008 at 10 μgml^{-1} to 1.016±0.02 at 100 μgml^{-1} and finally appreciated to 1.566±0.004 at 1000 μgml^{-1} in a concentration dependent manner. At tested concentrations the oil possessed the ability to reduce Fe^{3+}. It was observed that the seed oil of *E. toreliana* showed higher Fe^{3+} reducing power comparable to Ascorbic acid activity. The reducing power of the seed oil increased with concentrations in a strongly linear manner. The reducing power assay measures the electrondonating ability of antioxidants using the potassium ferricyanide reduction method. Antioxidants cause the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form and activity is measured as the increase in the absorbance at 700 nm.

Overall, IC_{50} values of the seed essential oils of the *E. toreliana* examined was more effective than oils of some other related species: *E. toreliana* (leaf from Republic of Benin) IC_{50}: 2.90 gl^{-1} (Alain *et al.*, 2012), *E. oleosa* with IC_{50}: >1000 (Marzoug *et al.*, 2011), *E. globulus*(leaf) IC_{50} : 57.00 μgml^{-1} (Noumi *et al.*, 2011). These findings in DPPH and FRAP assays are in agreement with Vardar-Unlu *et al.*, 2003, who reported that the entire essential oil showed greater antioxidant activity than individual components, indicating the possible synergistic interaction of the essential oil constituents. These results showed that the seed oil of *E. toreliana* potentially exert its radical scavenging effects at a much lower concentration. This observed effect is certainly associated with high phenolic content and sesquiterpenoids components in the oil. The results clearly showed that the seed oil of *E. toreliana* possesses strong antioxidant activity and can be considered as good sources of natural antioxidants for medicinal purposes such as reactive oxygen species ailments including chronic inflammatory joint disease such as rheumatoid arthritis. Studies have showed that the electron donating capacity, reflecting the reducing power of bioactive compounds, is associated with antioxidant activity.

Table 2 : IC_{50} of the DPPH and FRAP Antioxidant Activity of the Seed Essential Oil of *E. toreliana*

Oil and Reference Compound	DPPH IC_{50} μgml^{-1}	FRAP EC_{50} μgml^{-1}
E. toreliana	9.00	9.00
Ascorbic acid	9.00	20.00

Data are presented as triplicate of the mean ± S.E.M

ii. *In vivo Anti-inflammatory Activity*

The anti-inflammatory effects of the seed oil of *E. toreliana* on carrageenan induced oedema in rats hind paws is presented in Table 3. The anti-inflammatory activity of oil was found to have effect in time manner. There was a significant decrease in oedema paw volume of rats in the test group. However, there was no reduction in inflammation found in case of control group. The results showed that the seed oil of *E. toreliana* causes significant reduction in inflammation i.e. 99.61% (1000 μgkg^{-1} *p.o*), while the standard anti-inflammatory drug indomethacin gave 93.75% (25 $mgkg^{-1}$).

The seed oil of *E. toreliana* proves its anti-inflammatory potential in *in vivo* study by controlling biphasic inflammatory events induced by carrageenan. The carrageenan induced oedema shows to be a multi-mediated phenomenon that liberates diversity of mediators which could be in two phases. Degree of inflammatory immune responses is controlled by involvement of inflammatory cells into inflammatory lesions (Solanki and Jain, 2010). The early phase (one hour) of the inflammation is due to the release of serotonin, histamine and related substances. The later phase (over one hour) is mediated by prostaglandins, proteases and lysosome (Ayoola *et al.*, 2009). The seed oil extract promptly controlled both the phases of inflammation.

Table 3 : In vivo Anti-inflammatory Activity the Seed Essential Oil of *E. toreliana*

Oil and Reference Compound	2 Hour	% I (2 Hr)	4 Hour	% I (4 Hr)	Mean Paw Diameter (mm)	Mean % I
E. toreliana	4.5±0.50	99.50	4.5±0.12	99.88	4.50±0.39	99.61
Indomethacin (Standard)	4.7±0.21	87.50	4.6±0.35	99.65	4.65±0.29	93.75
10% DMSO (Control)	5.5±0.07	-	5.5±0.00	-	5.50±0.31	-

Data are presented as triplicate of the mean with standard deviation

iii. *In Vivo Antinociceptive Activity*

The antinociceptive activity of the seed oil of *E. toreliana* measured on abino rat by using injection of formalin solution is shown in Table 4. The extracts exhibited significant dose related reduction of hind paw licking caused by formalin. Interestingly, the seed oil at the concentration of 1000 μgkg^{-1} exhibited high inhibitory effect 88.69 and 75.90% in early and late phases respectively, while the standard anti-inflammatory drug indomethacin gave 64.23 and 54.70% in first and second phase respectively. The results showed that the seed oil is more active than the synthetic drug (indomethacin) commonly used in pain and inflammatory problems. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase. The early phase is probably a direct result of stimulation of nociceptors in the paw which reflects centrally mediated pain while the late phase is due to inflammation with a release of serotonin, histamine, bradykinin and prostaglandins. These phases represented neurogenic and inflammatory pain responses, respectively (Chen *et al.*, 1995).

Table 4 : In Vivo Analgesic Activity the Seeds Essential Oil of E. toreliana

Oil and Reference Compounds	Time of Licking and Biting Percentage Inhibition			
	Early Phase (0-5) min	Percentage Inhibition	Late Phase (5-30) min	Percentage Inhibition
E. toreliana	11.20±0.00	88.69	28.20±3.61	75.90
Indomethacin (Standard)	34.33±2.121	64.23	53.00±2.12	54.70
10% DMSO (Control)	96.00	-	117	-

Data are presented as triplicate of the mean ± S.E.M

During and after the *in vivo* experiment, no apparent behavioural side effects were observed in the animals; they were very active. This shows that the seed oil was relatively non-toxic and safe. This is in agreement with the report on the *Eucalyptus* leaf essential oil (Silva *et al.*, 2003).

Generally, phytocompounds found in the seed essential oil are very useful for various pharmacological

purposes such immunoinhibition; pinene derivatives are used as antioxidant, anticancer, antiinflammatory, antinociceptive, antibacterial, antifungal, antinoxious, insecticides, fungicides, inhibitors in breast cancer (Mercier *et al.*, 2009). Oleamide commonly use as analgesic, mood and sleeping agent, while 2-fluoro-*β*-3,4-trihydroxy-N-isopropylbenzeneethanamine which is a fluoro-substituted Ethylnorepinephirine (Trihydroxyl-subtituted 2-fluoroamphitamine) drugs used in the treatment of asthma, chronic bronchitis, emphysema and relaxing the smooth muscle in the lungs and dilates airways to improve breathing. Therefore the seed oil extract could have sympathomimetic, bronchodilating, analgesic and anorexiant potentials to handle problem such as arthritis, tumour, goitre and cancer. Bisabolol was also reported to have spasmolytic effects on intestinal smooth muscle, anti-inflammatory, antipyretic, ulcer protective, anti-inflammatory, anti-allergic, anti-pruritic, healing, decongestive and antispasmodic properties (Presibella *et al.*, 2010; Alves *et al.*, 2010). Eucalyptol which is one of the principal components of the seed oil and by far the most known naturally occurring oxide as it is the most common in essential oils as an oxygenated monoterpenoid has can readily penetrate tissue, one of the reasons for its efficacy in various decongestants and pain relief products and has anticatarrhale, mucolytic, antimicrobial, antiviral and as a stimulating expectorant in cases of chronic bronchitis (Ben-Hadj *et al.*, 2011; Caballero-Gallardo *et al.*, 2011).

IV. Conclusion

This research represents the first comprehensive study of the seed essential oil *E. toreliana*. The analysis of the seed essential oil from the plant indicates terpenes, terpenoids and phenoloids are the major constituents of this medicinal plant. Pharmacological activities of the oil may be due to the synergetic effects of these chemical constituents. Therefore, the seed essential oil of *E. toreliana* can be used as natural therapeutic product that may serve as leads for the development of new pharmaceuticals that can handle many health problems.

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The Comparison of the Amino Acids Profiles of Whole Eggs of Duck, Francolin and Turkey Consumed in Nigeria

By E.I. Adeyeye
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Abstract - The amino acids profiles were determined in the whole eggs of duck, francolin and turkey consumed in Nigeria on a dry weight basis. The protein content (g/100 g) had the trend: francolin (80.1) > turkey (77.6) > duck (67.9). The highest concentrated amino acid in the three eggs was glutamic acid with values of (g/100 g): duck (13.1) > francolin (13.0) > turkey (12.1); with total amino acid following similar trend as duck (82.4) > francolin (80.8) > turkey (79.9). The essential amino acids had a trend of (g/100 g): duck (39.5) > turkey (37.0) > francolin (36.0); others like this trend were basic amino acids, sulphur amino acids and essential amino acid index. Aromatic amino acid had a trend of (g/100 g): duck (10.2) > francolin (9.96) > turkey (9.26) and the predicted protein efficiency ratio also followed a similar trend. On scores based on whole hen's egg, serine was the limiting amino acid in all the samples having values of 0.48 (duck), 0.38 (francolin) and 0.37 (turkey). Scores based on pre-school children requirements, leucine (0.99) was limiting in duck, lysine (0.95) in francolin and leucine (0.90) in turkey. However, valine was limiting under the provisional amino acid scoring pattern: duck (0.80), francolin (0.77)

Keywords : *amino acids profiles, whole eggs, duck, francolin, turkey.*

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Keywords : amino acids profiles, whole eggs, duck, francolin, turkey.

I. Introduction

The egg is the astonishing and unintentional gift from birds to human beings, the acme offood packaging, and a prime resource of occidental and oriental cooks alike. It is also the ultimate measure of ignorance and incompetence in the kitchen; 'he/she can't even boil an egg', she/he will say, whether fondly or recentfully (Davidson, 1999).

A reference to 'an egg', with no qualification is assumed almost everywhere to mean a hen's egg, which is what, forms the first part of this introduction. The hen's egg is usually the one which carries symbolic significance (the renewal of life, e.g. in spring festivals and EASTER FOODS). Symbolic meanings and folklore and associated topics are admirably dealt with by Newall (1971).

The egg proteins are what make an egg so important a source of nutritional and such a versatile ingredient for the cook. Consider the composition of an egg as follows, white: 87.77 % (water), 10.00 % (protein), 0.05 % (fat), 0.82 % (ash); yolk: 49.00 % (water), 16.70 % (protein), 31.90 % (fat), 1.90 (ash). It will be apparent that the white, apart from its large water content, is almost pure protein; and that the yolk contains proportionately less water, more protein, and much more fat. White and yolk can therefore be expected to, and do, behave differently when cooked. Moreover, the proteins in the yolk are not the same as those in the white, and coagulate at distinctly higher temperature (Davidson, 1999).

(Many books refer to egg white as 'albumen,' which has the same meaning. However, this term can be confusing because there is also the word 'albumin' which refers to a class of proteins, all soluble in water, which includes albumen and others too.)(Davidson, 1999).

The protein in egg whites starts to coagulate in temperature range 55-60 °C (131-140 °F)and definitely coagulates at 65 °C (150 °F) or a little less. Those of egg yolks begin to thicken at 65 °C (150°F) and coagulate at just over 70 °C (158 °F). Thus the yolk always sets after the white, whether an egg is being boiled (when this would be bound to happen anyway because the heat reaches the yolk later than white) or being fried (Davidson, 1999).

Poultry eggs are eaten in most areas of the world with fewer social taboos associated with them than with pigs and cattle. In Asia ducks are sole source of livelihood of a considerable number of people who may own large flock for meat and egg production. Of the world duck population of 52.8 million, 90 % is found in Asia.

However, in Nigeria, more emphasis is laid on domestic fowl to the neglect of other classes of poultry. As a result domestic fowl dominated the poultry industry. Of the 150 million poultry population, 120 million (80 %) were indigenous. Domestic fowl constituted 91 % of this while guinea fowl, duck, turkey and others were 4 %, 3 % and 2 % respectively. The population of ducks in Nigeria had been put as 1.21 million as against 133.5 million local/exotic chicken. According to a report (Federal Government of Nigeria, 1988), 69 % of total meat, and 12 % of total eggs were supplied by domestic fowl in 1987.

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Despite abundant water, pasture land and the fact that 10 % of Nigerian households keep duck (Adenowo *et al*., 1999), consumption of its meat and especially eggs, was still low. A survey (Adenowo*et al*., 1999) showed that ducks were neither raised for egg production nor for consumption. Thus duck eggs were seldom eaten or sold. The reason obtained by the survey, basically on taboo, partially explains why duck eggs have not found favour with consumers.

The double-spurred francolin, *Francolin-usbicalcaratus*, is a game bird in the pheasant family Phasianidae of the order Galliformes, gallinaceous birds. Like most francolins, it is restricted to Africa. It is a resident breeder in tropical West Africa, but there is a small and declining isolated population in Morocco. This bird is found in open habitats with trees. It nests in a lined ground scrape laying 5-7 eggs. Double-spurred francolin takes a wide variety of plant and insect food.

The male is mainly brown, with black and whiteflank streaking. The face is pale, and the head features a chestnut crown and white supercilium. It has a chestnut neck colour, white cheek patches and brown wings. The male has two spurs on each leg. The female is similar, apart from the double spurs, but slightly smaller, and the young birds are drabber versions of the adult. This is a very unobtrusive species, best seen in spring when the male sings a mechanical *krak-krak-krak* from a mound. It has a pheasant's explosive flight, but prefers to creep away unseen. (Retrievedfromhttp://en.-wikipedia.org/wiki/Double-spurred_Francolin)

The domesticated turkey is a large poultry bird raised for food. The modern domesticated turkey descends from the wild turkey (*Meleagrisgallopavo*). The turkey is reared throughout the temperate parts of the world, and is a popular form of poultry, partially because industrialized farming has made it very cheap for the amount of meat it produces. The female domesticated turkey is referred to as a *hen* and the chick as *poult*. In the United States, the male is referred to as a *tom*, whilst in Europe, the male is a stag [htt://en.wikipedia.org/wiki/Turkey-(domesticated)]. In Nigeria, turkey meat is becoming a delicacy particularly at Christmas.

A comparative study on the characteristics of egg shells of some bird species had been carried out. The total egg weight for each of the birds was (g): francolin, 25.2 (23.5-27.1); duck, 74.9 (62.3-76.8) and turkey, 70.9 (62.3-79.5) and the edible portion was francolin, 19.9 (18.3-21.6); duck, 64.6 (54.0-67.3) and turkey, 62.7 (54.0-71.4) (Adeyeye, 2009).

There are no reports on the comparative study on the amino acids profiles of duck (*Cairinamoschata*, Linnaeus 1758), francolin (*Francolin bicalcaratus* Linnaeus 1766)and turkey (*Meleagrisgallopavo*, Linnaeus 1758) eggs. Due to the emphasis placed on the nutritive value of food by consumers a great need exists for information on nutritional composition of these eggs. The present study was therefore undertaken in an attempt to gain some information on the amino acids profiles and their comparison with other eggs like those from guinea fowl (*Numidameleagris*) and domestic fowl to evaluate their nutritional qualities.

II. Resources and Techniques

a) Materials

The francolin eggs were collected in the month of November in the bush (it is a taboo to rear the bird at home) while the eggs of local duck and white plumage turkey were directly obtained from poultry keepers. Five eggs were involved in each study and they were collected at once.

The edible portion was removed; oven dried, milled into flour and kept in a laboratory freezer pending analysis.

The amino acid profile in the known samples was determined using methods described by Spackman *et al* (1958). The known sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-sample Amino Acid Analyzer (TSM).

b) Defatting of Sample

A known weight of dried sample was weighed into extraction thimble and the fat was extracted with chloroform/methanol (2:1 v/v) using Soxhlet extraction apparatus as described by AOAC (2005). The extraction lasted for 15 hours.

c) Hydrolysis of the Sample

A known weight of the defatted sample was weighed into glass ample. 7 ml of 6 MHCl was added and oxygen was expelled by passing nitrogen into the ampoule. (This is to avoid possible oxidation of some amino acids during hydrolysis.) The glass ample was then sealed with Bunsen burner flame and put in an oven preset at 105 °C±5 °C for 22 h. The ampoule was allowed to cool before broken opened at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40 °C under vacuum in a rotary evaporator. The residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

d) Loading of the Hydrolysate into the TSM Analyser

The amount of sample loaded was between 5-10 micro litre. This was dispensed into the cartridge of the analyser. The TSM analyser is designed to separate and analyse free, acidic, neutral and basic amino acids of the hydrolysate. The period of analysis lasted for 76 minutes.

e) Method of Calculating Amino Acid Values from Chromatogram Peaks

The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the

chart was found and the width of the peak on the half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half-height.

The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

NE = Area of norleucine peak/Area of each amino acid (Norleucine was the internal standard.) A constant S was calculated for each amino acid in the standard mixture:

Sstd = NEstd x Mol. weight x μMAAstd

Finally the amount of each amino acid present in the sample was calculated in g/16N or g/100 g protein using the following formula:

Concentration (g/100 g protein) = NH x W@ NH/2 x Sstd x C

Where : C = Dilution x 16/Samplewt(g)xN % x 10 vol.loaded ÷ NH x W (nleu)

Where: NH = Net height

W = Width @ half height

Nleu = Norleucine

f) Estimation of Isoelectric Point (PI)

The theoretical estimation of isoelectric point (pI) was determined using the equation of Olaofe and Akintayo (2000) and information provided by Finar (1975).

$$IPM = \sum_{i=1} IPiXi$$

WhereIPm is the isoelectric point of the i^{th} amino acid in the mixture and Xi is the mass or mole fraction of i^{th} amino acid in the mixture.

g) Estimation of Dietary Protein Quality

The predicted protein efficiency ratio (P-PER) was estimated by using the equation given by Alsmeyer*et al.* (1974).

P-PER = -0.468 + 0.454 (Leu) - 0.105 (Tyr).

h) Estimation of Dietary Protein

The amino acid scores were calculated quality in three different procedures:

- The total amino acid scores were calculated based on the whole hen's egg amino acid profiles (Paul and Southgate, 1976).
- The essential amino acids scores were calculated using the formula (provisional amino acid scoring pattern) (FAO/WHO, 1973):

Amino acid score = Amount of amino acid per test protein [mg/g] / Amount of amino acid per protein in reference [mg/g].

- The essential amino acids scores (including His) based on pre-school childsuggested requirement (FAO/WHO/UNU, 1985).

i) Essential Amino Acid Index [EAAI]

The essential amino acid index (EAAI) was calculated by using the ratio of test protein to the reference protein for each eight essential amino acids plus histidine in the equation (Steinke *et al.*, 1980):

$$\text{Essential amino acid index} = \frac{\text{mgLysine in 1 g test protein etcfor all essential amino acid + His}}{\text{9mgLysine in 1 g reference protein}} \times$$

j) Leu/isoleucine Ratio

The leucine/isoleucine ratios, their differences and their percentage differences were also calculated.

k) Calculation of other Protein Quality Parameters

Determination of the ratio of total essential amino acids (TEAA) to the total amino acids (TAA), i.e. (TEAA/TAA), total sulphur amino acids (TSAA), percentage cystine in TSAA (% Cys/TSAA), total aromatic amino acids (TArAA), total neutral amino acids (TNAA), total acidic amino acids (TAAA) and total basic amino acids (TBAA) were estimated from the results obtained for amino acids profiles.

l) Statistical Analysis

Statistical analysis (Oloyo, 2001) was carried out to determine the mean, standard deviation and coefficient of variation in per cent, a summary of the amino acids profiles into factors A and B was also carried out.

III. Results

a) General Amino Acids Profiles

In Table 1, the general amino acids profiles were shown. The most concentrated amino acid was Glu (12.1-13.1 g/100 g) with a trend of turkey < francolin < duck. Next to Glu was another acidic amino acid, Asp in all the samples with values of 8.93-10.2 g/100 g. The highest concentrated essential amino acid (EAA) was Lys (duck, 6.65 g/100 g), Leu (francolin, 6.45 g/100 g) and Lys (turkey, 6.24 g/100 g). The protein levels were duck (67.9 g/100 g) < turkey (77.6 g/100 g) < francolin (80.1 g/100 g). An observation in Asp/Glu showed that the level of Asp appeared to affect the level of Glu and vice versa: the lower the Asp, the much higher the Glu and the much higher Asp, the less higher the Glu; for examples the Asp/Glu in the samples were (g/100 g): duck, 8.93/13.1; francolin, 9.01/13.0 and turkey, 10.2/12.1. The coefficient of variation per cent (CV %) values were generally low ranging between 1.58-27.4 showing that the samples were close in values for all the parameters determined.

In Table 2 were shown the various calculated values derived from Table 1. The total amino acids were (g/100 g crude protein, cp): duck (82.4) > francolin (80.8) > turkey (79.9). The EAA (with His) were duck (39.5 g/100 g, 47.9 %); francolin (36.0 g/100 g cp, 44.6 %) and turkey (37.0 g/100 g cp, 46.4 %). Total neutral

amino acid levels were duck (45.5 g/100 cp, 55.3 %); francolin (45.1 g/100 g cp, 55.8 %) and turkey (43.6 g/100 g cp, 54.6 %). Total aromatic amino acids ranged from 9.26-10.2 g/100 g cp (or 11.6-12.4 %); the total sulphur amino acid (TSAA) levels were low at 3.56-5.30 g/100 g cp (or 4.46-6.44 %). The protein efficiency ratio (P-PER) were better in duck (2.01) and francolin (2.14) but low in turkey (1.92). The isoelectric point (pI) was at acid range with values of 4.59-4.76 and the essential amino acid index ranged from 1.12-1.22. All CV % values were low.

In Table 3, the scores of the samples relative to whole hen's egg (Paul and Southgate, 1976) were shown .In the duck, the following amino acids had scores greater than 1.0: Lys (1.07), Glu (1.09), Gly (1.30) and Met (1.26); for francolin: Glu (1.08), Pro (1.04), Gly (1.40) andAla (1.07) whereas in turkey they were Lys (1.01) and Gly (1.54). The limiting amino acid here for each egg sample was Ser: duck (0.48), francolin (0.38) and turkey (0.37). The CV % levels were also low.

In Table 4, amino acid scores of the samples in relation to pre-school children requirements were depicted. In the duck, all the EAA values were better than the pre-school children requirements (scores > 1.0) except in Leu (score of 0.99); similar observation holds for turkey except in Leu (0.90) and in francolin, only scores for Lys (0.95) and Leu (0.98) were less than 1.0.

In Table 5, the sample amino acid scores relative to the provisional amino acid scoring pattern were shown. Valine was the limiting amino acid in the three different samples; in duck score was 0.80, in francolin score was 0.77 and in turkey it was 0.83. The summary of the essential and non-essential amino acids is shown in Table 6into Factors A and B means; both columns ended at 40.5. In Table 7 was shown the comparative compositions of the amino acids values (g/100 g) of duck, francolin, turkey, guinea fowl and domestic fowl.

IV. Discussion

a) General Amino Acids Profiles

Table 1 presents the amino acid composition of the samples. Virtually all the amino acids were high in value. Glu was the most concentrated amino acid (AA) in all the three samples with values of 13.1 g/100 g crude protein (cp) in the duck, 13.0 g/100 g cp in francolin and 12.1 g/100 g cp in turkey. A look at Table 1 will show that AA of the duck was most concentrated (on pair wise comparison) in 8/17 or 47.1 % of the AA; francolin was most concentrated in 4/17 or 23.5 % and in turkey it is 5/17 or 29.4 % best. Of these series, 5.8 (62.5 %) of the most concentrated AA in the duck were essential AA; it was ¼ (25.0 %) in francolin and 3/5 (60.0 %) turkey. The Asp was the second largest AA in the three samples. The most concentrated essential AA (EAA) in the samples was Lys (6.65 g/100 g) in the duck, Leu (6.49 g/100 g) in francolin and Lys (6.24 g/100 g) in turkey. The coefficient of variation per cent (CV %) ranged between 1.58-27.4 in the AA, with Arg having the least CV % and Cys the highest CV %. The calculations above would be an indication that the duck egg would be better in quality and quantity than francolin (in that order). So, whilst the AA levels (and quality) had this trend: duck > turkey > francolin; protein levels had opposite trend: francolin (80.1 g/100 g) > turkey (77.6 g/100 g) > duck (67.9 g/100 g). The Glu and Asp appeared to have opposite character levels in each sample; from Table 1, the much higher the Glu, the less higher is the Asp. For example: values of Glu/Asp (g/100 g cp) were : duck (13.1/8.93), francolin (13.0/9.01) and turkey (12.1/10.2). In the samples, highest Glu (13.1) was in duck and lowest Asp (8.93) was in Asp; second highest of 13.0 (Glu) was observed in francolin and second highest of 9.01 (Asp); in turkey third highest Glu (12.1) was observed and highest level of Asp (10.2) was observed. This type of observation had been noted for guinea fowl (Glu/Asp = 1.60/8.99 g/100 g) (Paul and Southgate, 1976).

Aspartic acid was first discovered in 1827 by deriving it from asparagine, which in turn had been isolated from asparagus juice about 20 years earlier. Aspartic acid was eventually understood to be an amino acid. Like all amino acids, Asp is a chiral molecule. The L-isomer is one of the 20 AA or building blocks of protein-based structures in human beings. Muscle tissue, skin, hair, fingernails and enzymes are all made from amino acids. L-aspartic acid is found in food, but it can also be made in the body, which makes it a non-essential AA. While L-Asp is widely used in the body as a building block, the biological role of its counterpart or enantiomer, D-Asp, is much more limited. D-aspartic acid is known to accumulate in the pituitary gland, pineal gland and testes, and is involved in hormone production. More specifically, it stimulates the release of sex hormones from the pituitary gland and testosterone from the testes. Consequently, D-Asp has become a popular supplement among body builders, other serious athletes and elderly men who have low-circulating levels of testosterone. Both forms of Asp are found in food, exceptional animal-based sources of Asp include beef, wild game, salmon, shrimp and eggs. Good vegetable sources include sprouting seeds, legumes, most nuts, avocados and asparagus (Kliegman*et al.*, 2007).

L-glutamic acid and L-glutamine have similar structures and play important roles in your body's functions. They are both amino acids that are constantly assembled and broken down again to form different proteins and enzymes. These amino acids are needed to form muscle and provide energy to the cells in the body small intestine. L-Glu is made from a number of amino acids, including omithine and arginine.

Glu is a non-essential amino acid, a component of folic acid and a precursor to glutathione, a powerful antioxidant. The acid is also referred to as glutamate, which is its salt form. Glu is involved in the metabolism of sugar and FAs, and works as a neurotransmitter in the brain, transporting potassium and detoxifying ammonia. L- glutamine can be synthesized, or made, from Glu. Glutamine is the most abundant free amino acid in the body, with most of it in skeletal muscle cells. It is metabolized in the small intestine, where it is broken down and used as the main source of cellular fuel, making it important in the regulation of the body GI tract. The study also states that glutamine is needed to fuel the cells of your immune system, and at times the kidney utilizes glutamine as well. It can be converted into glucose, or sugar when the body requires it, and it also helps to maintain the acid/alkaline balance in the body. Glutamic acid and glutamine are interconvertible, meaning that they can each make the other. Both compounds have a similar base chain while glutamic acid has a hydroxyl group attached to its chain (Kasper *et al.*, 2004).

b) Amino Acids Quality

Table 2 presents parameters on the quality of the protein of the samples. Total AA ranged as 82.4 g/100 g (duck) > 80.8 g/100 g (francolin) > 79.9 g/100 g (turkey). The EAA ranged between 36.0-39.5 g/100 g cp with a variation of 4.80 %. The values were lower than the value of 56.6 g/100 g cp of the egg reference protein (Paul and Southgate, 1980). The total sulphur amino acid (TSAA) of the samples were 5.30 g/100 g (duck), 3.63 g/100 g (francolin) and 3.56 g/100 g (turkey). The value of 5.30 g/100 g was close to the value of 5.80 g/100 g cp while values of 3.63-3.56 g/100 g cp formed more than one-half recommended for infants (FAO/WHO/UNU, 1985). The aromatic AA (ArAA) range suggested for ideal infant protein (6.8-11.8 g/100 g cp) (FAO/WHO/UNU, 1985) was very favourably comparable with the current report of 9.26-10.2 g/100 g cp showing that the samples protein could be used to supplement cereal flour. The percentage ratio of EAA to the total AA (TAA) in the samples ranged between 44.6-47.9 %. These values were well above the 39 % considered adequate for ideal protein food for infants, 26 % for children and 11 % for adults (FAO/WHO/UNU, 1985). The percentage of EAA/TAA for the samples could be favourably compared with other animal protein sources – 48.6 % in guinea fowl (Adeyeye, 2010), 51.1 % in domestic fowl (Paul and Southgate, 1976), 48.6-53.2 % in African giant pouch rat (muscle, skin) (Adeyeye and Falemu, 2012), 46.2 % in *Zonocerusvariegatus* (Adeyeye, 2005a), 43.7 % in *Macrotermes bellicosus* (Adeyeye, 2005b), 54.8 % in *Gymnarchus niloticus* (Trunk fish) (Adeyeye and Adamu, 2005), egg (50 %) (FAO/WHO, 1990). The TEAA in these results were close to the value of 44.4 g/100 g cp in soya bean (Altschul, 1958). The percentage of total neutral AA (TNAA) ranged from 54.6-55.8, indicating that these formed the bulk of the AA; total acid AA (TAAA) ranged from 26.8-27.8 which was lower than % TNAA, whilst the percentage range in total basic AA (TBAA) was 13.7-14.8 which made them the third largest group among the samples.

The predicted protein efficiency ratio (P-PER) was 2.16 (duck), 2.14 (francolin) and 1.92 (turkey). These results can be compared to these literature results: 2.25 (muscle) and 1.81 (skin) of guinea fowl (Adeyeye, 2011); 2.27 (skin) and 1.93 (muscle) of turkey hen (Adeyeye and Ayejuyo, 2007); 2.22 (*Clarias anguillaris*, 1.92 (*Oreochromis niloticus*) and 1.98 (*Cynoglossus senegalensis*) (Adeyeye, 2008a); 3.4 (whole body), 3.1 (flesh) and 2.6 (exoskeleton) of fresh water female crab (Adeyeye, 2008b); fresh water male crab: 2.9 (whole body), 2.8 (flesh), 2.4 (exoskeleton) (Adeyeye and Kenni, 2008); 4.06 (corn *ogi*) and reference casein with PER of 2.50 (Oyarekua and Eleyinmi, 2004). Some other literature values were 1.21 (cowpea) and 1.82 (pigeon pea) (Salunkhe and Kadam, 1989). 1.62 (millet *ogi*) and 0.27 (sorghum *ogi*) (Oyarekua and Eleyinmi, 2004); 3.21 in guinea fowl egg (Adeyeye, 2010) and domestic fowl (Paul and Southgate, 1976).The Leu/Ile ratio was low at 1.61-2.09, this is much less than in turkey hen (2.09-3.33) (Adeyeye and Ayejuyo, 2007) but close to the muscle and skin of guinea fowl at 1.191-1.98 (Adeyeye, 2011), hence no concentration antagonism might be experienced in the three samples. The essential AA index (EAAI) ranged from 1.12-1.22 as compared to the guinea fowl egg of 1.54 and domestic fowl of 1.55 (Adeyeye, 2010). The EAAI is useful as a rapid tool to evaluate food formulations for protein quality, although it does not account for differences in protein quality due to various reactions (Nielsen, 2002). It should be noted that Leu/Ile ratio in guinea fowl was 1.64 (Adeyeye, 2010) and 1.48 in domestic fowl (Paul and Southgate, 1976). The isoelectricpoints (pI) were acidic in values for all the results with range of 4.59-4.76 but higher in guinea fowl (5.93) (Adeyeye, 2010) and domestic fowl (5.93) (Paul and Southgate, 1976). The calculation of pI from AA would assist in the production of the protein isolate of an organic product.

Most animal proteins are low in Cys, for examples (Cys/TSAA) %: 36.3 % in *M. bellicossus* (Adeyeye, 2005a); 25.6 % in *Z. variegatus* (Adeyeye, 2005b); 35.5 % in *Archachatinamarginata,* 38.8 % in *Archatinaarchatina* and 21.0 % in *Limicolaria* sp. respectively (Adeyeye and Afolabi, 2004); 27.3 %-32.8 % in female fresh water crab body parts (Adeyeye, 2008b); 23.8 %-30.1 % in three different Nigeria fishes (Adeyeye, 2008a); 13.3 %-15.9 % in male fresh water crab body parts (Adeyeye and Kenni, 2008); 26.0-26.5 % in turkey hen meat (Adeyeye and Ayejuyo, 2007); 14.0 % in guinea fowl (Adeyeye, 2010) and 44 % in domestic fowl (Paul and Southgate, 1976); 26.2-30.3 % in the muscle and skin of guinea fowl (Adeyeye, 2011). The present

results of 19.8-31.2 % corroborated these literature observations. In contrast, many vegetable proteins contain substantially more Cys than Met, examples: 62.9 % in coconut endosperm (Adeyeye, 2004) and in *Anacardiumoccidentale* it is 50.5 % (Adeyeye *et al.*, 2007); however, in bambara groundnut seeds, the following were observed: testa (55.3 %), dehulled (46.1 %) and whole (44.5 %) (Adeyeye and Olaleye, 2012). Thus for animal protein diets, or mixed diets containing animal protein, Cys is unlikely to contribute up to 50 % of the TSAA (FAO/WHO, 1991). The percentage of Cys in TSAA had been set at 50 % in rat, chick and pig diets (FAO/WHO, 1991). Cys has positive effects on mineral absorption particularly zinc (Mendoza, 2002; Sandstrom *et al.*, 1989).

c) Amino Acid Scores

Table 3 contains the AA scores (AAS) of the samples based on the whole hen's egg profile (Paul and Southgate, 1976). The scores had values greater than 1.0 in Lys (1.07), Glu (1.09), Gly (1.30) and Met (1.26) in the duck egg; in francolin egg, they were Glu (1.08), Pro (1.04), Gly (1.40) and Ala (1.07) whereas it was 1.00 in His; in turkey, the followings were observed: Lys (1.01), Gly (1.54) whereas it was 1.00 in Glu. Gly had the highest score in duck (1.30), in francolin (1.40) and in turkey (1.54). Gly score in guinea fowl under this standard was 2.23 (Adeyeye, 2010); the least score was Ser: 0.48 (duck), 0.38 (francolin) and 0.37 (turkey). The eggs under discussion showed very good comparison with the AA profile of the whole hen's egg. The CV % between AA levels for the scores in the eggs ranged between 1.60-25.8. Table 4 shows the essential AA scores (EAAS) based on the suggested requirement of the EAA of a pre-school child (FAO/WHO/UNU, 1985). It is interesting to note that all the EAAS in duck were greater than 1.0 (except Leu, 0.99); all in francolin except Lys (0.95) and Leu (0.98); all in turkey (except Leu, 0.90). Under this comparison, Met +Cys had the best score in duck (2.12), in francolin (1.45), in turkey (1.42). The limiting AA (LAA) in duck was Leu (0.99), in francolin it was Lys (0.95) and in turkey it was Leu (0.90). To correct for the LAA: in duck it is 100/99 or 1.01 x protein of duck; in francolin it is 100/95 or 1.05 x protein of francolin; in turkey it is 100/90 or 1.11 x protein of turkey. The CV % values were close at 4.40-23.8. Table 5 shows the EAAS based on the provisional essential amino acid scoring pattern (FAO/WHO, 1973). EAAS greater than 1.0 in duck were Lys, Met +Cys, Phe + Tyr and total EAA; in francolin, scores greater 1.0 or equal to 1.0 were Lys, Met + Cys and Phe + Tyr; in turkey, EAAS greater or equal to 1.0 were Lys, Thr, Met + Cys, Phe + Tyr and total EAA. The LAA in duck was Val (0.80), in francolin it was Val (0.77) and in turkey it was Val (0.83); all the corrections follow the trend as done for Table 4. The highest EAAS was Met + Cys (1.51) in duck; it was Phe + Tyr (1.26) in francolin and it was Phe + Tyr (1.20) in turkey.

The following values would show the position of the quality of egg samples protein; the EAA requirements across the samples were (values with His) (g/100 g protein): infant (46.0), pre-school (2-5 years) (33.9), school child (10-12 years) (24.1) and adult (12.7) and without His: infant (43.4), pre-school (32.0), school child (22.2) and adult (11.1) (FAO/WHO/UNU, 1985). From the present results based on these standards, we have: 39.5 g/100 g protein (with His) and 37.2 g/100 g (no His) protein in the duck; 36.0 g/100 g protein (with His) and 33.6 g/100 g protein (no His) in the francolin; 37. 0 g/100 g protein (with His) and 35.0 g/100 g protein (no His) in the turkey.

d) Summary of the Amino Acids Profiles

Table 6 gives a brief summary of the AA profiles in the three samples. Column under Factor B means showed that the values there were close with a range of 37.5-43.5; similar observation could be made in Factor A column with AA profile range of 39.9-41.2. it should however be noted that both columns A and B means terminated at 40.5.

e) Comparison of Amino Acid Profiles of Many Eggs

Table 7 contains the whole egg amino acids profiles of duck, francolin, turkey (from present study), guinea fowl and domestic fowl (from literature). It is only meant for easy reference on quality variation among the eggs.

V. Conclusion

The findings of this study showed that the samples demonstrated amino acid profiles of three different whole eggs being different from each other. The eggs are virtually adequate for pre-school children because they were all having scores greater than 1.0 in the principal limiting amino acids of Lys (first limiting, 0.95-1.15), Met + Cys (second limiting, 1.42-2.12), Thr (third limiting, 1.03-1.23), Try (fourth limiting, not determined). This means, all these eggs should be encouraged and taken (any of them) as choice eggs.

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Table 1 : The amino acid composition (g/100g crude protein edible portion) of duck, francolin and turkey eggs (dry weight)

Amino acid	Duck	Francolin	Turkey	Mean	SD	CV%
Lysine (Lys)*	6.65	5.49	6.24	6.13	0.59	9.60
Histidine (His)*	2.24	2.41	2.08	2.24	0.17	7.36
Arginine (Arg)*	5.89	5.83	5.71	5.81	0.09	1.58
Aspartic acid (Asp)	8.93	9.01	10.2	9.38	0.71	7.58
Threonine (Thr)*	3.55	3.50	4.18	3.74	0.38	10.1
Serine (Ser)	3.80	3.01	2.91	3.24	0.49	15.0
Glutamic acid (Glu)	13.1	13.0	12.1	12.7	0.55	4.33
Proline (Pro)	3.02	3.94	3.39	3.45	0.46	13.4
Glycine (Gly)	3.91	4.21	4.61	4.24	0.35	8.28
Alanine (Ala)	4.21	5.79	4.01	4.67	0.98	20.9
Cystine (Cys)	1.27	0.72	1.11	1.03	0.28	27.4
Valine (Val)*	4.02	3.83	4.16	4.00	0.17	4.14
Methionine (Met)*	4.03	2.91	2.45	3.13	0.81	26.0
Isoleucine (Ile)*	3.24	3.11	3.69	3.35	0.30	9.09
Leucine (Leu)*	6.51	6.49	5.95	6.32	0.32	5.03
Tyrosine (Tyr)	3.09	3.25	3.00	3.11	0.13	4.07
Phenylalanine (Phe)*	4.88	4.30	4.18	4.45	0.37	8.41
Tryptophan (Try)*	-	-	-	-	-	-
Protein[a]	67.9	80.1	77.6	75.2	6.44	8.57

** Essential amino acid; [a] Dry weight and fat free basis.*

Table 2 : Essential, non-essential, acidic, neutral, sulphur, aromatic (g/100g crude protein edible portion) of duck, francolin and turkey eggs (dry weight)

Amino acid	Duck	Francolin	Turkey	Mean	SD	CV%
TAA	82.4	80.8	79.9	81.0	1.27	1.56
TNEAA	42.9	44.8	42.8	43.5	1.13	2.59
% TNEAA	52.1	55.4	53.6	53.7	1.65	3.08
TEAA – with His	39.5	36.0	37.0	37.5	1.80	4.81
– no His	37.2	33.6	35.0	35.3	1.81	5.15
% TEAA – with His	47.9	44.6	46.4	46.3	1.65	3.57
-no His	45.2	41.6	43.8	43.5	1.81	4.17
TNAA	45.5	45.1	43.6	44.7	1.00	2.24
% TNAA	55.3	55.8	54.6	55.2	0.60	1.09
TAAA	22.0	22.0	22.2	22.1	0.12	0.52
% TAAA	26.8	27.2	27.8	27.3	0.50	1.85
TBAA	14.8	13.7	14.0	14.2	0.57	4.01
% TBAA	17.9	17.0	17.6	17.5	0.46	2.62
TSAA	5.30	3.63	3.56	4.16	0.99	23.7
% TSAA	6.44	4.49	4.46	5.13	1.13	22.1
% Cys/TSAA	24.0	19.8	31.2	25.0	5.77	23.1
TArAA	10.2	9.96	9.26	9.81	0.49	4.98
% TArAA	12.4	12.3	11.6	12.1	0.44	3.60
P-PER[a]	2.16	2.14	1.92	2.07	0.13	6.42
Leu/Ile	2.01	2.09	1.61	1.90	0.26	13.5

% Leu-Ile (diff)	50.2	52.1	38.0	46.8	7.65	16.4
pI[b]	4.76	4.65	4.59	4.67	0.09	1.85
EAAI[c]	1.22	1.12	1.16	1.17	0.05	4.31

[a]P-PER = predicted protein efficiency ratio; [b]pI =isoelectric point; [c]EAAI = essential amino acid index.

Table 3 : Amino acids scores of the samples with respect to whole hen's egg (amino acids values were in g/100g)

Amino acid	Duck	Francolin	Turkey	Mean	SD	CV%
Lys	1.07	0.89	1.01	0.99	0.09	9.26
His	0.93	1.00	0.87	0.93	0.07	6.97
Arg	0.97	0.96	0.94	0.96	0.02	1.60
Asp	0.83	0.84	0.95	0.87	0.07	7.62
Thr	0.70	0.69	0.82	0.74	0.07	9.82
Ser	0.48	0.38	0.37	0.41	0.06	14.8
Glu	1.09	1.08	1.00	1.06	0.05	4.67
Pro	0.79	1.04	0.89	0.91	0.13	13.9
Gly	1.30	1.40	1.54	1.41	0.12	8.53
Ala	0.78	1.07	0.74	0.86	0.18	20.9
Cys	0.71	0.40	0.62	0.58	0.16	27.7
Val	0.54	0.51	0.55	0.53	0.02	3.90
Met	1.26	0.91	0.77	0.98	0.25	25.8
Ile	0.58	0.56	0.66	0.60	0.05	8.82
Leu	0.78	0.78	0.72	0.76	0.03	4.56
Tyr	0.77	0.81	0.75	0.78	0.03	3.93
Phe	0.96	0.84	0.82	0.87	0.08	8.67
Total	0.84	0.82	0.81	0.82	0.02	1.86

Table 4 : Amino acids scores of the samples with respect to pre-schoolchildren requirements (amino acids value were in g/100g)

Amino acid	Duck	Francolin	Turkey	Mean	SD	CV%
Lys	1.15	0.95	1.08	1.06	0.10	9.57
His	1.18	1.27	1.09	1.18	0.09	7.63
Thr	1.04	1.03	1.23	1.10	0.11	10.2
Met + Cys	2.12	1.45	1.42	1.66	0.40	23.8
Val	1.15	1.09	1.19	1.14	0.05	4.40
Ile	1.16	1.11	1.32	1.20	0.11	9.17
Leu	0.99	0.98	0.90	0.96	0.05	5.16
Phe +Tyr	1.27	1.20	1.14	1.20	0.07	5.41
Total	1.20	1.06	1.09	1.12	0.07	6.60

Table 5 : Amino acids scores of the samples with respect to provisional amino acid scoring pattern (amino acids values were in g/100g)

Amino acid	Duck	Francolin	Turkey	Mean	SD	CV%
Lys	1.21	1.00	1.13	1.11	0.11	9.52
Thr	0.89	0.88	1.05	0.94	0.10	10.1
Met + Cys	1.51	1.04	1.02	1.19	0.28	23.3
Val	0.80	0.77	0.83	0.80	0.03	3.75
Ile	0.81	0.78	0.92	0.84	0.07	8.81
Leu	0.93	0.93	0.85	0.90	0.05	5.11
Phe +Tyr	1.33	1.26	1.20	1.26	0.07	5.15
Total	1.06	0.96	1.00	1.01	0.05	5.00

Table 6 : Summary of the amino acids profiles into factors A and B

Amino acid composition(Factor B)	Samples(Factor A)			Factor B means
	Duck	Francolin	Turkey	
Total essential amino acid	39.5	36.0	37.0	37.5
Total non-essential amino acid	42.9	44.8	42.8	43.5
Factor A means	41.2	40.4	39.9	40.5

Table 7 : Whole egg amino acid compositions of duck, francolin and turkey compared with those of whole egg amino acid compositions of guinea fowl and domestic fowl (g/100g crude protei

Amino acid	Duck	Francolin	Turkey	Guinea fowl[a]	Domestic fowl[b]
Lys	6.65	5.49	6.24	6.91	6.2
His	2.24	2.41	2.08	2.62	2.4
Arg	5.89	5.83	5.71	6.55	6.1
Asp	8.93	9.01	10.2	8.99	10.7
Thr	3.55	3.50	4.18	5.20	5.1
Ser	3.80	3.01	2.91	3.80	7.9
Glu	13.1	13.0	12.1	1.60	12.0
Pro	3.02	3.94	3.39	5.08	3.8
Gly	3.91	4.21	4.61	6.68	3.0
Ala	4.21	5.79	4.01	5.77	5.4
Cys	1.27	0.72	1.11	0.55	1.8
Val	4.02	3.83	4.16	6.58	7.5
Met	4.03	2.91	2.45	3.39	3.2
Ile	3.24	3.11	3.69	5.55	5.6
Leu	6.51	6.49	5.95	9.10	8.3
Tyr	3.09	3.25	3.00	4.29	4.0
Phe	4.88	4.30	4.18	5.70	5.1
Try	-	-	-	-	1.8

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Keywords : pour point, rheology, flow improver, pour point depressant, vinyl acetate methyacrylate copolymer.

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SYNTHESIS AND CHARACTERIZATION OF POLYMERIC ADDITIVES AND THEIR EFFECT ON FLOW PROPERTIES OF WAXY EGYPTIAN CRUDE OIL

Strictly as per the compliance and regulations of :

Synthesis and Characterization of Polymeric Additives and their Effect on Flow Properties of Waxy Egyptian Crude Oil

Ayman M. Atta[α], Rasha A. El-Ghazawy[σ], Fatma A. Morsy[ρ], Ali Mohamed Salah Ali [ω] & Abdullah Elmorsy [¥]

Abstract - The main target of this work is to solve the transportation problems associated with the waxy crude oil particularly the deposition of paraffin wax during transportation and storage if the temperature of the crude drops below certain temperatures. This work aims to prepare different copolymers based on vinyl acetate and methacrylic acid monomers. The reactions involve copolymerization of vinyl acetate with methacrylic acid in different monomer ratios. The prepared copolymers then esterified using stearyl and behenyl alcohols. The chemical structure of the prepared copolymers and their characteristics were evaluated using FTIR and ^{1}HNMR spectroscopic techniques. The molecular weight of the polymeric additives was measured using GPC. The efficiency of the prepared copolymers were studied for their capability as pour point depressants (PPD) and flow improvers for Egyptian waxy crude oil to solve the problem of wax deposition during transportation and storage. The study showed that generally the CoVASMA esters were efficient in the role of both pour point depressant and flow improver for the tested crude oil.

Keywords : pour point, rheology, flow improver, pour point depressant, vinyl acetate methyacrylate copolymer.

I. Introduction

In the last quarter of the 20th century, global demand for crude oil had a very stable yearly growth rate averaging 1%. This has changed radically in the first years of the 21st Century due to "Emerging Countries" like China and India whose dynamic economies resulted in a remarkable 1.8% global growth in demand for crude oil in 2009. Serious international studies still foresee that in the next 20 years, at least 80% of the world's energy requirements will come from petroleum, natural gas and coal. Consequently, oil will remain the dominant source of energy for the next half century. Crude oils are complex hydrocarbon mixtures containing nonpolar *n*-paraffins and polar components such as asphaltenes. According to some estimates from the International Energy Agency (IEA), heavy oil represents at least half of the recoverable oil resources of the world. Heavy oil is defined as petroleum which has a density equal or lower than 20 API, but if petroleum has 10 API or less it is considered as extra heavy oil or bitumen, which is denser than water.

The incorporation of heavy oil to energy markets presents important challenges that require significant technological developments in the production chain. The transportation of heavy and extra-heavy oil presents many operational difficulties that limit their economical viability.

Pipelining is the most convenient mean for transportation of crude oils and derived products continuously and economically. However, transportation of heavy and extra heavy crude oils through pipelines is difficult due to the low mobility and flowability of the crude and wax and asphaltene deposition on pipeline wall surfaces.

Waxy crude oils always suffer serious problems during transportation and storage, particularly in cold environments; this arises from the presence of significant amounts of paraffin wax in the crude oil that impede the flow of crude oil due to wax precipitation. Regarding the composition of the waxy crudes, the high molecular weight *n*-alkanes (*n*-paraffins) are the main components in wax deposits.[1,2] Waxes are heavy paraffinic solids that settle out of a crude oil to form a gel structure. Wax formation is a liquid-solid phase transition from a liquid mixture which is largely sensitive to a drop in temperature.[3] In the petroleum industry, wax precipitation is undesirable because it may cause plugging of pipelines and process equipment. Wax precipitation is an old problem [4-7]. In this respect the wax deposition problem has been thoroughly studied both chemically and thermodynamically.[8] The crude oil pipelining at temperatures lower than the pour point temperature (PPT) of the crude oil change the flow behavior of the crude oil dramatically, below this temperature there is no any flow behavior of crude oil.

Solid wax deposition is a challenging problem in crude oil production, transportation, and storage and causes huge economic losses for the petroleum industry.[1] The wax crystals deposition leads to higher viscosity of crude oil, with high transportation costs arises from the increased energy consumption for pumping, the decreased pumping capacity, and the

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reduction of the effective cross sectional area of the pipe via wax deposition. Wax deposition also increases the pipeline roughness which results in an increase in pressure drop.[10] Avoiding and/or solving the wax deposition problems is therefore an economically beneficial target which can be highly achieved by introducing a polymeric chemical additive in the ppm level to the crude oil in order to reduce its pour point temperature.[11-14] Although, crude oil treatment with chemical additives is not the only known solution for inhibition of wax deposition, it remains the preferred solution over other options like pigging, heating, and biological treatments. Pour point depressants (PPD) and flow improvers are polymeric additives that used to decrease the pour point and enhance the flow characteristics of crude oil, respectively. In doing that PPD's and flow improvers should posse oil-loving long chains in addition to polar groups such as ester, amine, and hydroxyl groups.[15-21] This study involves the preparation and evaluation of vinyl acetate methacrylic acid copolymer as pour point depressant PPD by changing the feed ratios of the two monomers and incorporating different alkyl side chains into the polymer backbone via esterification. Egyptian waxy crude oil was used to evaluate the efficiency of the above mentioned esters through measurements of pour point and rheological properties of untreated and additive treated crude oil.

II. Experimental

a) Materials

Vinyl acetate (VA), methacrylic acid (MAA), stearyl alcohol (SA), behenyl alcohol (BA), dibenzoyl peroxide (BP), and P-toluenesulfonic acid (PTSA) were obtained as analytical grade from Aldrich chemicals Co. (Germany). Butanone, dimethyl formamide (DMF), methanol and xylene were obtained from Adweic Chemicals Co. Egypt. Egyptian Waxy crude oil produced from Norpetco (Egypt) and delivered without treatment from Fardous field. The physicochemical characteristics and composition of Norpetco crude oil are listed in Tables (1)

Table 1 : The Physicochemical properties of Norpetco crude oil

Test	Method	Value
API gravity at 60 F	ASTMD-1298	41.1
SPECIFIC GRAVITY @ 60/60 F	ASTM D-1298	0.820
Wax content, (Wt%)	UOP 46/64	8.4
Asphaltene content, (Wt%)	IP 143/84	3
Water content vol%	IP 74/70	0.23
Kinematic Viscosity(cSt)@ 50 60 °C	ASTM D-445	7 4.3
Pour Point °C	QPC procedure	30

b) Copolymerization

The copolymerization reaction was carried out in three-neck glass flask equipped with magnetic stirrer, thermometer, nitrogen gas inlet and a reflux condenser. The reaction carried out using butanone as solvent in which vinyl acetate monomer dissolved. Dibenzoyl peroxide (0.1% by weight of the total monomer weight) is dissolved in suitable amount of butanone and used as initiator. The solution of dibenzoyl peroxide is added in four proportions during the first two hours of the reaction. In the beginning of the reaction the reaction flask was swept with nitrogen gas, then the temperature of the reaction mixture is raised to 80 °C and methacylic acid monomer is continuously introduced during the reaction progress. At the end of the six hours of the reaction the copolymer was precipitated from solution in methanol. The precipitate was collected, washed with methanol, and dried in vacuum oven at 40 °C.

The above mentioned procedure were repeated with different mole ratios of the reacting monomers to produce copolymers with feed ratios namely (1:1), (1:2) and (2:1).

c) Esterification

The esterification reaction of the prepared copolymers was carried out in three-necked reaction flask fitted with reflux condenser, thermometer, and Dean stark separator at constant stirring. The reaction mixture is a solution of 0.02 mol of copolymer with one of the previously described ratios with 0.01 mole of alcohol (stearyl, or behenyl alcohol) was refluxed in 50 ml DMF in the presence of 0.1 % (wt/wt) PTSA as catalyst. The reaction was carried out at the refluxing temperature until theoretical amount of water was collected in the Dean stark trap. The resulting esters were washed out with water to remove the catalyst and any unreacted materials.

d) Characterization

The prepared esters, stearyl methacrylate- vinyl acetate copolymers (CoVASMA), and behenyl methacrylate- vinyl acetate copolymers (CoVABMA), were analyzed by FTIR as spectroscopic technique. Infrared (IR) spectra were performed on a Bio-Rad FTS 165 FTIR spectrophotometer using KBr pressed pellets or KBr salt plate for solid and liquid compounds, respectively.

^{1}HNMR using a 300 MHz Varion NMR 300 spectrometer using Dimethyl sulphoxide (DMSO) as solvent.

The molecular weight were characterized (in terms of Mw and Mn) and polydispersity index using Shimadzu's gel permeation chromatograph equipped with refractive index detector and polydivinylbenzene mix gel-D column. Tetra Hydro Furan (THF) with a flow rate 1 mL/min was used as mobile phase. Polystyrene was used as the standard.

e) Evaluation Tests

The additives consisted of 10 ml of toluene containing 3 g of each copolymer; each solution was stirred for 10 min at a doping temperature for homogenization.

Pour point test was made according to ASTM D97 test procedure at different concentrations of the prepared additives namely 1000, 2000, 3000, 4000, and 5000 ppm.

Viscosity and flow curves (Rheogram) were measured using Brookfield viscometer equipped with thermostated cooling system for temperature adjustment. To obtain consistent results with accurate rheological measurements, the memory of the evaluated crude oil samples has to be removed by heating at 80 °C while stirring. Tests started by heating the preconditioned untreated crude oil samples to 60 °C in an ultrasonic bath and then loading them into a hermetic bottle with an appropriate amount of flow improver. Finally, the temperature of the sample in the ultrasonic bath was maintained at 60 (1 °C for 30 min). The prepared additives were evaluated as flow improver for wax crude through rheological measurements at concentrations of 2000 ppm. Measurements were carried out at different temperatures below pour point of crude oils ranging from 21 to 12 °C. The experimental procedure started when the additives were mixed with crude oil at the prescribed concentration at 65 °C. Meanwhile, the viscometer cup is preheated to the same temperature, then loaded with 25 ml of the treated sample, and then the temperature is brought down to a constant temperature, at which the measurements will be achieved, at a low shear rate of 7.29 S^{-1} (dynamic cooling). Shearing was continued for 15 minute at the test temperature before evaluation. The shear stress-shear rate relationship was recorded for the tested samples.

III. Result and Discussion

Free radical polymerization is a common method for the synthesis of many polymers. One drawback of free radical polymerization is uncontrolled structure of the produced polymer. In the present work, solution copolymerization of VA and MAA was carried out in 2-butanone as a solvent to control radical polymerization. The type and amount of initiator, temperature, and delayed monomer feeds have all been used to control the final structure and size of the polymer particles. Random copolymers of MAA with VA are difficult to produce by free radical polymerization, since MAA has a much higher reactivity that the VA monomer.[22] In order to prepare PVAMA copolymer, MAA was added into the reactor fluid for a longer period of time during the reaction.

a) Chemical structures of the prepared additives

The FTIR spectra of PVAMA copolymer with different mole ratios, not given here for brevity, show bands at about 2950 cm^{-1} represent the stretching absorption of –CH aliphatic present in both vinyl acetate and methacrylic acid, and the bands at about 1700 cm^{-1} assigned for the absorption of carbonyl group. Also, the presence of broad band at in the range of 3400 cm^{-1} is indicative for the presence of OH group in free carboxyl group of methacrylic acid. The absence of bands in the range of 1640-1680 cm^{-1} which characterizes the C=C group indicate the completion of polymerization.

Also, the structure of PVAMA copolymer was confirmed by ^{1}HNMR as follow. **Figure 1** represents the ^{1}HNMR spectrum of PVAMA copolymer with composition ratio (1:2). The figure shows chemical shift at about 1.0 ppm due to the methyl protons present in MAA and signal at about 2.0 ppm assigned to the protons of methyl group from VA. Also, the figures contain bands at chemical shift 2.5 ppm which indicate the methylene protons from VA and signals at chemical shift about 3.5 ppm assigned to methylene protons from MAA, and the band at chemical shift about 5.3 ppm for CH protons adjacent to the acetate group in the vinyl acetate moieties. The clear signal at about 12.3 ppm is resulting from the free carboxyl group of MAA. The disappearance (or the presence of very weak bands) in chemical shift range of 6 – 6.5 ppm indicate the absence of vinyl protons and completion of polymerization.

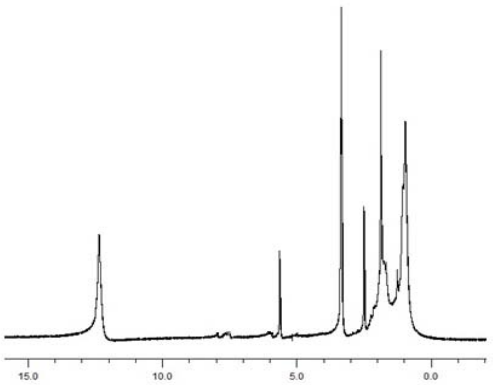

Figure 1 : ^{1}HNMR of VA: MAA 1:2 copolymer

Molecular weights data of the prepared polymers with the different feed ratios, number average molecular weight (Mn), weight average molecular weights (Mw) and polydispersity (PD= Mw/Mn), were determined using gel permeation chromatography (GPC) using THF as eluent and the results are summarized in **Table 2.** The molecular weight results indicated that the molecular weight increased by increasing either vinyl acetate or methacrylic acid

content. While the polydispersity increased with equimolar ratios of VA and MAA.

Table 2 : The average molecular weight of the prepared copolymer at different mole ratios

Polymer composition	PD	Molecular weight (g/mol)	
		Mw	Mn
PVAMA (1:1)	2.4	10,190	4,231
PVAMA (1:2)	1.69	170,015	100,382
PVAMA (2:1)	1.96	163,105	82,995

b) The influence of additive on pour point of crude oil

The solubility of paraffin in the lighter alkanes is greatly dependent upon temperature. When temperature drops below certain value, PPT, solid paraffin deposits appear and prevent the crude oil from flowing. The paraffin content of the tested crude oil was determined by urea adduction, and then subjected to gas liquid chromatographic analysis for determination of average molecular weight distribution. The structure and composition of wax in crude oil are very important to select the suitable structure of wax dispersant to increase its flowability. However, still in most cases the wax dispersants having highly polar functional groups are used for improving flowability of wax crude oil. The n-paraffin content in the crude oil was found 12 wt % by urea adduction. Further analysis of n-parrafins by GLC for Norpetco crude oil was carried out to determine the carbon numbers as shown in **Figure 2.** From data represented in **Figure 2,** it has been concluded that the average carbon number was 44 and the molecular weight distribution expressed in $W_{h/2}$ has a broad distribution. It is obvious that the concentration of 50 wt % of the n-paraffin content in the crude oil in a broad range and a high average carbon number (44.2) tends to precipitate suddenly in the form of a solid at a fairly high temperature above the pour point. These n-paraffins have the ability to construct rapidly a massive interlocking network that hinders the response of the crude to additive at a preceding stage of formation of fine crystals.

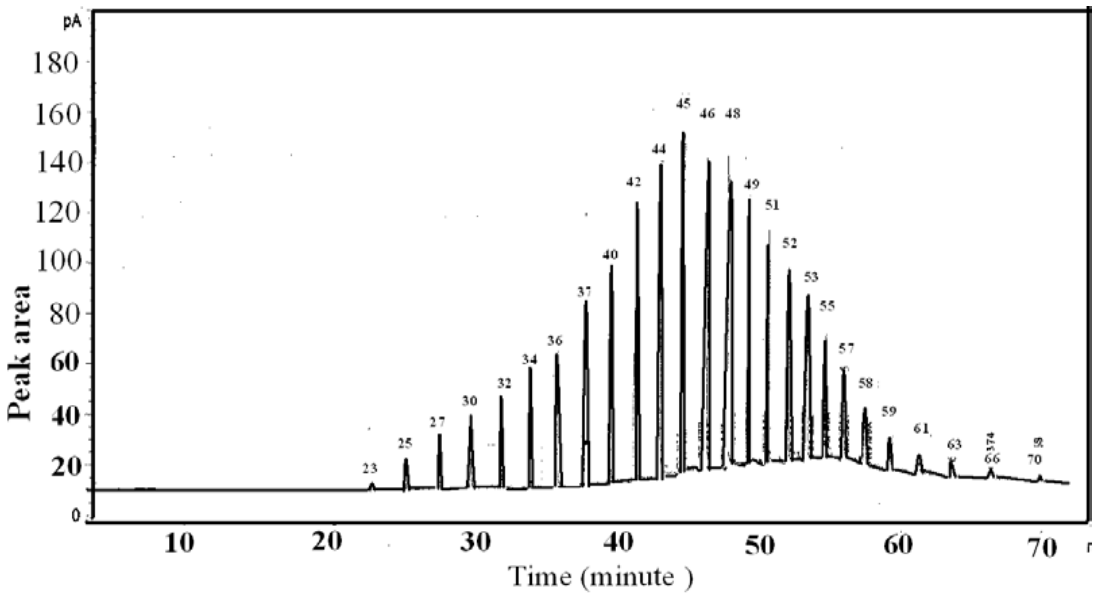

Figure 2 : Chromatogram for wax from Norpetco crude oil

The performance of PPD depends on the characteristics of crude oil including total wax content, the chain length and shape (linear or branched), quantity and type of wax present in crude[9,20] The mechanism of pour point depression has been well explained;[9, 21] the PPD in crude oil changes the wax crystal shapes from extensively interlocking plates to more compact crystals by co-crystallizing with the wax. The more similar the polymer structure to wax components, the better is its performance and the better its ability to attach to wax components and create a barrier for networking of wax particles[1].The efficiency of these copolymers as PPD was evaluated through the pour point test procedure. The six prepared additives were tested for reduction of pour point at different concentrations namely 1000, 2000, 3000, 4000, 5000 ppm. The pour point depression results for stearyl methacrylate-co-vinyl acetate (CoVASMA) were measured and listed in **Table 3** Also the results of behenyl methacrylate-co-vinyl acetate (CoVABMA) are listed in **Table 4.**

Table 3 : The pour point of untreated crude oil and treated crude oil with CoVASMA

Copolymer composition	Pour point temperature (°C) at concentrations (ppm)					
	Nil	1000	2000	3000	4000	5000
CoVASMA (1:1)	30	27	15	15	12	12
CoVASMA (1:2)	30	15	15	15	15	12
CoVASMA (2:1)	30	15	12	12	12	12

Table 4 : The pour point of untreated crude oil and treated crude oil with CoVABMA

Copolymer composition	Pour point temperature (°C) at concentrations (ppm)					
	Nil	1000	2000	3000	4000	5000
CoVABMA (1:1)	0	30	27	27	27	27
CoVABMA (1:2)	0	27	27	27	27	24
CoVABMA (2:1)	0	30	30	27	27	24

The pour point data given in **Tables 3** and **4** shows the most effective copolymers for depression of pour point of this crude oil was the CoVASMA with mole ratio (2:1). It was able to reduce the pour point to about 12°C at concentration of 2000 ppm. The interaction of these additives with crude oil may occur through well matching of the alkyl side chain and polar ester groups of additive with that of n-paraffin of crude oil. The function of the additive's two moieties is further explained. The large proportions of alkyl side chain in combination with the polarity of ester groups formed and the free acid groups impart the additives the function as pour point depressant where the polar groups introduce good dispersing action through interacting with the wax crystal and impeding the growth of wax crystals, and the long alkyl side chain of the copolymer will be merged into the wax crystal of the crude oil. It also forms as many as possible active points for excessive nucleation to form a large number of small crystals thereby may inhibit the crystal growth of wax crystals in three dimensional networks resulting in small proportions of free paraffin to prevent the oil flow with the reduction in temperature and, consequently, the pour point was reduced. On the other hand the poor efficiency of behenyl esters for this crude oil can be explained by the increased chain length which affects the interactions between wax crystals and polymeric additives. This effect result in, not only, poor interaction between the polymeric additive and the paraffins in crude oil which make it less efficient but also may introduce adverse effect and assist wax nucleation and deposition.

c) The influence of additive on rheology of crude oil

The rheological parameters for untreated and treated crude oil with 2000 ppm concentration of CoVASMA (2:1) were determined at different temperatures namely 12ºC, 15ºC, and 21ºC. **Figures 3, 4,** and **5** represent shear stress against shear rate relationships and **Figures 6, 7, and 8** represent the relationship between viscosity and shear rate. The yield values of of neat crude oil and crude oil in presence of 2000 ppm concentration of CoVASMA (2:1) are displayed in **Table 5**. Investigation of the previous data shows that the CoVASMA (2:1) was able to modify the flow and rheological behavior of the treated crude oil by decrease the yield shear stress value. The decrease in yield shear stress values increase with increasing the temperature. The higher efficiency of CoVASMA with mole ratio (2:1) in lowering the yield shear stress value of crude oil may be attributed to the fact that the polar groups present in the polymeric backbone of the additives were able to form some type of physical attraction with polar moieties of resins and asphaltenes present in the crude oil such as hydrogen bond. The high polarity of this additive may be rationalized by presence of polar ester groups in the polymer in addition to polar acetate groups from vinyl acetate.

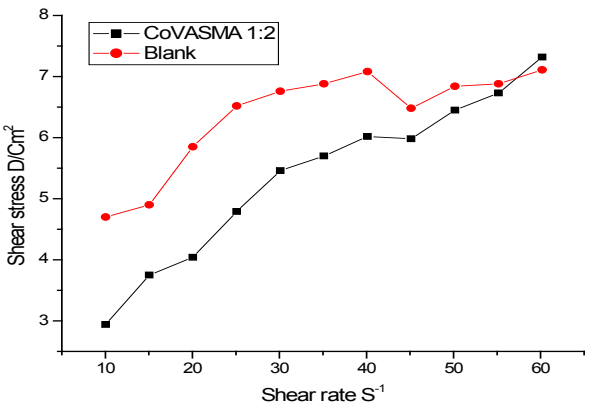

Figure 3 : Rheogram of untreated and treated crude oil with 2000 ppm of CoVASMA (1:2) at 12 °C

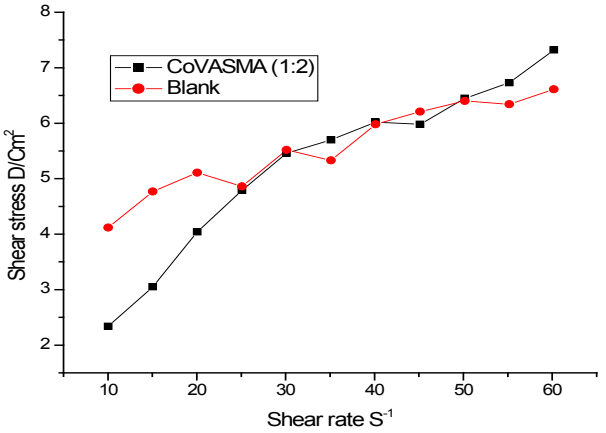

Figure 4 : Rheogram of untreated and treated crude oil with 2000 ppm of CoVASMA (1:2) at 15 ºC

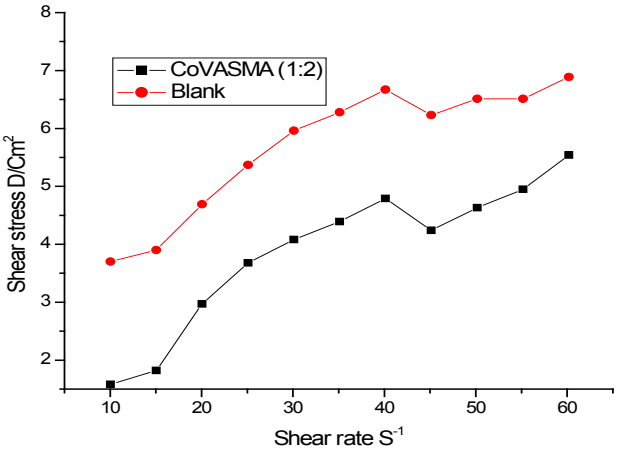

Figure 5 : Rheogram of untreated and treated crude oil with 2000 ppm of CoVASMA (1:2) at 21 °C

Table 5 : Yield value of untreated and treated crude oil with 2000 ppm Concentration of CoVASMA (2:1)

Oil sample	T° C	Yield value (D/cm^2)
Untreated	12	4.89
	15	3.93
	21	3.52
CoVASMA (2:1)	12	2.63
	15	2.09
	21	1.39

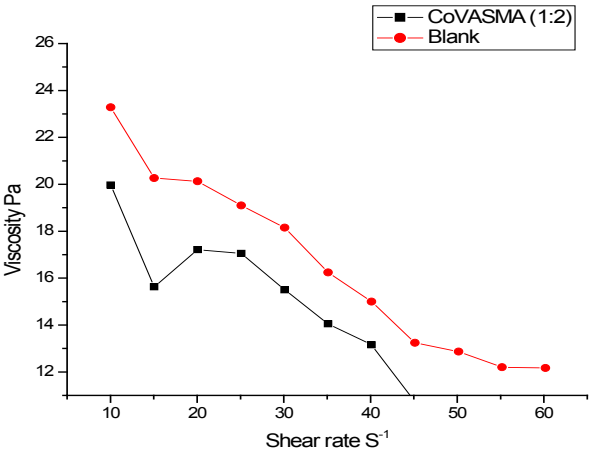

Figure 6 : Effect of shear rate on viscosity of untreated and treated crude oil with 2000 ppm of CoVASMA (2:1) at 12 °C

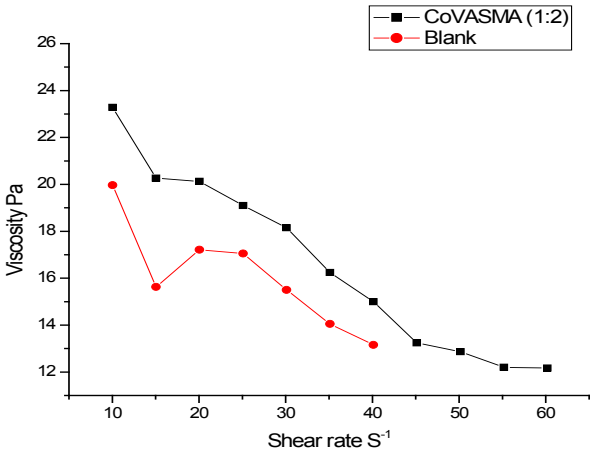

Figure 7 : Effect of shear rate on viscosity of untreated and treated crude oil with 2000 ppm of CoVASMA (2:1) at 15 °C

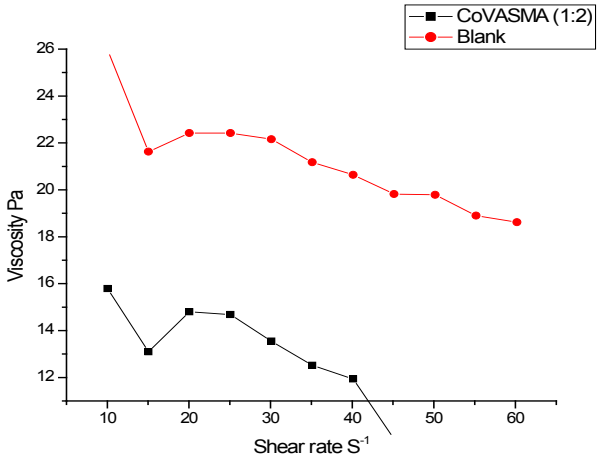

Figure 8 : Effect of shear rate on viscosity of untreated and treated crude oil with 2000 ppm of CoVASMA (2:1) at 21 °C

The obtained results for pour point and viscosity showed good correlation. This may be explained in terms of the ability of CoVASMA (2:1) to introduce strong interaction with the wax crystals modifying their crystal structure and forming small crystals. This in turn inhibits the probability of wax crystals to agglomerate and thereby depress the pour point. In case of rheological measurements the task of the additive was relatively easier since the rheology testing involves shearing that assist to break probable aggregations of wax producing particles whose size and shape are easier to disperse by the additive and the net results is decrease in the yield shear stress and viscosity of crude oil that correlate well with the results of pour point depression.

IV. Conclusion

In this work six polymeric additives with different composition were synthesized and evaluated as pour point depressants and flow improvers for Egyptian crude oil. The copolymers of CoVASMA, particularly the copolymer with monomer feed ratio (2:1), were satisfactorily able to act as pour point depressants (PPD's) and flow improvers. They were able significantly to improve the viscosity of the tested crude oil in as well as decrease in the crude oil pour point. In this study it was clear the effect of the length of alkyl side chain of the polymeric additive in the response of the crude oil to the additive introduced.

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Antagonist Effect of Theophylline and Caffeine on Some Transaminase Enzymes Activities

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Abstract - The purpose of this study is to show the effect of caffeine and theophylline on the activities of aspartate aminotransferase AST and alanine aminotransferase ALT, in the human sera. Serum AST and ALT were activated by caffeine, while inhibiting by theophylline, this effect increased with increasing the concentration of theophylline and caffeine. Kinetic properties of AST and ALT activities revealed by theophylline and that non-competitive inhibition type, and non-competitive activation by caffeine.

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Antagonist Effect of Theophylline and Caffeine on Some Transaminase Enzymes Activities

Salma Abdul Rudhaabbass [α], Manalsadeqhammood [σ], Wafa Raji Mohammed [ρ] & Amer Hasan Abdullah [ω]

Abstract - The purpose of this study is to show the effect of caffeine and theophylline on the activities of aspartate aminotransferase AST and alanine aminotransferase ALT, in the human sera. Serum AST and ALT were activated by caffeine, while inhibiting by theophylline, this effect increased with increasing the concentration of theophylline and caffeine. Kinetic properties of AST and ALT activities revealed by theophylline and that non-competitive inhibition type, and non-competitive activation by caffeine.

I. Introduction

Theophylline and caffeine are natural compounds that are made by plants. They are classified as a member of the xanthine family alkaloid[1]. Figure (1) shows their structure:-

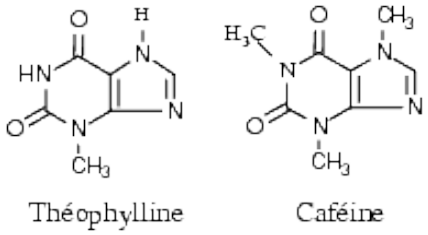

Figure (1) : Theophylline and caffeinestructure[2]

Coffee consumption is worldwide spread with few side effects. Interestingly, coffee intake has been inversely related to the serum enzyme activities gamma glutamyltransferase, and ALT in studies performed in various countries. In addition, epidemiological results, taken together, indicate that coffee consumption is inversely related with hepatic cirrhosis; however, they cannot demonstrate a causative role of coffee with prevention of liver injury figure (2) shows the negative and positive effect of caffeine [3,4].

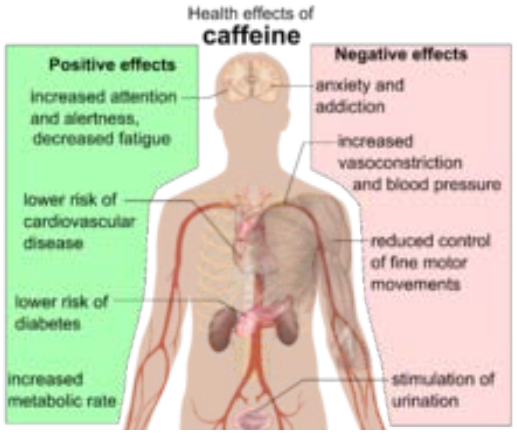

Figure (2) : Positive and negative effect of caffeine[5]

Theophylline also known as dimethylxanthine, it a beans structural and trace amounts (~1 mg/L) ,significantly less than therapeutic doses .It is found also in cocoa beans. Amounts as high as 3.7 mg/g have been reported in Criollo cocoa beans pharmacological similarity to caffeine. It is naturally found in tea, although in [6]. Theophylline and caffeine from coffee or other beverages are easily absorbed by the stomach and small intestine of ingestion, and it is rapidly distributed throughout all tissues of the body. The volume of distribution may increase in neonates and those suffering from cirrhosis or malnutrition, whereas the volume of distribution may decrease in those who are obese.[7]

Theophylline and caffeine are metabolized in the liver, through demethylation and oxidation, it forms three dimethylxanthines, and each of these metabolites is further metabolized and then excreted in the urine. Methylation caffeine is also important in the infant population. Smokers and people with hepatic impairment metabolize it differently. Caffeine causes an increase in blood flow to the kidneys and an increase in the production of urine. It also decreases the tubular reabsorption of sodium and water, resulting in more dilute urine. Caffeine stimulates skeletal muscle by increasing the strength of contraction and decreasing fatigue. It also stimulates the breakdown of glycogen and lipids to enhance endurance [8]. In view of the importance of transferees enzymes reactions like GOT, and GPT which form links between the metabolism of amino acids, carbohydrates and fats [9].

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a) How Caffeine Work

Adenosine helps prepare the body for sleep by curbing the chatter between nerve cells and by widening blood vessels to increase the flow of oxygen. Receptors on the surface of brain cells can't tell the difference between adenosine and caffeine. So when you consume caffeine, it attaches itself to the receptors and adenosine is shut out. Without adenosine to make you sleepy, your brain activity perks up and you're more alert. By blocking adenosine, caffeine also constricts your blood vessels, which makes your headache disappear [10].

b) Aim of Study

Peoples in the world consume large quantities of theophylline and caffeine through having main sources of theophylline and caffeine in general life which are: coffee, tea, chocolate, drugs…etc, therefore This study was designed to show the effects of caffeine and theophylline on some transaminase enzymes such as GOT and GPT.

II. Materials and Methods

a) Effect of theophylline and Caffeine on GOT and GPT activities

Colorimetric determination of GOT or GPT activity according to the following reactions:-

$$\text{L-Aspartate} + \alpha\text{-ketoglutrate} \xrightarrow{\text{GOT}} \text{Oxaloacetic} + \text{glutmate}$$

$$\text{Alanine} + \alpha\text{-ketoglutrate} \xrightarrow{\text{GPT}} \text{pyruvate} + \text{glutmate}$$

The pyruvate or oxaloacetate formed was measured in its derivates form 2, 4-dinitropheny-lhydraone, which was absorbed at wave length 505 nm [11] .

A-.A stock solution (0.1M) of caffeine and theophylline compounds was prepared and the following concentration of ($1x10^{-2}$, $1x10^{-3}$, $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$,$1x10^{-7}$,$1x10^{-8}$) M were prepared by diluting with distilled water.

The enzymes GOT and GPT activities were measured in human serum by using the same methods of these enzymes with replace 100µl of buffer with 100µl of compound.

The inhibition percentage was calculated by comparing the activity with and without the compound and under the same conditions, according to the equation:-

$$\% \text{ Inhibition} = 100 - 100 \times \frac{\text{The activity in the presence of inhibitor}}{\text{The activity in the absence of inhibitor}}$$

The activation percentage was calculated by comparing the activity with and without the activator and under the same conditions, according to the equation:-

$$\% \text{ Activation} = 100 \times \frac{\text{The activity in the presence of activator}}{\text{The activity in the absence of activator}} - 100$$

B- A constant concentration of compound (10^{-1}, $10^{-2}$and 10^{-3}) M were used with different substrate concentrations of (40, 80,120,160, 200) mmol/L for GOT and GPT, to study the type of inhibition .Buffers were used to prepare different substrates concentrations of these enzymes, (phosphate buffer pH 7.4, 100 mmol/L)

The enzymes activities were determined with and without compound, by using the Lineweaver-Burk equation and plotting 1/v against 1/[s] were evaluated values [12]:-

a) ki, b) Apparent v_{max} (v_{mapp}), c) Apperent k_m (k_{mapp}), d) Type of inhibition.

III. Results and Discussion

This research addresses investigation of the effects of caffeine and theophylline on GOT and GPT enzymes. The biochemical tests revealed that activatory effects of caffeine on GOT and GPT enzymes activities, while theophylline caused inhibitory effects on GOT and GPT enzymes activities ,Figures 3A, 3B, 5A,and 5B respectively .

The normal value of the GOT enzyme activity was (77 U/L). The relationship between caffeine concentration versus and the activity of this enzyme as shown in figure 3A, these results observed that any increase in compound concentrations caused increase

in percentage of activation of enzyme. The greater activation of caffeine was demonstrated at concentration (0.1M) (20.78 %) .

While the normal value of the GOT enzyme activity was (105 U/L). to theophylline results observed that any increase in theophylline concentrations caused increase in percentage of inhibition of GOT enzyme ,this relationship illustrated in figure 5A . The greater inhibition was demonstrated at concentration (0.1M) (40.9 %) .

The normal value of the GPT enzyme activity was (55 U/L). The relationship between caffeine concentration versus and the activity of enzyme as shown in figure 3B, these results observed that any increase in compound concentrations caused increase in percentage of activation of enzyme. The greater activation of caffeine was demonstrated at concentration (0.1) M was (22.72 %).

The normal value of the GPT enzyme activity was (33 U/L). The relationship between theophylline concentration versus and the activity of enzyme, these results observed that any increase in theophylline concentrations caused increase in percentage of inhibition of GPT enzyme illustrated in figure 5B. The greater inhibition was demonstrated at concentration (0.1M)(54.5 %) .

Competitive, noncompetitive and uncompetitive inhibition can be easily distinguished with the use of double reciprocal plot of the Lineweaver-Burk plot. Two sets of rate determination in which enzyme concentration was held constant, were carried out. In the first experiment the velocity of enzyme without inhibitor was established, in the second experimental constant amount of inhibitor is included in each enzyme assay. Varieties of substances have the ability to reduce or eliminate the catalytic activity of specific enzyme [13-16].

Table (1) and figure 6A, showed that the type of enzyme activation using Lineweaver-Burk plot for caffeine on serum GOT activity. The V_{max} and K_m with (10^{-1} and 10^{-8})M of caffeine and without it, V_{max} and K_m without caffeine was 67 U/L, 200 M respectively. A liquate 10^{-1} and 10^{-8} M of caffeine were non-competitive activation for enzyme activity. Non-competitive activation changed the V_{max} of the enzyme but not the K_m. When concentration of caffeine (10^{-1}, 10^{-8}) M the V_{max} were (200,100) M respectively. By using Lineweaver-Burk equation was calculated the Ki values of enzyme for compound which was studied in different concentration. The Ki of caffeine in (10^{-1}, 10^{-8}) M were (0.15, 3 x 10^{-8}) M respectively.

Table (2) and figure 7A showed that the type of enzyme inhibition using Lineweaver-Burk plot for theophylline on serum GOT activity. The Vmax and Km with (10^{-1}, 10^{-2} and 10^{-3}) M of theophylline and without it , Vmax and Km without theophylline was 125 U/L ,20 M respectively. A liquate 10^{-1}, 10^{-2} and 10^{-3}M of theophylline were non-competitive inhibition for enzyme activity. Non-competitive inhibition changed the Vmax of the enzyme but not the Km. When concentration of theophylline (10^{-1}, 10^{-2} and 10^{-3})M the V max were (76.9,90.9,111.1) U/L respectively. By using Lineweaver-Burk equation was calculated the Ki values of enzyme for theophylline which was studied in different concentration. The Ki of theophylline in (10^{-1},$10^{-2}$and 10^{-3}) M were (16x10^{-2}, 26 x 10^{-4} , 8x10^{-4}) M respectively.

Table (1) and figure (4) B1 showed that the type of enzyme inhibitor using Lineweaver-Burk plot for caffeine on serum GOT, GPT activity. The V_{max} and K_m with (10^{-1} and 10^{-8}) M of caffeine and without it, V_{max} and K_m without caffeine was 37.037U/L, 0.40 M respectively. A liquate 10^{-1} and 10^{-8} M of caffeine were competitive inhibtion for enzyme activity. Competitive inhibition changed the K_m of the enzyme but not the V_{max}. When concentration of caffeine (10^{-1}, 10^{-8}) M the K_m were (1.429, 0.714) M respectively. By using Lineweaver-Burk equation was calculated the Ki values of enzyme for compound which was studied in different concentration. The Ki of caffeine in (10^{-1}, 10^{-8}) M were (0.0388, 1.27 x 10^{-8}) M respectively.

Table (2) and figures 7A and 7B showed that the type of enzyme inhibition using Lineweaver-Burk plot for theophylline on serum GOT, GPT activity. The Vmax and Km with (10^{-1}, 10^{-2} and 10^{-3}) M of theophylline and without it, the GOT Vmax and Km without theophylline was (76.9 ,90.9 ,111.1) U/L, and (16x10-^{2},26x10^{-3},8x10^{-4}) M respectively. A liquate 10^{-1}, 10^{-2} and 10^{-3}M of theophylline were non-competitive inhibition for GPT enzyme activity, when concentration of theophylline (10^{-1},10^{-2} and 10^{-3})M the Vmax were (27.7,29.4,30.3,) U/L respectively. By using Lineweaver-Burk equation was calculated the Ki values of enzyme for theophylline which was studied in different concentration. The Ki of theophylline in (10^{-1},10^{-2} and 10^{-3}) M were (62x10-^{2},10 x10^{-2},15x10^{-3}) M respectively.

The enzymes play important role in amino acid metabolism and in the urea and tricarboxylic acid cycles. Therefore activation or inhibition of GOT and GPT enzymes may affect of metabolism of carbohydrates, proteins and lipids. So it was useful to study effect of any compound which was intake by foods or drugs on these enzymes and other enzymes that related to the metabolism. We suggested that theophylline molecule has (N-and O=) groups by which, it inhibits the active sides of GOT and GPT enzymes by decreasing affinity of active sides of enzymes to react with the substrate. The results of our study is in agreement with before studies of same enzymes[17-20], and the results is in disagreement with before study of same enzymes[21],which was study the effect of caffeine on GOT and GPT enzymes activities, the study showed activation of GOT and GPT by caffeine. Theophylline and caffeine exist in plants such as coffee, tea,cocoa beans. Theophylline and caffeine are rapidly and completely absorbed, distributedin the extracellular fluid, in the central nervous system and metabolized in the

liver [22]. Theophylline was inhibition of GOT and GPT, caffeine was activation of these enzymes in the same plant and in the same time. Therefore we suggest that the theophylline and caffeine have no effect on GOT and GPT when the plants (coffee, tea, cocoa beans) are intake.

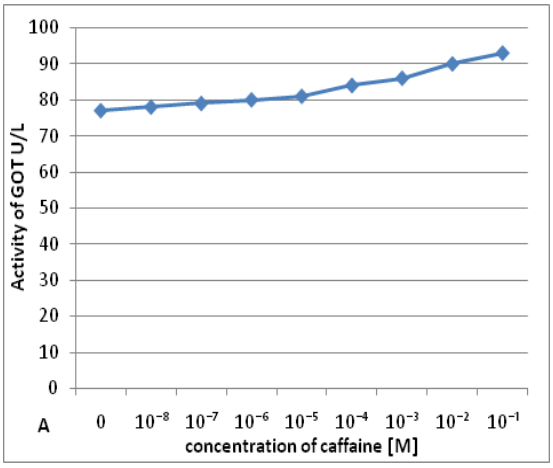
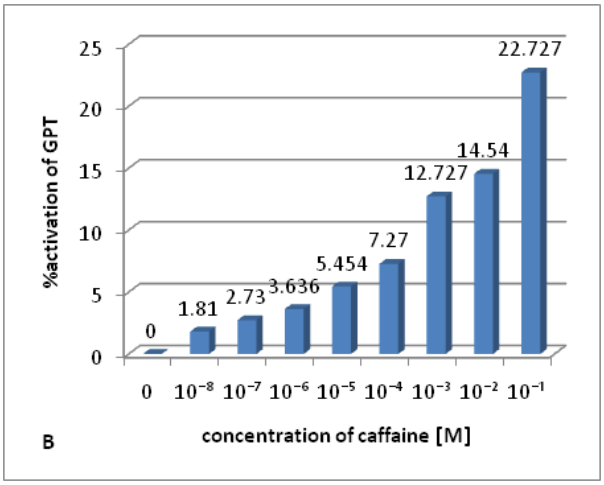
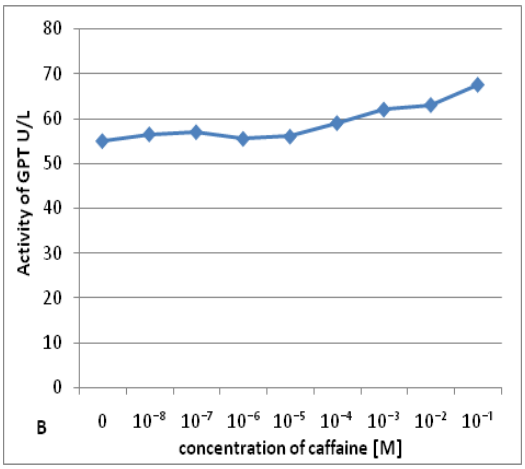


Figure (3A) : The relationship between caffeine and GOT activity



Figure(4B) : The relationship between caffeine and GPT% activity



Figure(3B) : The relationship between caffeine and GPT activity

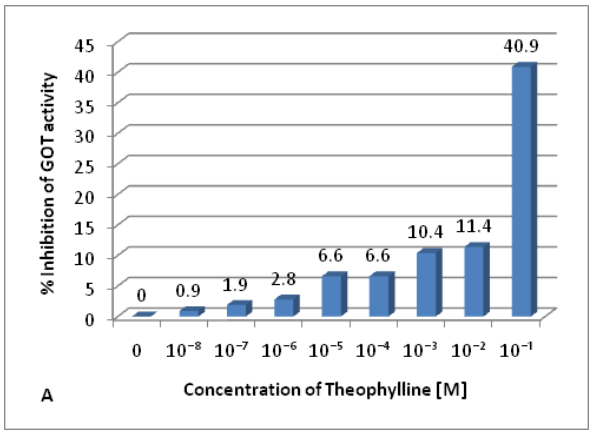


Figure (5A) : The relationship between theophylline and GOT % activity

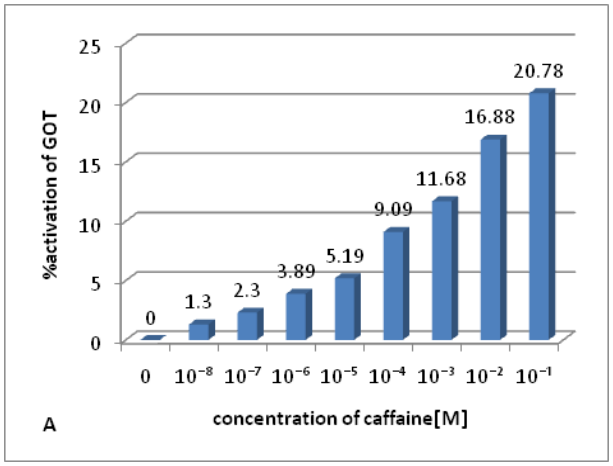
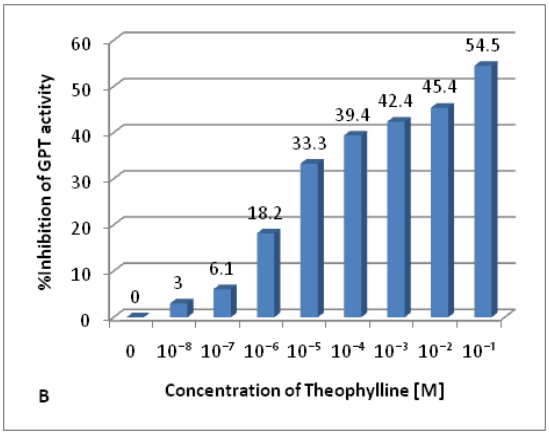


Figure (4A) : The relationship between caffeine and GOT % activity



Figure(5B) : The relationship between and theophylline GPT% activity

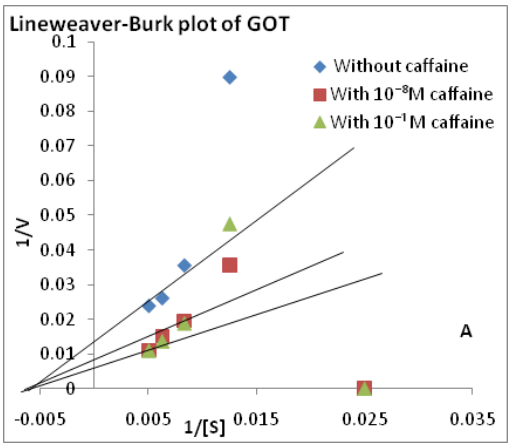


Figure (6A) : Lineweaver-Burk plots for caffeine effects on GOT

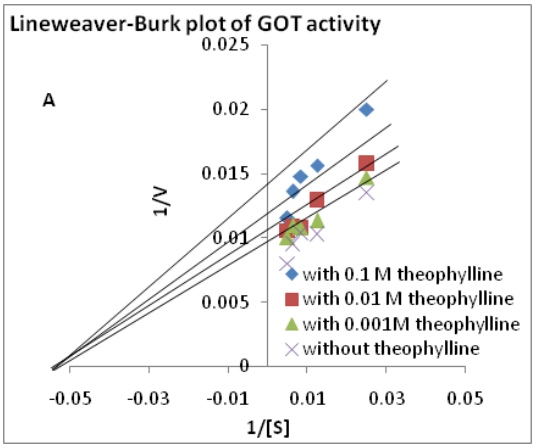


Figure (7A) : Lineweaver-Burk plots for theophylline effects on GOT

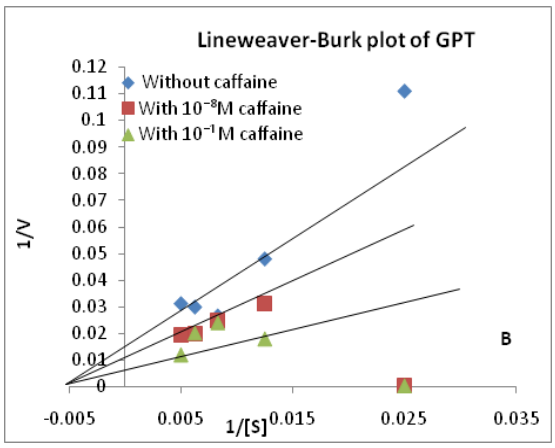


Figure (6B) : Lineweaver-Burk plots for effects on G caffeine GPT

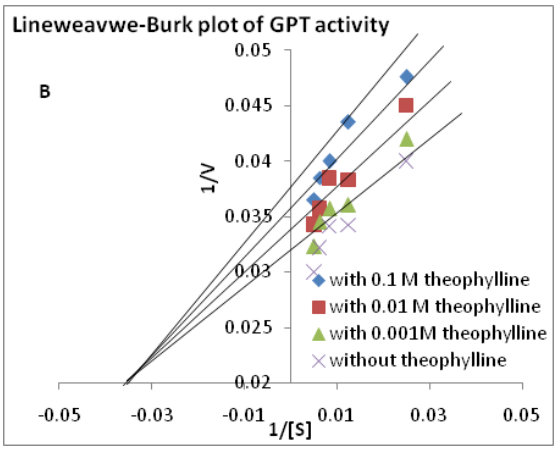


Figure (7B) : Lineweaver-Burk plots for theophylline effects on GPT

Table1 : The kinetic properties of GOT,GPT with caffeine

Enzymes	Con.of caffeine	K_{map} (M)	V map U/L	Ki (M)	Type of effect
GOT	10^{-1}	200	200	0.15	Non-competitive
	10^{-8}	200	100	3×10^{-8}	
GPT	10^{-1}	400	250	0.1666	Non-competitive
	10^{-8}	400	111.111	1×10^{-7}	

Table 2 : The kinetic properties of GOT and GPT, with theophylline.

Enzymes	Con.of theophylline	V map U/L	Ki (M)	Type of inhibition
GOT	10^{-1}	76.92	16×10^{-2}	Non- competitive
	10^{-2}	90.90	26×10^{-3}	
	10^{-3}	111.11	8×10^{-4}	

GPT	10^{-1}	27.7	$62x10^{-2}$	Non-competitive
	10^{-2}	29.411	$10x10^{-2}$	
	10^{-3}	30.30	$15x10^{-3}$	

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Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

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Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

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Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

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Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

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9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

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14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

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18. **Pick a good study spot:** To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. **Think technically:** Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

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Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

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- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
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This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
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- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

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- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

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- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

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The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
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- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

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- If you desire, you may place your figures and tables properly within the text of your results part.

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- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

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The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

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- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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