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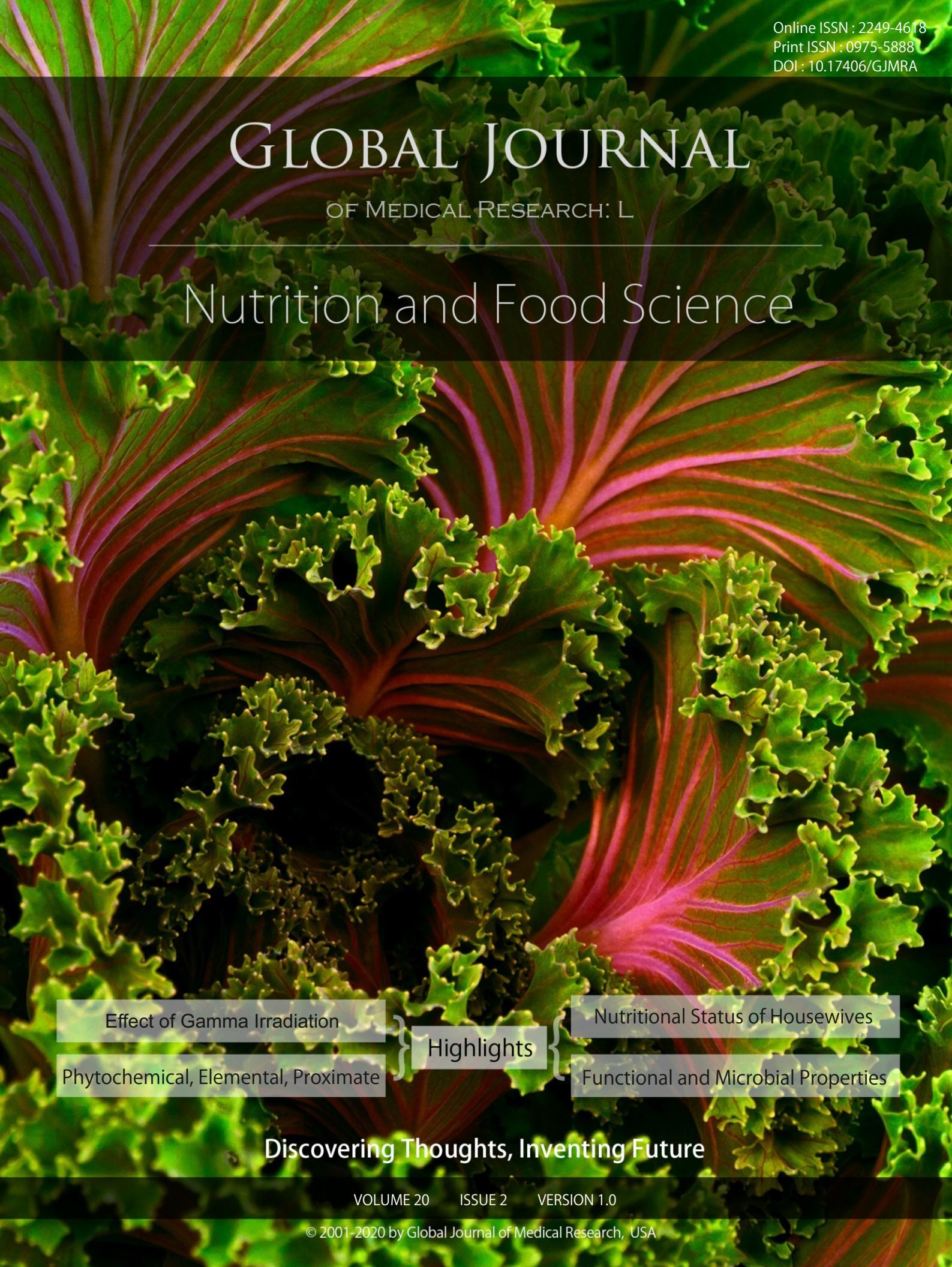

Effect of Gamma Irradiation

Phytochemical, Elemental, Proximate

Highlights

Nutritional Status of Housewives

Functional and Microbial Properties

**Discovering Thoughts, Inventing Future**

VOLUME 20 ISSUE 2 VERSION 1.0

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Nutrition & Food Science

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# Effect of Gamma Irradiation on the Pasting, Functional and Microbial Properties of Flours from white Yam (*Dioscorearotundata*) and Water Yam (*Diocoreaalata*)

By Ameh Elijah Ugbedeojo, Momoh Clement Owoicho, Gerna Dickson & Felicia AdizaIdoko
*University of Agriculture*

*Abstract-* In this study, flours prepared from white yam and water yam tubers were treated using gamma-irradiation at 0, 0.15 kGy and 2 kGy dosages, giving rise to four samples. The samples were analysed for functional, pasting and microbiological properties using standard analytical methods. The functional properties of the flour samples showed significant differences ($p<0.05$) for irradiated samples. The pasting properties of the irradiated water yam and white yam flour sample ranged from 206.11 ± 1.99 RVU to 162.44 ± 2.12 RVU for doses of 0.15 kGy and 2 kGy (peak viscosity), trough ranged from 108.54 ± 1.98to 90.68±1.56 RVU, breakdown ranged 102.18 ± 1.00 to 62.67 ± 1.87 RVU, while the pasting temperature ranged from 83.10 ± 1.10 to 77.29 RVU.

*Keywords:* *gamma, irradiation and yam.*

*GJMR-L Classification:* *NLMC Code: WB 400*

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*Keywords: gamma, irradiation and yam.*

## I. Introduction

Yam is the second most important tuber crop in Africa next to cassava. Nigeria is the main producer of yam globally with 71% of world production (FAO, 2009). Yams are annual or perennial tuber-bearing and climbing plants with over 600 species in which only few are cultivated for food and medicine (IITA, 2006). The most cultivated species in Nigeria are the white yam (*Dioscorea. rotundata*), yellow yam (*Dioscoreacayenensis*), water yam (*Dioscorea. alata*) and trifoliate yam (*Dioscorea. dumetorum*) (Amusa *et al.,* 2003). The crop is of major importance in the diet and economic life of people in West Africa (Giranidin, *et al.,* 1998). Yam is an elite crop, preferred over other root and tuber crops of West Africa and a choice during ceremonies and festivities. *Dioscorea. alata*is also referred to as greater yam. It is more important as food in West Africa and the Caribbean than in Asia and the Americas where it originated, and has been competing with the most important native species, *Dioscorearotundata*. *Dioscoreaalata* is also known for its high nutritional content (Osagie, 1992). *Dioscoreaalata* tubers have variable shapes, the majority being cylindrical. The flesh of the tuber ranges in colour from white to purplish (FAO, 2009). The texture of its flesh is usually not as firm as that of white yam and less suitable compared to other species for the preparation of the most popular food products from yam (fufu and pounded yam especially) in the West Africa region.

The production of the yam is seasonal, so storage is necessary before subsequent planting or for use as food. Fresh yam tubers are often difficult to store and are subject to deterioration by sprouting and microbial rot during storage. Post-harvest losses usually range from 25 to 60% (Afoakwa and Sefa-dede, 2001).

Loss of yam in storage is very high. Several inhibitory chemical growth regulators such as maleic hydrazide, Tetrachloronitro benzene, acetic acid, naphthalene have been used to retard sprouting in stored yam tubers. Apart from unavailability of the right type of chemicals and their toxicity nature, widespread adulteration of the available ones especially in Nigeria is a serious problem (David, 2009). Processing of yams into various products including flour is a viable alternative for checking postharvest losses and promotes year round availability. Radiation technology can complement existing technologies to ensure food security and safety (ICGFI, 1999).

Radiation processing could be used for anti-infestation and spoilage of food grains, flours and pulses; inhibition of sprouting in onions, potatoes, garlic, yam and ginger, preventing microbial contamination of spices; extending shelf-life under recommended conditions of storage of flours; and overcoming quarantine barriers in international trade. Ionizing radiations have the potentials of reducing considerable storage losses through inhibition of sprouting, inactivation of food spoilage micro-organism, control of

*Author α σ ρ: Department of Food Science &Technology, University of Agriculture, Makurdi, Benue State, Nigeria.*
*Author ω: Department of Food Science & Technology Kaduna Polytechnic, Kaduna. e-mails: momohclement86@gmail.com, amehelijah@gmail.com*

insects and sterilization of food crops. Irradiation technology is easy to apply, clean, and environment friendly. It is a direct, simple and efficient on-line process (Bansa and Appiah, 1999).

Unlike most previous studies which have focused on other species or cultivars of root and tuber crops, this study investigated the potential of applying gamma irradiation for improving the quality of two yam tuber varieties in Makurdi, Benue State. It was carried out to determine the effect of gamma irradiation on the physico-chemical and microbial properties of flours from *Dioscorearotundata* and *Dioscoreaalata*

## II. Materials and Methods

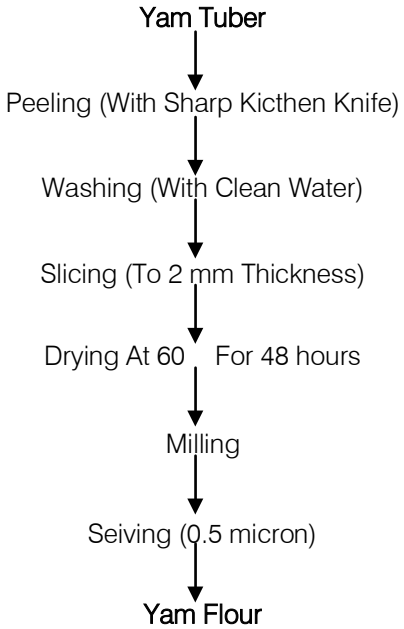
*a) Procurement of Materials*

White yam (*Dioscorearotundata*), and Water yam (*Dioscoreaalata*), were purchased from Modern market in Makurdi, Benue State, Nigeria.

*b) Sample Preparation*

Fresh White yam (*Dioscorearotundata*), and water yam (*Dioscoreaalata*) were properly cleaned and sorted to remove dirt, and other extraneous matters, before they were processed into flour. The method used was a modification of the method described by En were, (1998). The yam tuber was washed, peeled, sliced, heated and dried, which was then milled into flour. The flow diagram for the production of yam flours is shown in Figure 1.

The yam tubers were processed into flour and then subjected to gamma irradiation doses, with one un-irradiated batch to serve as control. Irradiation of samples were done using $^{60}CO$ source. Target doses were150 kGy and 2 kGy




*Source: Modified Enwere (1998)*

*Figure 1:* Yam flour processing flow diagram

## III. Analytical Methods

Pasting properties, functional properties and microbiological properties were done using standard methods as described by AOAC, 2012.

*a) Statistical Analysis*

Statistical Package for Social Sciences (SPSS) V23 computer software was used to analyze the data. Means and Standard deviation were calculated where appropriate. One way Analysis of variance (ANOVA) was used to determine the treatment that was different from others in the various parameters tested; differences were considered significant at 95% ($p<0.05$) significant level and 99% ($p<0.01$) significant level where mentioned.

## IV. Result and Discussion

*a) Effect of Gamma Irradiation on the Pasting Properties of White Yam Flour and Water Yam Flour*

Peak viscosity and trough viscosity of the two varieties of yam flours were relatively low. The values of the peak viscosity were 162.44 RVU for the white yam (*D. rotundata*) flour at irradiation dose of 2 KGy and 198.88 RVU for the control sample. Peak viscosity is the maximum viscosity attained by the paste during the heating cycle (i.e from 50 to 95°C) due to starch granules swelling and leaching out of the soluble components into the solution. It reflects the ability of starch granules to swell freely before their physical breakdown (Sing *et al*., 2003) and often correlates with product quality. Peak viscosity had values of 198.33 and 225.56 RVU for white yam flour and 162.44 and 198.88 RVU for water yam flour. The relatively longer time taken for most (*D. alata*) flour to paste further indicates stronger bonding forces in their starch granules. Yam flours generally have some level of resistance to swelling which in this case is more pronounced in *(D. alata)*. Richard *et al.* (1991) reported that cassava starch has a high peak viscosity because it exhibits a high degree of swelling. The low value of the peak viscosity in water yam (*D. alata*) and white yam (*D. rotundata*) flour indicates strong internal forces between their starch granules which resulted in lower swelling power pasting viscosity. High amylose content has been linked to low swelling power due to greater reinforcement of the internal network by amylose molecules (Hoover, 2001). Highly associated starch granules with an extensive and strongly bonded structure also exhibit resistance towards swelling. According to Richard *et al*., (1991) pasting viscosity and swelling are positively related. The higher the swelling power of a flour sample, the higher the pasting viscosity.

Kulkanir (1996), observed that (*D. alata)* flour has relatively low viscosity but high gel strength when compared with *D. rotundata* which was in agreement with the present study under irradiation. Therefore, *D.*

*alata* flour may be more suitable for products which require high gel strength and a low viscosity or they could be parboiled and processed to products such as amala. Flour with a higher peak viscosity is also required for making food products such as jelly or binder while dose with lower viscosity are desirable for preparing weaning foods and lighter gruels (Kulkani *et al.,* 1996).

Trough is considered as a measure of the breakdown of hot paste. The ability of a paste to withstand the heating and shear stress is an important factor for most food processing operations and is also a factor in describing the quality of starch gels (Madens and Christensen, 1996). High paste stability is a requirement for industrial users of starch (Bainbridge *et al.,* 1996). This is because drastic changes in paste during and after processing could lead to textural changes that may be undesirable. Trough values obtained were 96.38 RVU and 140.40 RVU for white yam (*D. rotundata*) flour also 90.68RVU and 146.21RVU for water yam (*D. alata*) flour. The *D. alata* flour had the highest trough and lowest breakdown values, which indicates greater ability to withstand shear at high temperatures and higher cooked paste stability (Farhat *et al.,* 1999). Flour with low trough values would have greater need for cross-linking than one with high value, *D. alata* flour could therefore be targeted for industrial uses because of its hot paste stability.

The breakdown viscosity increased with the increasing radiation dose in the white yam flour *(D. rotundata*) and water yam (D. alata) flour. The values ranged from 95.82±0.70 to 103.68±1.32 RVU for the white yam flour, while the water yam flour ranged from 89.92±0.50 to 109.89±0.02 RVU. Mohd *et al,* (2009) observed significant changes in breakdown viscosity by the radiation dose indicates considerable disruption or weakening of the bonding forces (hydrogen bonds) in the starch granules by the radiation. The lower breakdown of viscosity indicates good stability of the starch granules, Celik *et al.* (2004) observed a reduction in the cooking period of irradiated legumes.

The viscosity after cooling cooked paste to 50 °C is the final viscosity**.** Anonymous (1990) reported that starch paste increases in viscosity when cooled. The increase in viscosity is not only caused by simple kinetic effect of cooling but also by re-association of molecules (particularly amylose). Final viscosity is the most commonly used parameter to determine the quality of starch-based samples because it indicates starch/flour ability to form a gel after cooking.

Yam flour is thixotropic and, as observed in this study, has a higher cooled paste viscosity than hot paste viscosity. The observation supports the general fact that yam pastes form firm gels rather than viscous gels after cooking and cooling. This has implications for the kind of products yam flour could be used for, such as weaning diets or crackers. Yam flour is noted for high retro gradation during cooling and this might have accounted for the increase in final viscosities during cooling. This is brought about by the high degree of association between the starch-water systems and their high ability to re-crystallize, resulting in progressively higher viscosities during cooling of yam starches (Anonymous, 1990). Ayernor (2012) reported that the rate at which the development of rigidity occurs in yam flour is dependent on the degree of starch-water binding which can be affected through processes that influence the interaction between the flour particles and water.

Setback shows the cold paste viscosity's tendency to retro- grade. Therefore, the decreased viscosity of the irradiated samples reveals the lack of ability of amylopectin to hold the granule during the imbibition of water (Mohd *et al.,* 2009). Therefore, from the values obtained for the white and water yam flour decreasing as the irradiation dose increase show lack of ability of amylopectin to hold granules during imbibition of water.

Setback viscosity is an important factor for starch used as a food ingredient in processing and preservation, because the quality of the food's texture and physical properties deteriorate due to retro gradation as time passes (Nunoo, 2009). A decrease in setback viscosity was observed with increase in the irradiation dose. The lower setback viscosity of the paste indicated that the reassociation of the starch polymers is not as great as in the control. Pomeranz (1991) established that high setback (retrogradation) will cause undesirable gel texture or high freeze-thaw stability. This means the protein and starch in the flour obtained from the two irradiated yam flour would be unable to form strong gel matrices or networks, perhaps, due to poor ionic interactions between the individual molecules. Gamma irradiation is capable of hydrolyzing chemical bonds, there by cleaving large molecules of starch into smaller fragments of dextrin that maybe either electrically charged or uncharged as free radicals (Wu *et al*., 2002). These changes may affect the physical and rheological properties of irradiated starches, resulting in changes in properties of starch paste (Mohd*etal.,*2009). Decreases in pasting properties, such as breakdown and setback values, may result primarily from irradiation-induced starch degradation and may present opportunities such as ease of cooking and reduced starch retrogradation, respectively (Sabularse *et al.,* 1991).

There was a reduction in the peak temperature among the two varieties of yam flour, although the reduction was not dose dependent as there were little variation in the values.

The peak temperature for the white yam (*D. rotundata*) flour with irradiation doses of 0.15 kGy and 2 kGy had a reduced value whereas in the water yam (*D. alata*) flour, the minimum peak temperature was observed in the 2 KGy irradiated samples. It can be deduced that irradiation caused the reduction. The

reduction will result in low temperature and time of cooking of the flours from irradiated yam flour and temperature may be prepared when all other properties are equal.

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*b) Effect of Gamma Irradation on the Functional Properties of White Yam flour and Water Yam Flour*

In this study, it was observed that water absorption capacity (WAC) and oil absorption capacity (OAC) of the two varieties of yam flours were significantly ($p<0.05$) influenced by the irradiation.

Significant increase in water absorption capacity in the irradiated white yam (*D.alata)* and water yam (*D. rotumdata*) was observed, the control of white yam flour had the lowest value for the oil absorption capacity and the irradiated water yam flour at 2 kGy had the highest value of water absorption capacity 1.93ml/g. Abu *et al*., (2006a,b) observed similar trends. This suggests that the irradiation increases water affinity of resulting flours. The increase in the WAC may in part be due to irradiation-induced damage or degradation of cowpea starch to simpler molecules such as dextrins and sugar that have higher affinity for water than starch (Abu *et al.,* 2006a,b). High WAC of flour is advantageous in the preparation of food items like bread and sausages to maintain freshness and for easy handling. Oshodi and Adeladun (1993) reported that high WAC of seed flour is useful in the preparation of soups and gravies. The phenomenon could be attributed to a degree of cross-linking, which may have occurred simultaneously with chain scission at the doses (1.0 and 1.5 KGy) hence the observed results. Similar results were reported by Abu *et al.,* (2006b).

OAC is an important functional trait in food industries for retention of flavor and improvement of shelf-life and palatability (Bhat *et al.,* 2008). The OAC of the flour obtained from the irradiated samples of yam flours of the white yam (*D. rotundata*) and water yam (*D. alata*) were higher than the control samples. This was in agreement as reported by Abu *et al. (*2006a,b) that OAC of cowpea irradiated increased as doses increased. The increase in the OAC may be related to the radiation. The mechanism of the increase in the OAC upon irradiation involves the increased ability of degraded and/or cross-linked starch to physically entrap more oil and the high affinity of non-polar protein side chains for lipids (Salbe *et al.,* 1982). As reported by Urbein, (1986) increase in OAC of seed flour has been attributed to the unmasking of non-polar protein residues as a result of irradiation-induced denaturation. White yam (*D. rotundata*) and water yam (*D. alata*) flour had relatively significant lowest OAC at 0.15 kGy with 1.11±0.01 ml/g.

Water solubility index of the irradiated samples was significantly lower than the control across the two varieties of yam flour. This is an indication that the flours obtained from irradiated yam flour samples were less soluble in water to an extent. The irradiation may have cused the soluble substances in the flour to idolize making their binding sites for water inaccessible. Medoua *et al.,* (2005) reported that the decrease in water solubility could be due to the mobilization of soluble substances. The water solubility of the yam flours for both white yam (*D. rotundata*) and water yam (*D. alata*) decreased significantly with the radiation doses but was not dose dependent. The values for control for both yam flours ranged from 8.58±0.02 ml/g and 6.15±0.03 ml/g as compared to the irradiated samples with values of 7.5604±1.04 ml/g for white yam flour and 5.13±1.31 ml/g for water yam flour. This result was similar to those observed by Azim*et al.*(2009). Abu *et al.* (2006a,b), however, observed reduction in WSI with increasing radiation dose.

Bulk density of the white yam (*D. rotundata*) and water yam (*D. alata*) flour of irradiated or non-irradiated were not significantly different ($p< 0.05$) from each other, even at 2 kGy the bulk density was not significantly different, which may not be attributed to the irradiation. This means that the bulk density of the yam flour was not significantly ($p<0.05$) affected by the irradiation. Abu *et al.,* 2006 a, b. Azim *et al.* (2009) reported that the bulk density of groundnut flour was not affected significantly by gamma irradiation.

Reconstitution index of the yam flour samples were not significantly ($p< 0.05$) different from the irradiated and non-irradiated from each other. The control values for the white yam (D. alata) and water yam (D. rotundata) flour ranged between 6.76±0.02 ml/g and 6.09±0.02 ml/g as compared with the irradiated samples with values of 6.40±0.01ml/g and 5.58±0.01 ml/g. though statistical differences were observed in the values obtained for reconstitution index in the white yam flour, their functionality in sample may not differ.

*c) Effect of Gamma Irradiation on the Microbial Content of White Yam Flour and Water YamFlour*

The microbial analysis for the white yam sample for aerobic plate count for the control was $1.93x10^3$cfu/ml and that for water yam sample was 2.4 $x10^3$. The bacteria present in the samples before irradiation would have been picked up during processing (Jay *et al*., 2005), but the low moisture content (due to the flour nature) might have prevented the growth of some bacteria even before irradiation and when there is low moisture in stored products, *Bacillus* species are usually the one ones that grow (Jay *et al*

2005); this might have accounted for the low presence of *Bacillus* species in the flour samples after irradiation. Most of the bacteria isolated from the flour samples (white yam and water yam) before irradiation have been obtained by previous workers (Ojokoh and Gabriel, 2010). Report of occurrence of many *Lactobacillus* and *Bacillus* species were found to be dominant during the production of a fermented cassava product (Achi and Akomas, 2006). The same situation was obtained for the fungi whereby the samples contained less fungi qualitative after irradiation than before irradiation. *Aspergillusniger* which was the most predominant fungus in the samples before irradiation. This should be desirable because *A. niger* is one of the aflatoxin-producing moulds (Frazier and Westhoff, 2004) whose presence in foods will constitute health hazard to consumers. *A. flavus* and *A. niger* which are sporadic (Adegoke, 2004). Toxigenic strains of *A. flavus* have been known to produce aflatoxin, a potent hepatotoxic and carcinogenic agent (Uraih and Ogbadu, 2019).

## V. Conclusion

The pasting temperature was not significantly affected by gamma irradiation which indicates the ability to withstand shear stress and high temperature. Gamma irradiation n reduced significantly bacteria and fungi count in the two varieties of yam flour, thereby lowering the activity of micro-organism. The use of gamma irradiation in the treatment of water yam flour and white yam flour showed that irradiation can preserve and maintain the quality of yam flour

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# Phytochemicals, Elemental, Proimate Analysis and Anti-Nutrient Composition of *Citrus Aurantifolia* Seeds

By Ezekiel T. Williams, Buba Julius & Nachana'a Timothy
*Adamawa State University Mubi*

*Abstract-* In this study the phytochemicals, elemental, proximate analysis and anti-nutrient composition of citrus aurantifolia seeds was investigated. Fresh matured and unaffected fruits of citrus aurantifolia was bought in Mubi market. They fruits were cut opened and the seeds were removed, dried and pulverized it to powder. Phytochemical composition, proximate analysis and anti- nutrients in seed were determined using standard laboratory procedure. While elemental composition were determined using flame photometer and atomic absorption spectrophotometer. The result of the phytochemical analysis revealed that alkaloids, flavonoids and polyphenols were highly present.

*Keywords:* *phytochemicals, elemental, proimate, anti-nutrient, citrus aurantifolia and seeds.*

*GJMR-L Classification:* *NLMC Code: QU 145*


*Strictly as per the compliance and regulations of:*

# Phytochemicals, Elemental, Proimate Analysis and Anti-Nutrient Composition of *Citrus Aurantifolia* Seeds

Ezekiel T. Williams [α], Buba Julius [σ] & Nachana'a Timothy [ρ]

*Abstract-* In this study the phytochemicals, elemental, proximate analysis and anti-nutrient composition of *citrus aurantifolia* seeds was investigated. Fresh matured and unaffected fruits of *citrus aurantifolia* was bought in Mubi market. They fruits were cut opened and the seeds were removed, dried and pulverized it to powder. Phytochemical composition, proximate analysis and anti- nutrients in seed were determined using standard laboratory procedure. While elemental composition were determined using flame photometer and atomic absorption spectrophotometer. The result of the phytochemical analysis revealed that alkaloids, flavonoids and polyphenols were highly present. The result of proximate analysis showed that Moisture (12.54 ± 0.02 %), crude fibre (5.01 ± 0.01%), crude fat (29.22 ± 0.04 %), ash content (6.05 ± 0.00 %), protein (8.78 ± 0.01 %) and carbohydrate (37.95 ± 0.05 %) were present in *C. aurantifolia* seed. Also the result of anti- nutrients revealed that Oxalates (3.45±0.01 mg/100g), Phytates (7.49 ± 0.02 mg/100g), Tannins (6.13 ± 0.02 mg/100g), Lectins (0.28 ± 0.01 mg/100g) and Saponins ( 9.45 ± 0.02 mg/100g) were also present. The result of the mineral analysis also show the presence of Ca, Fe, P, Zn, Mg, Na, Se. The results obtained indicate that *C. aurantifloia* seed could be good for human consumption. Also the seed could be used as a source of minerals in diet and source of drugs in pharmaceuticals industries.

*Keywords: phytochemicals, elemental, proimate, anti-nutrient, citrus aurantifolia and seeds.*

## I. Introduction

Herbal medicine is currently in demand and their popularity is increasing day by day. In the health care sector, WHO recommends and encourages the use of traditional herbs or remedies because huge amount of raw materials easily available. Plants are very complex in nature; their therapeutic activity varies according to species, geographical location and harvesting processes. Improper authentication of herbs, adulteration by micro-organisms, pesticide residue has made standardization of herbal drug of primary importance. According to the world health organization, the macroscopic and microscopic description of a medicinal plant is therefore step towards establishing the identity and the degree of purity of such material and should be carried out before any test are undertaken (WHO, 2013).

Many important drugs used in medicine today are directly or indirectly derived from plant. The most important of this bio active constituent of plants are alkaloids, tannins, steroids, terpenoids and phenolic compounds (Mohammed *et al*., 2016).

Several published report had describe the anti-microbial activity of various crude extract of plant either in single or in combination (Igoli *et al.,* 2005). It has been estimated that about 2.5 million species of higher plants and their therapeutic values are yet to be determined. However, herbal extract are becoming popular as natural medicine, preservatives and additives (Cox *et al.,* 2010). According to WHO (2002) medicinal plants contain substances in one of its organs such as stem, roots, leaves, rhizomes, fruits, flower and seed that can be used for therapeutic purposes or which are precursor for chemo- pharmaceutical semi- synthesis.

*Citrus aurantifolia* (christm) swingle (C. aurantifolia) is a polyembryonic plant cultivated in many countries all over the world and grows in hot sub - tropical or tropical regions (Akinwunmi and Omotayo, 2016). *C. aurantifolia* is a small shrubby tree, about 5 m tall. It is an ever green an ever daring tree that is densely and irregularly branched and possess short and stiff spumes (thorn). The leaves are alternating elliptical to oblong-obviate (4–8 cm by 2–5 cm) shaped and has a crenulated margin. The fruit are globose to ovoid berry of about 3–6 cm in diameter and sometimes have apical papilla. It is yellow when ripe but usually picked green commercially. The fruit and flower appear throughout the year but are most abundant from May to September in the Northern hemisphere. The fruits peels are very thin with densely glandular segment with yellow –green pulp vesicles. The fruit juice is acidic and fragrant, sour as lemon juice but more aromatic. It is usually valid for its unique flavor compared to other lime. The seed are small, plump, ovoid, pale and smooth with white embryo (Quilly *et al*., 2017; Golob, 2013; Okwu, 2008).

*C. aurantifolia* in its natural state is widely used in west Africa particularly in Nigeria where it is employed in herbal medicine to treat several illness, it forms an essential ingredient in the preparation of most herbal concoctions' (Aibinu *et al* ., 2007). Different parts of the

*Author α σ ρ: Department of pure and applied chemistry, faculty of science, Adamawa State University Mubi, Nigeria.*
*e-mail: tagwiezekiel@gmail.com*

tree have been used traditionally to cure some illness: the decoction pounded leaves is drunk for stomach ache, used as eye wash and to bath feverish patient. Poultice of leaves are applied to ulcer wounds, used for skin diseases and also applied to abdomen after child bath. Crushed leaves are applied to fore head to treat headache and it is squeezed near the nostril for irritant inhalation to treat nausea and resuscitate fainting individual (Rwarinda, 2016). Infussion of *C. aurantifolia* leaves have been given to treat fever with jaundice, sore throat and oral thrush (Mustafa, 2016). A decoction of the flower is believed to help to induce sleep for those with insomnia (Quilly *et al.,* 2017). Decoction of roots is used to treat dysenthery, diarrhea, colic, gonorrhea and fever (Mustafa, 2016). In Nigeria *C. aurantifolia* fruit juice is added to sugar and palm oil or honey to relief cough. In Malayan medicine, the juice is considered as tonic for elibido and as antidote for poison. It is also used to increase stamina, treat dysfunctional uterine bleeding, used as facial wash to rejuvenate the skin and remove stain (Williams *et al*., 2019; Mariana *et al.,* 2017).

The fruit *C. aurantifolia* is of unique economic importance as all portions contain potentials for diverse industrial usability (Oikeh *et al.*, 2013). Those far researches into Citrus wastes have been concentrated on the peels (flavedo) with little interest in the seeds. This study aims to draw attention to the possible waste – to – wealth utilization of *C. aurantifolia* seed with added advantage of providing novel sources of nutraceuticals, phytochemical and pharmaceuticals by investigate the phytochemical, elemental, proximate and anti- nutrient composition of *C. aurantifolia* seed.

## II. Materials and Methods

*a) Sample collection and identification*

The matured ripped unaffected fruit of *C. aurantifolia* were bought from Mubi market, identified and authenticate by Baba Yahaya Kirri in the Department of crop production Adamawa State University Mubi.

*b) Sample preparation*

The matured ripped an unaffected fruit were cut opened and the seed were removed then dried and pulverized in powder.

*c) Chemicals and reagents*

All Chemicals and reagents used were of analytical grade

*d) Preparation of the extract*

Twenty five grams (25 g) of powdered seed were extracted separately in a soxhlet apparatus and solvent were removed. The percentage yield was determined by following the method described by Harborne (1998): The yield percentage=weight of extract recovered x 100 / weight of dry powdered and the extract was used for phytochemical screening, proximate analysis, anti- nutrient and elemental composition.

*e) Elemental Analysis*

The mineral content of the sample were determined using atomic absorption spectrophotometer and flame photometer following the procedure adopted by AOAC (2000)

*f) Phytochemical screening*

*Citrus. aurantifolia* seed was tested for the presence of bioactive compounds. The phytochemicals of the seed samples were estimated following the procedure adopted by Williams *et al*. (2020 a).

*g) Test for Terpenoids*

Organic extract of 2 ml was dissolved in 2 ml of chloroform and evaporated to dryness. Concentrated sulphuric acid of 2 ml was added and heated for two minutes. A grayish color was observed.

*h) Test for Flavonoids (Alkaline Reagent Test)*

Extract of 200 mg was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour formed which turned colorless on addition of few drops of diluted acid was observed.

*i) Test for Steroids*

To 2 ml of acetic anhydride was added 0.5 g of the sample followed by an addition of 2ml $HR_2RSOR_4R$. The color changed from violet to blue green was observed (AOAC, 2003).

*j) Test for Alkaloids*

Extract of 200 mg was mixed with 10 ml of methanol. To 2 ml of the filtrate was added 1% HCl and then steamed. To 1ml of the filtrate was added 6 drops of Wagner reagent. Brownish-redprecipitate was observed.

*k) Phenol*

A small amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

*l) Proximate analysis*

The proximate composition (moisture, crude fibre, crude fat, ash content, protein and carbohydrate) of powdery sample of C. aurantifolia was determined following the method described by AOAC (2005).

*m) Anti-nutritional Content Analysis*

The anti-nutrient contents (oxalates, phytates, tannins, lectins, saponins and glycosides) were determined using high performance liquid chromatography (HPLC) following the procedures adopted by AOAC (2003).

*n) Statistical Analysis*

All determinations were replicated three times and results were reported in mean (±) standard deviation.

## III. Results and Discussion

*Table 1:* The result of elemental analysis of C. aurantifolia seed (mg/100g)

| Element | Concetration |
|---|---|
| Ca | 162.36 ± 0.03 |
| Fe | 4.12 ± 0.01 |
| P | 108.16 ± 0.02 |
| Zn | 1.76 ± 0.01 |
| Na | 72.34 ± 0.02 |
| Mg | 73.63 ± 0.03 |
| Se | 0.22 ± 0.01 |

The elemental composition of *C. aurantifolia* seed were shown in Table 1. It contains considerable amount of Zn (1.76 ± 0.01), Fe (4.13 ± 0.01) and Se (0.22 ± 0.01). Williams *et al.* (2020 a) reported that Zn is vital in protein synthesis, cellular differentiation and replication, immunity and sexual functions. Fe facilitates the oxidation of bio molecules to control obesity which pre disposes an individual to various diseases. It is also essential for hemoglobin formation (Thomas and Krishan kumari, 2015) and plays a role in energy transfer within the plant and also an essential constituent of certain enzymes and proteins. This justifies the use of *C. aurantifolia* seed in folklore medicine as blood tonic because of its blood boosting effect (Njoku – oji *et al.,* 2016). Selenium serves as bioactive constituent present in the *C. aurantifolia* seed due to its therapeutic properties.

Moderate quantity of Na (72.34 ± 0.02) and Mg (73.63 ± 0.03) were present in the *C. aurantifolia* seed and these are principal cat ions of extracellular and intracellular fluids and aid in maintaining electrolyte balance in the body (Mustafa, 2016). In humans, Mg is required in the plasma and extracellular fluid, where it helps maintain osmotic equilibrium (Thomas and Krishnakumari, 2015). It can also prevent some heart dis order and lower blood pressure in human.

Minerals found to be present in high amount are Ca (162.36± 0.03) and P (108.16 ± 0.02), Ca reported to be essential for blood clotting, bone and teeth formation and as a co factor in some enzyme catalysis (Mustafa, 2016). Phosphorous maintain blood sugar levels and normal heart contraction (Williams et al., 2020b). It is also important for normal cell growth and repair, bone growth and kidney function. It plays an important role in maintaining the bodies' acid–alkaline balance (Ramadass and Subramanin, 2018).

*Table 2:* Result of the phytochemical screening of *C. aurantifolia*

| Phytochemical | Qualitative | Quantitative (mg/100g) |
|---|---|---|
| Alkaloids | + + + | 12.46 ± 0.03 |
| Flavonoids | + + + | 8.42 ± 0.01 |
| Polyphenols | + + + | 19.87 ± 0.03 |
| Terpenoids | + | 0.87 ± 0.01 |
| Steroids | + | 0.42 ± 0.01 |

+ = present
+ + = moderate present
+ + + = highly present

The result of the phytochemical screening of *C. aurantifolia* seed was presented in Table 2.The result revealed that Alkaloids, Flavonoids, Polyphenols, Terpenoids and Steroids were present in *C.aurantifolia* seed.

Alkaloids was highly present (12.46 ± 0.03 mg/100g) in the *C. aurantifolia* seed. The presence of alkaloids supports the findings of Oyeleke *et al. (*2008), that the microbial activity of this plant may be attributed to the presence of alkaloids. Alkaloids have been reported to possess various pharmacological activities including antihypertensive effects, antiarrhythmic effect, antimalarial and anticancer activity (Saxena *et al.,* 2013). Pure isolated alkaloids and their synthetic compounds have been used in medicine as an analgesic, antispasmodic and bactericidal agents (Williams *et al*. ,2019; Zida et al., 2016).

Flavonoid was found to be highly present (8.42 ± 0.01 mg/100g) in *C. aurantifolia* seed. Flavonoids have an influence on arachidonic acid metabolism and have been known to be a good antimicrobial agent against a wide array of micro- organism. This activities may be due to the ability of flavonoids to form complexes with extracellular and soluble proteins and to complex with bacteria cell walls (Tona *et al*., 2001); Ayoola, 2008).

Also Polyphenols was highly present (19.87 ± 0.03 mg/100g) in *C. aurantifolia* seed. Polyphenols are generally germicidal, usually used in formulating disinfectant and some possess estrogenic or endocrine disrupting activity. They are also the active ingredient in species that contribute to it flavor, taste and medicinal properties (FAO/WHO, 2011). Hence, it can be said that *C. aurantifolia* seed has antioxidative, anticarcinogenic, anti inflamentory, antiallergic, antithrombotic and antibacterial effect.

Terpenoids was present (0.87 ± 0.01 mg/100g) in *C. aurantifolia* seed. The presence of terpenoids supports its use in the treatment and manages of cancer, ulcers and malaria. Plants produce volatile terpenes either to attract specific insects for pollination or otherwise to expel certain preys which consume this plant as food (Akinwunmi and Omotayo, 2016). In addition, terpenoids possess medicinal perties such as anti-carcinogenic, antimalarial, antiulcer, antimicrobial or diuretic activity (Mariana *et al*., 2017).

Steroids also was present (0.42 ± 0.01 mg/100 g) in C. auranifolia seed. Steroids are phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs (Timothy, 2018). Trace amount of steroids compound in the seed could be useful in promoting nitrogen retention in osteoporosis and in animals with wasting illness (Maurya *et al*., 2008; Madziga *et al*., 2010).

*Table 3:* Result of the proximate analysis of C. aurantifolia (%)

| Composition | Value |
|---|---|
| Moisture | 12.54 ± 0.02 |
| crude fibre | 5.01 ± 0.01 |
| crude fat | 29.22 ± 0.04 |
| ash content | 6.05 ± 0.00 |
| protein | 8.78 ± 0.01 |
| carbohydrate | 37.95 ± 0.05 |

The result of the proximate analysis was presented in Table 3. The result revealed that moisture, crude fibre, crude fat, ash, protein and carbohydrate was present in *C. aurantifolia* seed The moisture content recorded was 12.54 ± 0.02 %. The low moisture content of the seed suggests it can be stored for long periods of time after harvest. The crude fibre (5.01 ± 0.01 %) of the seed could aid in the absorption of trace element in the gut and therefore increases intestinal bowel movement (Abolaji *et al.,* 2007). Consuming vegetables in the diet could aid in managing constipation problems (Olowokuedjo *et al.,* 2008). Dietary fibres also lower cholesterol, triglycerides and protect against carncer and disgestive disorder (Ubwa *et al.*, 2015). The crude fat (29.22 ± 0.04 %), the ash content (6.05 ± 0.00 %) of C. aurantifloria seed provide a measure of total amount of mineral matter in the seed. Measuring ash content is important because mineral matter may the cause of a pharmacological effect (Ubwa *et al.,* 2015). The protein (8.78 ± 0.01 %) higher content recorded in *C. aurantifolia* can serve as reason for its use in malnourished communities. Protein vital for various body functions such as body development, maintence of fluid balance, formation of hormones, enzymes and sustaining strong immune function (Emebu and Anyika, 2011). The estimated carbohydrate content (37.95 ± 0.0 %5) in the *C. aurantifolia* seed was high and carbohydrate are known to produce energy require for the body because they are essential nutrients for adequate diet and supplies energy to cells such brain, muscle and blood (Ejelonu *et al.*, 2011).

*Table 4:* The result of the anti - nutrient analysis of the *C. aurantifolia*

| Anti - nutrient | Concentration (mg/100g) |
|---|---|
| Oxalates | 3.45 ± 0.01 |
| Phytates | 7.49 ± 0.02 |
| Tannins | 6.13 ± 0.02 |
| Lectins | 0.28 ± 0.01 |
| Saponins | 9.45 ± 0.02 |
| Glycosides | 4.12 ± 0.01 |

The result of the anti-nutrientanalysis of *C. aurantifoia* seed was presented in Table 4, the result revealed that Oxaletes, Phytates, Tannins, Lectins, Saponins and Glycosides were present in *C. aurantifolia* seed.

The oxalates (3.45 ± 0.01 mg/100g) in *C. aurantifolia* seed is a concern because of its negetaive effect on mineral availability. High oxalate diet can increase the risk of renal Ca absorption and has been implicated as kidney stones (Igidi and Edene, 2014; Williams *et al* 2018). Phytates (7.49 ± 0.02 mg/100g) often considered an anti-nutrient because it binds minerals in the digestive tract, making them less available to the body. Yet this same anti-nutrient property can also help in the prevention of chronic disease. Tannins were present in the seed (6.13 ± 0.02 mg/100g). Tannins are known to possess anti- microbial activities because of that it plays an important role in wound healing (Zida *et al.,* 2016). Lectins (0.28 ± 0.01 mg/100g) in *C. aurantifolia* seed are type of protein that can bind to cell membranes. They are sugar binding and become they "glycol" portion of glyconjugates on the membranes. Lectins offer a way for molecules to tick together without getting immune system involved, which can influence–cell interaction. Saponins (9.45 ± 0.02 mg/100g) in *C. aurantifolia* seed, Saponins in fruit and vegetables are important dietry supplements and are known to exhibit antimicrobial activity and protect plant from microbial pathogens (Mohammed *et al*., 2016). They could be beneficiary in modulating blood lipids, lower cancer risk and improve blood glucose responsible as well as possess antioxidant activity (Igidi and Edene, 2014). Glycosides (12±0.01 mg/100g) in *C. aurantifolia* seed could be used as flavoring agents in pharmaceutical preparation (Sarkere and Nahar, 2007). Therefore the presence of glycoside in the seed of *C. auratifolia* support its pharmacological use as flavoring agents and in management of cancer.

## IV. Conclusion

In conclusion the study revealed the presence of phytochemicals, elemental composition, the proximate and anti- nutrient composition in *C. aurantifolia* seed which are nutritional requirement for both human and livestock and can be useful as feed supplement to improve health and growth performance of human and livestock and can also be used in the pharmaceutical industries for the production of drugs.

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# Serum Biochemistry Profile, Haematological Indices and Body Weight Gain of Albino Rats Fed Cookies Produced from Wheat (*Triticum spp*), Supplemented with Soyabeans (*Glycine Max*) and Pro-Vitamin a Cassava (*Manihot Utilisima Crants*) Flour Blends

By Momoh Clement Owoicho, Aliyu Juliet Abibat & Ikya Julius
*University of Agriculture*

*Abstract-* In this study, the serum biochemical, haematological indices and body weight gain of albino rats fed cookies produced from, supplemented with soya beans and pro-vitamin A cassava flour blends were determined, A preliminary work was carried out to ascertain the optimal acceptable level of soybeans using 0 to 20% (w/w) addition to wheat flour cookies production. Soybeans yielded the most acceptable cookies at 10% (w/w). Consequently in the main study, the level of soybean was maintained at 10% (w/w) while wheat and pro-vitamin A cassava were varied in the ratios of 100:0:0, 80:10:10, 70:10:20 and 60:10:30 giving a total of four samples. The cookies produced by standard methods were fed to 20 albino rats for thirty (30) weeks and were characterized for serum biochemistry profile, haematological indices and bodyweight gain using standard analytical methods.

*Keywords:* *cookies, haematological, biochemistry and weight.*

*GJMR-L Classification:* *NLMC Code: QV 180*

*Strictly as per the compliance and regulations of:*

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*Keywords: cookies, haematological, biochemistry and weight.*

## I. Introduction

Cookies are nutritive snacks produced from unpalatable dough that is transformed into appetizing product through the application of heat in an oven (Anozie, 2014). Cookies are traditionally made from soft wheat and are nutritious and convenience foods with long shelf life. Soft wheat flour has been the major ingredient used in the production of cookies and other pastry products, but they can also be made with non-wheat flours such as sorghum, maize, pearl millet, acha grain, Soybean etc. (Okpala, 2013). In Nigeria, reliance on wheat flour in the bakery industries has over the years restricted the use of other cereals and tuber crops available for domestic use (Racheal and Margaret, 2016).

In recent years, government has through intensive collaboration with research institutes encouraged the use of composite flours in the production of cookies and related food products such as bread. The adoption of these locally produced flours in the bakery industry increased the utilization of indigenous crops cultivated in Nigeria and also reduces the cost of bakery products (Ayo and Gaffa, 2002).Wheat grains are relatively low in total protein and vitamins (Vitamin A), generally low in lysine and certain other amino acids, which could be supplemented by the use of soybeans and Pro-vitamin A cassava in cookies production (Agu, 2007). The most obvious result of such blending is that the mixture is higher in protein than the cereal component alone. Soybeans usually improve the quality of cereal protein by supplementing them with limiting amino acids such as lysine and sometimes tryptophan and threonine (Chinma, 2012). Soybean *(Glycine max)* a grain legume, is one of the richest and cheapest sources of plants protein that can be used to improve the diet of millions of people especially the poor and low income earners in developing countries because it produces the greatest amount of protein used as food by man (Lui, 2004). It is also known to be a good source of the trace elements copper, zinc and manganese (Ampofo, 2009). It is the only source that contains all essential amino acids such as Lysine, tryptophan etc. Protein content of soybean is about two times that of other pulses, four times that of wheat, six times that of rice grain (Akubor, 2005). Pro-vitamin A Cassava (*Manihotesculenta Crantz*) is currently been used as an aid in reducing the prevalence of dietary

*Author α: Department of Food Science & Technology, University of Agriculture, Makurdi, Benue State, Nigeria.*
*e-mail: momohclement86@gmail.com*

Vitamin A deficiency (VAD) due to its high content of B-carotene (Sogo *et al.,* 2016) Haematology refers to the study of the numbers and morphology of the cellular elements of the blood, and this results is used in diagnosing and monitoring of disease (Merck, 2012). Haematological indices generally provide information on inflammation, various infections of the visceral organs and the presence of stress factors (Jurick *et al.,* 2007). Haematological Indices i. e the packed cell volume (PCV) is an index of toxicity factor in the blood and it suggests presence of toxic factors in the food eaten which has adverse effect on blood formation (makinde, 2016). RBC serves as a carrier of haemaglobin, the haemoglobin in turn react with oxygen in the blood during respiration to form oxy-haemaglobin (Johnson and Morris, 1996). White blood cells (WBC) performs a defensive actions in the body. These indices are necessary to be able to conclude that the cookies produced does not have adverse effect on the rat being fed. Serum biochemistry refers to the chemical analysis of the blood serum (Dessouky, 1992). nvestigation of blood serum constituents can provide valuable benefits and indication about the general health of animals and humans (Dessouky, 1992). Observation of a deviation of certain blood parameters from their normal limits could be an indication for diagnosis or differential diagnosis of a diseased condition (Dessouky, 1992). Information on the effect of these selected food products on the serum biochemical profile and haematological indices of albino rats is limited hence the impetus of this study.

## II. Materials and Methods

*a) Procurement of Materials*

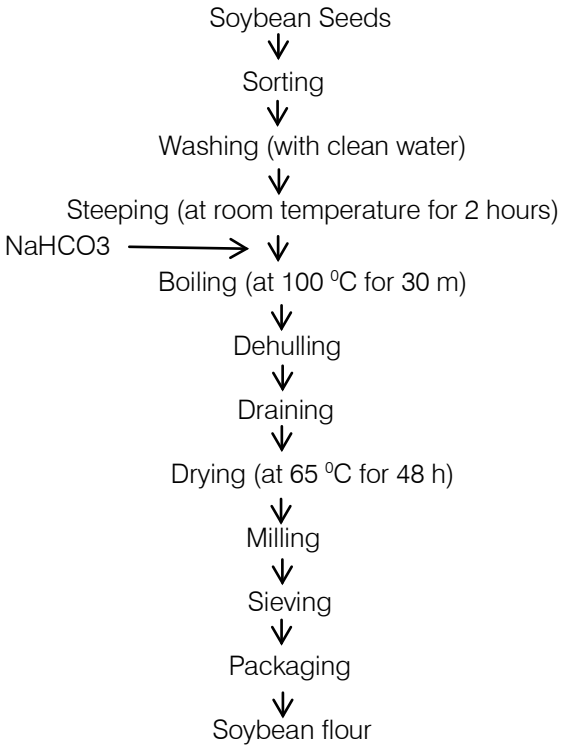
Wheat flour and soyabean seeds were purchased from wurukum market in Makurdi, Benue State. Pro-Vitamin a Cassava (TMS 1368) tubers (*Manihotutilisimacrantz*) was purchased from BERNADA in Makurdi, Benue State.

*b) Reagent*

Chemical used such as Sodium bicarbonate to facilitate removal of beany off-flavor and dehulling of soy bean seeds is of analytical grade.

*c) Sample Preparation*

Fresh pro-vitamin A cassava roots were peeled with knife, grated and pressed using a laboratory scale hydraulic press (Fisher, USA Hydro DL, 2017) and oven dried at 60°C for 4 h, they were then ground into flour using laboratory grinder (M/S Sujata: New Delhi India) and sieved through a 0.5mm size mesh and were packaged in Low density polyethylene bags prior to analyses (Ubbor *et al.,* 2009). Soy bean flour was prepared as shown in Figure 1. The flour blend formulation is captured in Table 1. The flour blends were conditioned to 25% moisture content, mixed manually with other ingredients for cookies production and baked following the methods of Igbabul *et al.,* 2013*)*. The recipe for the production of cookies from flour blends of wheat, soybeans and pro-vitamin A cassava is captured in Table 2.




*Source: (Modified Ndife et al., 2014). NaHCO3: Sodium metabisulphite*

*Figure 1:* Flow chart for the production of soybeans flour

*d) Feeding Trial*

A total of twenty (20) mixed breed, albino rats of both sex were obtained from a reputable disease free albino rat farm and used for ten (10) week experiment. The cages, feeders and drinkers were properly cleaned and disinfected using izal, seven days before the arrival of the rats. The rats were reared in four (4) cages made of wood/wire netting measuring 60 cm x 40cm x 40 cm having wire mesh flour, raised 60 cm above the floor. Rats in cage 1, 2, 3 and 4 were fed with cookies A, B, C and D respectively. The rats were allowed to acclimatize to the new environment for seven days, after which 3 rats were randomly selected from each cage and allocated to each treatment group, live weight differences between treatment groups was minimized. Individual rats were given the experimental diet (cookies) and fresh water. Standard rat husbandry practices including medication, recommended sanitary measures and other health practices were strictly observed throughout the experimental period.

## III. Analytical Methods

Haematological indices and serum biochemistry profile was done using the method as

described by Adekojo *et al*. (2014). While weight gain was done following the method as described by Mmoreole *et al.* (2011)

*a) Statistical Analysis*

Data Obtained was subjected to Analysis of Variance (ANOVA) followed by Duncan Multiple Range test to compare treatment means; differences was considered significant at 95% ($P \leq 0.05$) (SPSS V21 software).

## IV. Result and Discussion

Haematological parameters generally provide information on inflammation, necrosis, various infections of visceral organs and the presence of stress factor (Jurcik *et al.,* 2007). Serum biochemical investigations have been explored extensively to distinguish normal state from from stress and disease conditions in animals (Awosanya *et al.,* 2000). The haematological indices and serum biochemical indices of rat fed cookies produced from wheat, soybeans and pro-vitamin A cassava composite flour is presented in Table 3, while Table 4 presented the weight gained by Albino Rat fed with cookies produced from wheat, soybeans and pro-vitamin A cassava composite flour. Total protein ranged from 5.22 to 6.55 g/dL with sample Dhaving the highest value while the control had the least. The high serum total protein is an indication of nutritional adequacy since high serum They were no significant difference ($p \leq 0.05$) in albumin and White Blood Cell (WBC) content with increased addition of soybeans flour in rat fed cookies. WBC performs a defensive action in the body, its values were within the normal range reported by Hewitt *et al.,* (1986), therefore, the feeding of rats with the experimental diet did not affect the ability of the rats to fight infections and distribute antibodies for immune response. Glucose, ASTU/L, ALTU/L, PCV and RBC increased while cholesterol decreased with increasing quantity of soybeans flour in rats fed cookies, PCV is an index of toxicity factor in the blood and it suggest a presence of a toxic factor which has adverse effect on blood formation (Makinde, 2007), the PCV values in this study ranged from 32.0 to 41.0 % which was within the reference range (33-50%) reported by Medi-rat (2007) for healthy rats. This is an indication that the cookies fed were not toxic to the health status of the rats. Serum glucose values range of 69.50 to 74.33 mg/dL observed in this study was in line with standard reference range of 75 to 150 mg/dL recommended by RAR (2011), which implies that the experimental diets met the requirements for growth and development. Normal cholesterol level (10-80 mg/dL) reported by Karen (2002) was in line with the cholesterol values (85.10-85.50 mg/dL). Possibilities of anorexia, diabetes, liver dysfunction and mal-absorbtion of fat which are symptoms of abnormal glucose and cholesterol levels in blood (Bush, 1991) were ruled out. These results were similar to the study by Ogu *et al. (*2006) for quality evaluation of *Pannicum maximum* and *Leucaenaleucocephala* leaves in rabbit feeding.

*Table 1:* Flour blends formulation

| | SAMPLES | | | |
|---|---|---|---|---|
| | A (Control) | B | C | D |
| Wheat flour | 100 | 80 | 70 | 60 |
| Pro-vitamin A cassava flour | 0 | 10 | 20 | 30 |
| Soybeans flour | 0 | 10 | 10 | 10 |

*Table 2:* Recipe for preparation of cookies from wheat, soybeans and pro-vit a cassava flour blends

| Ingredient | Quantity(g) |
|---|---|
| Flour | 100 |
| Salt | 1.0 |
| Sugar | 20 |
| Baking fat | 7.5 |
| Skimmed milk | 80 |
| Water | 76-80 (mL) |

*Source: Igbabul et al., 2013*

*Table 3:* Hematological indices and Serum Biochemical Indices of Rat Fed with Cookies Produced from Wheat, soybeans and Cassava Composite Flour

| | SAMPLES | | | |
|---|---|---|---|---|
| | A (Control) | B | C | D |
| Wheat flour | 100 | 80 | 70 | 60 |
| Pro-vitamin A cassava flour | 0 | 10 | 20 | 30 |

| Soybeans flour | 0 | 10 | 10 | 10 |
|---|---|---|---|---|
| Total protein (g/dL) | $5.20^{b}\pm0.1$ | $6.55^{a}\pm0.0$ | $6.56^{a}\pm1.1$ | $6.55^{a}\pm2.3$ |
| Albumin (g/dL) | $4.15^{a}\pm1.0$ | $4.16^{a}\pm1.2$ | $4.13^{a}\pm2.3$ | $4.14^{a}\pm1.3$ |
| Glucose (mg/dL) | $69.50^{c}\pm1.$ | $73.79^{b}\pm1.1$ | $73.87^{b}\pm1.6$ | $74.33^{a}\pm1.4$ |
| Cholesterol (mg/dL) | $85.10^{c}\pm1.1$ | $88.89^{b}\pm3.1$ | $86.82^{a}\pm2.1$ | $85.80^{a}\pm2.2$ |
| ASTU/L | $28.33^{c}\pm1.1$ | $28.65^{c}\pm3.1$ | $32.48^{b}\pm2.4$ | $38.19^{a}\pm2.6$ |
| ALTU/L | $21.83^{b}\pm1.1$ | $24.53^{a}\pm3.1$ | $25.48^{a}\pm2.1$ | $25.80^{a}\pm2.2$ |
| PCV (%) | $32.0^{d}\pm1.1$ | $35.25^{c}\pm3.1$ | $37.00^{b}\pm2.1$ | $41.0^{a}\pm2.2$ |
| RBC ($10^{12/L}$) | $6.70^{c}\pm1.1$ | $7.89^{b}\pm3.1$ | $6.82^{a}\pm2.1$ | $6.88^{a}\pm2.2$ |
| WBC ($10^{9/L}$ | $4.45^{a}\pm1.1$ | $4.49^{a}\pm3.1$ | $4.32^{a}\pm2.1$ | $4.30^{a}\pm2.2$ |

Values are Mean± Standard deviation of triplicate determination. Mean values with common superscript letter(s) along each row are not significantly (P>0.05) different

Key: PCV= Packed Cell Volume
RBC= Red Blood Cell
WBC= White Blood Cell

*Table 4:* Weight Gained by Albino Rat Fed with Cookies Produced from Wheat, Soybeans and Pro-Vitamin A Cassava Composite Flour

| | SAMPLES | | | |
|---|---|---|---|---|
| | A (Control) | B | C | D |
| Wheat flour | 100 | 80 | 70 | 60 |
| Parameter Pro-vitamin A … cassava flour | 0 | 10 | 20 | 30 |
| Soybeans flour | 0 | 10 | 10 | 10 |
| Initial Weight (g) | $120.34^{a}\pm0.2$ | $121.40^{a}\pm0.1$ | $120.20^{a}\pm0.3$ | $123.32^{a}\pm0.2$ |
| Final Weight (g) | $370.21^{b}\pm0.1$ | $385.45^{a}\pm0.1$ | $388.22^{a}\pm0.1$ | $391.33^{a}\pm0.1$ |
| Total Weight Gain (g) | $249.89^{b}\pm0.1$ | $264.05^{a}\pm0.1$ | $268.02^{a}\pm0.1$ | $268.01^{a}\pm0.1$ |
| Feed Conversion Ratio | $3.20^{a}\pm0.1$ | $3.27^{a}\pm0.1$ | $3.26^{a}\pm0.1$ | $3.30^{a}\pm0.1$ |

Values are Mean± Standard deviation of triplicate determination. Mean values with common superscript letter(s) along each row are not significantly (P>0.05) different

## V. Conclusion

In conclusion, response of the rabbits to the test diet (Cookies) revealed that weight was affected positively. The high conversion ratios are indications that adequate nutrients available for growth and maintenance. The value of haematology and serum biochemistry were comparable with normal reference values, an indication of nutritional adequacy and safety of the experimental diet (cookies)

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# Phytochemical, Elemental, Proximate, and Antinutrient Composition of Custard Apple Seed (Annona Reticulata) from Maiha Adamawa State, Nigeria

By Ezekiel T. Williams, Kwaye Luka & Nachana'a Timothy
*Adamawa State University*

*Abstract-* The Proximate, phytochemicals, elemental and anti-nutrients of annona reticulata seed collected from Michika were investigated. The Proximate, phytochemicals, and anti-nutrients were determined using standard methods, while the elemental composition was determined using Atomic absorption spectrophotometer (AAS) and flame photometer. The result of the proximate analysis shows the presence of Moisture (15.20± 0.02%), crude fibre (32.00 ± 0.01%), crude fat (30.34 ± 0.04 %), ash content (4.5 ± 0.00%), protein (17.12 ± 0.01%) and carbohydrate (37.91± 0.05 %) in the seed. The result of the mineral composition showed Ca (14.40 ± 0.03 mg/ 100 g), Fe (0.32 ± 0.01 mg / 100g), P (46.26 ± 0.02mg /100g), Zn (0.85 ± 0.01mg / 100 g), Na (17.34 ± 0.02mg /100 g), Mg (18.18 ± 0.03mg /100 g) Se (0.12 ± 0.00 mg /100g) were present in the annona reticulate seed.

*Keywords:* *phytochemical, elemental, proximate, anti-nutrient and annona reticulata.*

*GJMR-L Classification:* *NLMC Code: QU 145*


*Strictly as per the compliance and regulations of:*

# Phytochemical, Elemental, Proximate, and Antinutrient Composition of Custard Apple Seed (Annona Reticulata) from Maiha Adamawa State, Nigeria

Ezekiel T. Williams [α], Kwaye Luka [σ] & Nachana'a Timothy [ρ]

*Abstract-* The Proximate, phytochemicals, elemental and anti-nutrients of *annona reticulata* seed collected from Michika were investigated. The Proximate, phytochemicals, and anti-nutrients were determined using standard methods, while the elemental composition was determined using Atomic absorption spectrophotometer (AAS) and flame photometer. The result of the proximate analysis shows the presence of Moisture (15.20± 0.02%), crude fibre (32.00 ± 0.01%), crude fat (30.34 ± 0.04 %), ash content (4.5 ± 0.00%), protein (17.12 ± 0.01%) and carbohydrate (37.91± 0.05 %) in the seed.. The result of the mineral composition showed Ca (14.40 ± 0.03 mg/ 100 g), Fe (0.32 ± 0.01 mg / 100g), P (46.26 ± 0.02mg /100g), Zn (0.85 ± 0.01mg / 100 g), Na (17.34 ± 0.02mg /100 g), Mg (18.18 ± 0.03mg /100 g) Se (0.12 ± 0.00 mg /100g) were present in the *annona reticulate* seed. The result of phytochemical test showed the presence of oxalates, phytates, lectins, saponins and glycosides in the annona reticulata seed. The proximate composition is an indication that the *annona reticulate* seed can be an important nutrient to both human and livestock, the phytochemical is an indication of bioactive component that is used as a source for drugs production and the mineral analysis showed that the *annona reticulate* seed had the potential to be used as a source of nutrient in human diet.

*Keywords: phytochemical, elemental, proximate, anti-nutrient and annona reticulata.*

## I. Introduction

Plants serve as the sources of raw materials for diverse industrial products such as dyes, perfume, textile, fibre and building material. Plants provide a variety of resources that contribute to the fundamental need of both human being and animal such as food, clothing and shelter. Among plants of economic importance are medicinal plants. Plants have been utilized as therapeutic agents since time immemorial in both organized and unorganized forms. The healing properties of many herbal medicines have been recognized in many ancient cultures (Zhang, 2015). Medicinal plant has been the main sources of traditional herbal medicine among rural dwellers worldwide (Mustapha, 2016).

*Author α σ ρ: Department of pure and applied chemistry, faculty of science, Adamawa State University, Mubi Nigeria.*
*e-mail: tagwiezekiel@gmail.com*

Plant is a source of a large number of drugs comprising of different groups such as antispasmodics, emetics, anti-cancer, anti-microbial, anti-inflammatory, anti-malaria, anti-oxidant etc (Quilly *et. al.,* 2017; Williams *et al.,* 2019). A large number of plants are claimed to have the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another, as well as nutrition for both human and animals (Williams *et al*., 2019). Plants have been known to relieve various diseases in India, Africa, Panama and America. Medicinal plants are the richest bio-resources of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, Pharmaceutical intermediates and chemical entities for synthetic drugs (Akinwunmi and Omotayo, 2016; Du, 2016).

According to the world health organization, a medicinal plant is any plant which, in one or more of its organs contain substances that can be used for therapeutic purposes or which are pre cursors for chemo-pharmaceutical synthesis. Parts of such plant including leaves, roots rhizomes teams, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical component that are medically active (Mohammed *et al.,* 2016; Williams *et al.,* 2020a).

*Annona reticulate*is semi–ever green and small deciduous tree from the plant family Annonaceae (Baskar *et al.,* 2007). It is well known for its fruit commonly called custard apple having flavored sweet and pleasant (Chang et al., 1993). The plant is native of Caribbean region and has also been spread across central and South America, Africa and Asia. *Annona* species are cultivated allover India for their edible fruit belonging to custard apple family. All parts of *annona* are used in natural medicine in the tropic (Maeda *et al*., 1993; Ogunwade and Olusegun, 2006; Pandy and Barve, 2011; Thang *et al*., 2013; Kaladhar *et al* 2014). It is considered to be a good source of natural, antioxidant for various diseases (Baskar *et al.*, 2007; *Bhalke et al*., 2011. *Annona reticulate* seed is often discarded after

taking the pulp of the fruit. Research has shown that the custard apple seed is good source nutrition (Chang *et al*., 1998; Jirovetz *et al*., 1998; Joy *et al*., 2004). Since these nutrient composition vary not only with the varieties and species of plant but also with the environment in which the plant are grown. It is important to determine the nutritional composition of a given plant grown in different localities. In this part of the world the information on the nutritional composition of custard apple grown locally is not available. The purpose of the study is to investigate the nutritional composition of seed of custard apple grown in the study area.

## II. Materials and Methods

*a) Sample collection and identification*

The matured ripped unaffected fruit of custared apple (*Annona reticulata*) was collected from Michika Adamawa, identified and authenticate by Baba Yahaya Kirri in the Department of crop production Adamawa State University Mubi.

*b) Sample preparation*

The fruits were cut opened and the seed were removed then washed with distilled water and dried at room temperature and pulverized in to powder.

*c) Chemicals and reagents*

All Chemicals and reagents used were of analytical grade.

*d) Preparation of extract*

Twenty five grams (25 g) of powdered seed were extracted separately in a soxhlet apparatus and solvent were removed. The percentage yield was determined by following the method described by AOAC (2005): The yield percentage = weight of extract recovered x 100 / weight of dry powdered and the extract was used for phytochemical screening, proximate analysis, anti-nutrient and elemental composition.

*e) Elemental Analysis*

The mineral content of the sample were determined using atomic absorption spectrophotometer and flame photometer following the procedure described by AOAC (2003).

*f) Phytochemical screening*

*Annona reticulata* seed was tested for the presence of bioactive compounds. The phytochemicals of the seed samples were estimated following the procedure adopted by Williams *et al.,* (2020b).

*g) Test for Terpenoids*

Organic extract of 2 ml was dissolved in 2 ml of chloroform and evaporated to dryness. Concentrated sulphuric acid of 2 ml was added and heated for two minutes. A grayish color was observed.

*h) Test for Flavonoids (Alkaline Reagent Test)*

Extract of 200 mg was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour formed which turned colorless on addition of few drops of diluted acid was observed.

*i) Test for Steroids*

To 2 ml of acetic anhydride was added 0.5 g of the sample followed by an addition of 2ml $HR_2RSOR_4R$. The color changed from violet to blue green was observed (AOAC, 2005).

*j) Tests for tannin and phenolic compounds ferric chloride test*

A small amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

*k) Test for Alkaloids*

Extract of 200 mg was mixed with 10 ml of methanol. To 2 ml of the filtrate was added 1% HCl and then steamed. To 1ml of the filtrate was added 6 drops of Wagner reagent. Brownish-red precipitate was observed.

*l) Proximate analysis*

The proximate composition (moisture, crude fibre, crude fat, ash content, protein and Carbohydrate) of powdery sample of *Annona reticulata* was determined following the method described by AOAC (2003).

*m) Anti-nutritional Content Analysis*

The anti-nutrient contents (oxalates, phytates, lectins, saponins and glycosides) were determined using high performance liquid chromatography (HPLC) following the procedures adopted by AOAC (2003).

*n) Statistical Analysis*

All determinations were replicated three times and results were reported in mean (±) standard deviation.

## III. Results and Discussion

*Table 1:* The Result of the Elemental analysis of *Annona reticulata* seed (mg/100g)

| Element | Concentration |
|---|---|
| Ca | 14.40 ± 0.03 |
| Fe | 0.32 ± 0.01 |
| P | 46.26 ± 0.02 |
| Zn | 0.85 ± 0.01 |
| Na | 17.34 ± 0.02 |
| Mg | 18.18 ± 0.03 |
| Se | 0.12 ± 0.00 |

The elemental composition of *Annona reticulata* seed was presented in Table 1. The result revealed the presence of Ca, Fe, P, Zn, Na, Mg and Se. The concentration of elements ranged from 0.12 ± 0.00 mg/ 100 g to 46.26 ± 0.02 mg / 100g. Se (0.12 ± 0.00 mg / 100g) has the lowest concentration, while P (46.26 ± 0.02 mg / 100g) has highest concentration. This result corroborates with the study of Ubwa *et al* . (2015).

Phosphorus (46.26 ± 0.02 mg/100g) is an important constituent of adenosine triphosphate and nucleic acid and is also essential for acid base balance, bone and tooth formation. Phosphorus deficiency can cause low appetite, irritation, numbness, bone pain, poor bone and tooth development. The concentration of magnesium recorded was 18.18 ± 0.03 mg/100 g. Magnesium is an important component of chlorophyll in plant. Mg is an important mineral element in connection with a circulatory disease such as heart disease (Tona *et al*., 2001).The value of sodium obtained in *annona reticulata* seed was found to be 17.34 ± 0.02 mg /100 g. Insufficient Na causes low blood pressure of the body. Na is important in the maintenance of osmotic balance between cell and the interstitial fluid. Calcium (14.40 ± 0.03 mg /100 g) is used in the construction, maintenance and normal function of the nerves and muscles (Williams *et al*., 2019). The concentration of Zn in *annona reticulatas* was found to be 0.85 ± 0.01 mg /100 g. Zinc is one of the important cofactor found in the structure of certain enzymes. It is involved in the normal function of the immune system. The value of Fe recorded was 0.32 ± 0.01mg/ 100 g. Iron is component of the cytochromes that function in cellular respiration. Selenium (0.12 ± 0.00 mg / 100 g) is an important co factor found in the structure of certain enzymes and are indispensible in numerous biochemical path way. It plays a critical role in metabolism and thyroid function and helps to protect the body from damage caused by oxidative stress. It also plays an important role in health of immune system.

*Table 2:* Result of the Phytochemical Screening of Annona reticulata

| Phytochemical | Qualitative | Quantitative (mg/100g) |
|---|---|---|
| Tannins | + + + | 9.12 ± 0.01 |
| Alkaloids | + + | 6.10 ± 0.00 |
| Flavonoids | + + | 6.78 ± 0.01 |
| phenols | + + + | 16.15 ± 0.02 |
| Terpenoids | + | 0.23± 0.01 |
| Steroids | + | 0.16 ± 0.01 |

+ = *present*
+ + = *moderate present*
+ + + = *highly present*

Table 2 contains the result of the phytochemical screening of *annona reticulata* seed. The result revealed the presence of tannins, alkaloids, flavonoids, phenols, terpenoids and steroids. The concentrations of the phytochemicals ranged from 0.16 ± 0.01mg / 100g to 16.15 ± 0.02 mg / 100 g. The lowest concentration recorded was Steroids (0.16 ± 0.01mg / 100/g), while highest was phenols (16.15 ± 0.02 mg / 100 g). This finding is in line with the report of Mariana *et al*. (2017), which describes the presence these phytochemicals.

Phenol (16.15 ± 0.02 mg / 100 g) was found to be highly present in the seed. It is used as defense against ultraviolet radiation or aggression by pathogens, parasites and predators as well as contributing to plant colors. Phenolic compounds are important for the quality of plant based foods, they are responsible for the color of red fruit, juices as well as wine and substrates for enzymatic browning and are also involved in flavor properties in particular, as stringency is ascribed to precipitation of salivary proteins by polyphenol, a mechanism possibly involve in defense against anti-nutrition effect (Ramadass and Subramanian, 2018).

Tannins was highly present in the seed, the concentration was found to be 9.12 ± 0.01mg / 100g. It plays an important role as a source of energy. It serves as a protective to the plant. In the food industry tannins are used to carify wine, beer and fruit juice (Funatagawa *et al.,* 2004).

Flavonoid (6.78 ± 0.01mg / 100 g) was found to be moderately present in the seed. In general flavonoids can play an important role in decreasing disease risk through various physiologic mechanisms. Some of these include anti-viral, anti-inflammatory, cytotoxic, anti-microbial and antioxidant effect. Mechanisms responsible for improvement in heart disease risk include: improve endothelial function, decreased blood pressure and improvement in lipid sand insulin resistance (Pandey and Barve, 2011).

Alkaloids was moderately present in the seed the concentration was found to be 6.10 ± 0.00 mg / 100 g. It is used in pharmaceutical industries in the production of drugs (James, 2012).

Terpenoids was found present in the seed and the concentration was 0.23± 0.01mg / 100 g. It has biological activity as anti - platelet (Pandey and Barve, 2011).

The concentration of steroids in seed was 0.16 ± 0.01mg / 100 g. It plays an important role in the proper function of the human body.

*Table 3:* result of the proximate analysis of (Annona reticulata %)

| Composition | Value |
|---|---|
| Moisture | 15.20± 0.02 |
| crude fibre | 32.00 ± 0.01 |
| crude fat | 30.34 ± 0.04 |
| ash content | 4.5 ± 0.00 |
| protein | 17.12 ± 0.01 |
| carbohydrate | 37.91± 0.05 |

The result of proximate analysis of *annona reticulata* seed was presented in Table 3. The result showed that moisture, crude fibre, crude fat, ash, protein and carbohydrate were present in the seed. The ash content has the lowest value (4.5 ± 0.00 %) among all the nutrient composition while carbohydrate was the highest (37.91± 0.05%). This is an average when compared with that of other legumes ranging from 23% to 66% in groundnut though this value is higher compared with that of other legumes which have been reported to range between 15.00–16.00% (FAO/WHO, 2011; Ubwa *et al*., 2015).

Carbohydrate (37.91± 0.05 %) provide energy, stored energy, build macro molecules and spare protein and fat for use. Glucose energy is stored as glycogen, with majority of it in muscle and liver. The ash content of seed was found to be 4.5 ± 0.00 % which plays an important role in the relief of fever, arthritis, constipation, fluid retention and bladder problems. It is also used as tonic (Zida *et al.,* 2016).

*Table 4:* The Result of the Anti - nutrient Analysis of the Annona reticulate seed

| Anti - nutrient | Concentration (mg/100g) |
|---|---|
| Oxalates | 2.12 ± 0.01 |
| Phytates | 9.45 ± 0.02 |
| Lectins | 0.20 ± 0.01 |
| Saponins | 6.12 ± 0.02 |
| Glycosides | 7.13 ± 0.01 |

The result of the anti- nutrient analysis of annona reticulate seed was shown in Table 4. The result revealed that oxalates, phytates, lectins, saponins, and glycosides were present in the seed.

The presence of these secondary metabolites in plants produces some biological activity in man and animals and it is responsible for their uses as herbs in primary health care (Ramadass and Subramanian, 2018). These compounds also serve to protect the plant against infections by microorganisms, predations by insects and herbivores, while their odor and flavor are responsible for their pigments (Galeane1, *et al.*, 2017).

The results indicate that phytates (9.45 ± 0.02 mg / 100 g) have the highest value while lectins (0.20 ± 0.01 mg / 100 g) has the lowest value. Phytates serve as a store of cat ions, of high energy phosphoryl groups, an chelating free ion, as a potent natural anti- oxidant it also bind mineral in digestive track, making them less available to the body but when it is in required amount it can help in the prevention of chronic disease (Mueller, 2001). Glycosides (7.13 ± 0.01 mg/ 100 g) are used in preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value.

The concentration of saponins in the seed was 6.12 ± 0.02mg /100 g. Saponins were recognized as anti- nutrient constituents due to their adverse effect such as growth impairment and reduce their food intake due to the bitterness and throat- irritating activity. In addition saponins were found to reduce the bioavailability of nutrients and decrease enzymes activity

and it affect protein digestibility by inhibit various digestive enzymes such as tryps in and chymotrypsin (Linner, 2005). Saponins are attracting considerable interest as a result of their beneficial effect in humans. Recent evidence suggests that saponins possess hypocholesterolemic, immunostimulatory and anti-carcinogenic properties. In addition it reduces the risk of heart diseases in humans. Saponins rich food are important in human diet to control plasma cholesteros preventing peptic ulcer, osteoporosis and to reduce the risk of heart disease. Saponins are used as adjuvants in viral (e.g quillaja saponaria- 21) and bacterial vaccine (e.g quillaja saponins) application (Williams et al., 2018). A high saponin diet can be used in the inhibition of dental caries and plate late aggregation in the treatment of hypercalciuria in humans and as an anti-dote against acute lead poisoning (Rwarinda, 2016).

Oxalates was found to be 2.12 ± 0.01mg /100 g in the seed. It plays an important role in the formation of kidney stone and urinary tract when the acid is excreted in the urine (Nachbar *et al.,* 2000).

The concentration of lectins in the seed was 0.20 ± 0.01mg / 100g. It has the ability to bind carbohydrate and also have potent in vivo effect. When consumed in excess, they can cause three primary physiological reactions: they can cause severe intestinal damage disrupting digestion and causing nutritive deficiency (Timothy, 2018; Tona, 2001). It can also bind to erythrocytes, simultaneously with immune factors causing hem agglutination and anemia (Fereidoon, 2014)Generally these anti-nutritional factors can easily be reduced to the tolerable limit by proper simple technics such as soaking, cooking and frying (Williams *et al.,* 2019).

## IV. Conclusion

From the study, it can be concluded that the *annona reticulata* seed analysed have great potential as nutrient sources, considering their proximate chemical composition and mineral composition. Thus the *annona reticulata* seeds can be used as food in narrowing the nutrients supply deficit that is prevalent in many developing countries. Furthermore, the proximate composition indicates the potential of the plant seed as sources of the important nutrients needed by the body. The mineral analysis of the *annona reticulata* seed indicated that the seed is rich in minerals considered. This indicates that *annona reticulata* seed could also be a source of minerals in diet as well as drugs in pharmaceutical industries.

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# A Study to Assess the Nutritional Status of Housewives of the Below Poverty Line Families Living in a Slum in the Kidderpore Area of Kolkata, India

By Ms. Diksha Sharma & Dr. Sampa Mitra
*All India Institute of Hygiene and Public Health*

*Abstract-* Housewives in the below poverty line (BPL) families are at a high risk of suffering from malnutrition, because of poverty, and the fact that they often give priority to the nutritional needs of other family members, neglecting their own. This study has attempted to assess the nutritional status of housewives of the BPL families living in a slum in the Kidderpore area of Kolkata (India), by employing binomial tests at 5% level of significance, and using three indicators viz., body mass index or BMI, presence of angular stomatitis, and presence of pallor, and one predisposing factor viz., frequency of consumption of green leafy vegetables. Chi-square tests at 5% level of significance have been utilized to check the associations between the frequency of consumption of green leafy vegetables and the occurrence of pallor, and between the physiological condition and the presence of pallor, among the housewives. The results show that a significant number of housewives of the concerned slum are neither suffering from, nor predisposed to, malnutrition, so far as the aforesaid indicators and predisposing factor are concerned, and that there is a significant association between the frequency of consumption of green leafy vegetables and the occurrence of pallor, whereby the likelihood of the latter decreases as the former increases. The outcomes also show that there is a significant relationship between the physiological condition and the presence of pallor, whereby the pregnant/lactating housewives (these women have higher nutritional needs) are more susceptible to pallor than the non-pregnant and the non-lactating ones.

*Keywords: nutritional status, housewife, BPL, slum, BMI, angular stomatitis, pallor, green leafy vegetables, physiological condition, binomial test, chi-square test.*

*GJMR-L Classification: NLMC Code: QU 145*

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*Keywords: nutritional status, housewife, BPL, slum, BMI, angular stomatitis, pallor, green leafy vegetables, physiological condition, binomial test, chi-square test.*

## I. Introduction

In India, monthly per capita consumption expenditure of Rs. 972 in rural areas and Rs. 1407 in urban areas, is considered as the poverty line (Rangarajan, Dev, Sundaram, Vyas, & Datta, 2014). Therefore, a person spending < Rs. 47 a day in urban areas and < Rs. 32 a day in rural areas, is living below the poverty line in India (Seth Sharma, 2014).

*Author α: Student of MSc (Applied Nutrition), Department of Biochemistry and Nutrition, All India Institute of Hygiene and Public Health (AIIH & PH), Kolkata, India.*
*Author σ: Corresponding Author; Director Professor, Department of Epidemiology, All India Institute of Hygiene and Public Health (AIIH & PH), Kolkata, India. e-mail: sampamitra2003@yahoo.co.in*

In urban areas, generally, the slum-dwelling people are living below the poverty line. A slum is a densely populated area (usually in a city) of substandard housing, unsanitary conditions and social disorganization (The Editors of Encyclopaedia Britannica, 2020).

A housewife is a woman (usually married) managing a household (Thompson, 1996). In other words, a housewife's main occupation is running or managing the home, caring for her children and other family members, cooking and storing food, buying necessary goods for the family etc.

Housewives in the below poverty line (BPL) families are at a high risk of suffering from malnutrition, because of poverty, and the fact that they often give priority to the nutritional needs of their children, husband and other family members, neglecting their own. Hence, it is necessary to know, whether the housewives of the BPL families are suffering from malnutrition, and, if so, to what extent.

So, this study has attempted to assess the nutritional status of housewives of the BPL families living in a slum in the Kidderpore area of Kolkata (India).

It is a cross-sectional study conducted on 96 housewives, in the age-group of 20-60 years (belonging to the BPL families living in a slum of the Kidderpore region of Kolkata); these 96 housewives had been selected using simple random sampling technique. The study period is 6 months, and the study was conducted between March 2018 and September 2018. During the study, house-to-house visit was undertaken with a predesigned and pretested questionnaire (for extracting relevant information) and suitable measuring instruments (for recording weight and height); besides, important physical signs and symptoms (indicating nutritional deficiency), if any, were also noted.

The sample size (n) was calculated using equation (1):

$$n = \frac{z^2 p1(1-p1)}{d^2} \quad (1)$$

where,

z = level of confidence as per the standard normal distribution = 1.96 (for 95% level of confidence),

p1 = estimated proportion of the population that represents the relevant characteristic i.e., malnutrition = 0.5 (since p1 is unknown, it is taken as 0.5),
d = tolerated margin of error = 0.1 (for 10% allowable error).
After calculation, one gets, n = 96.04 ≃ 96.

To understand the nutritional status of housewives, three indicators viz., body mass index or BMI (the weight in kilograms divided by the square of the height in metres), presence of angular stomatitis (inflammation of one or both corners of the mouth due to vitamin $B_2$ deficiency and other reasons), and presence of pallor (pale colouring of the skin, generally of the face and the palms, due to anaemia and other reasons), and one predisposing factor viz., frequency of consumption of green leafy vegetables (green leafy vegetables constitute an important source of nutrition), were considered. For studying the nutritional status with the help of the aforesaid indicators and predisposing factor, the binomial tests were done at 5% level of significance. The formula for calculating the p-value (p11) is given by equation (2):

$$p11 = 2\frac{n!}{(n-X)!\,X!}p^X q^{(n-X)} \qquad (2)$$

where,
n = total number of housewives = sample size = 96,
X = n/2 = 48,

p = proportion of cases corresponding to the first variable pertaining to an indicator (the value in the first column in any one of tables- 1 to 4),
q = proportion of cases corresponding to the second variable pertaining to the same indicator (the value in the second column in the same table).

If p11 < 0.05, for a criterion, then one can draw an inference about the nutritional status of a significant number of housewives, using the relevant indicator or factor; otherwise, no such inference can be drawn.

To ascertain whether there is a significant association between the frequency of consumption of green leafy vegetables and the occurrence of pallor among the housewives, and also whether there is a significant relation between the physiological condition (i.e., whether a housewife is pregnant/lactating or not) and the presence of pallor among the housewives, the chi-square tests were done at 5% level of significance. The formula for chi-square ($\chi^2$) is given by equation (3):

$$\chi^2 = \sum\frac{(O-E)^2}{E} \qquad (3)$$

where,
O = each observed value in any one of tables - 5 and 6,
E = each expected value in the same table = (row total X column total / grand total) corresponding to each value in that table,
p2 = p-value (calculated using relevant software system) corresponding to the chi-square value with 1 degree of freedom.

If p2 < 0.05, then it can be concluded that the relevant association is significant; otherwise, it (i.e., the pertinent association) is not significant.

It may be noted here that the nutritional needs of a pregnant/lactating woman is higher than those of a non-pregnant and non-lactating one.

Before undertaking this work, a brief literature survey was conducted, but no study related to the nutritional status of housewives of BPL families of Kidder pore area, was found.

## II. Methodology

The outline of the method adopted in this work, is shown in figure-1.

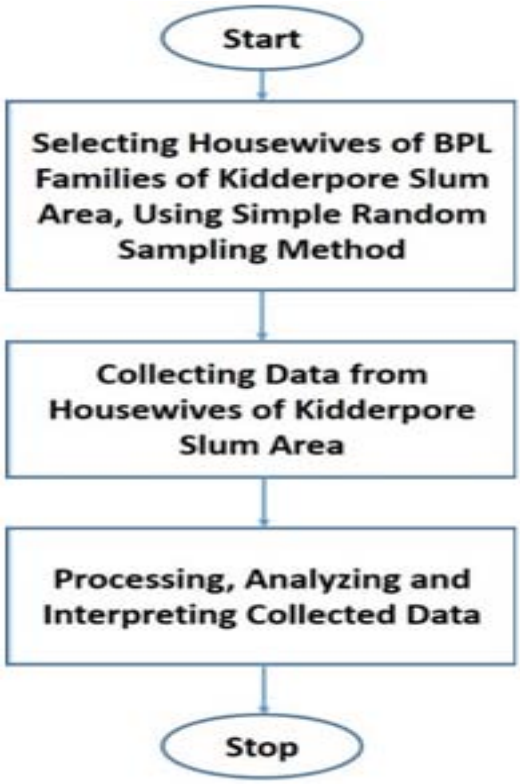


*Figure 1:* Flowchart depicting the outline of the method

## III. Results and Discussions

Table-1 categorizes the housewives into two classes according to BMI.

*Table 1:* Classification of housewives according to BMI

| Normal/overweight (BMI >= 18.00) | Underweight (BMI < 18.00) | Total |
|---|---|---|
| 57 | 39 | 96 |

According to table-1, 57 housewives are either normal or overweight. Here, the p-value for the binomial test is, p11 = 0.0292 < 0.05. Thus, a significant number

of the housewives are not suffering from malnutrition, so far as the BMI is concerned.

In table-2, the housewives are classified into two groups according to lip condition.

*Table 2:* Categorization of housewives according to lip condition

| Presence of angular stomatitis | Normal lip | Total |
|---|---|---|
| 24 | 72 | 96 |

As per table-2, 72 housewives have normal lip. Here, the p-value for the binomial test is, $p11 = 1.6355 \text{ X } 10^{-7} < 0.05$. Therefore, a significant number of housewives are not suffering from malnutrition, so far as the occurrence of angular stomatitis is concerned.

In table-3, the housewives are grouped into two categories according to skin colour.

*Table 3:* Classification of housewives according to skin colour

| Presence of pallor | No presence of pallor | Total |
|---|---|---|
| 38 | 58 | 96 |

In table-3, 58 housewives do not have pallor. Here, the p-value for the binomial test is, $p11 = 0.0193 < 0.05$. Hence, a significant number of housewives are not suffering from malnutrition, so far as the occurrence of pallor is concerned.

Table-4 categorizes the housewives into two groups according to frequency of consumption of green leafy vegetables.

*Table 4:* Grouping of housewives according to frequency of consumption of green leafy vegetables

| 0-3 days in a week | >3 days in a week | Total |
|---|---|---|
| 14 | 82 | 96 |

Table-4 shows that 82 housewives consume green leafy vegetables for more than 3 days in a week. Here, the p-value for the binomial test is, $p11 = 4.8793 \text{ X } 10^{-16} < 0.05$. So, a significant number of housewives are not predisposed to malnutrition, so far as the frequency of consumption of green leafy vegetables is concerned.

Table-5 shows the relation between the frequency of consumption of green leafy vegetables and the presence of pallor, among the housewives.

*Table 5:* Association between the frequency of consumption of green leafy vegetables and the presence of pallor, among the housewives

| Frequency of consumption of green leafy vegetables | Presence of pallor | No presence of pallor | Total |
|---|---|---|---|
| 0-3 days in a week | 12 | 2 | 14 |
| >3 days in a week | 26 | 56 | 82 |
| Total | 38 | 58 | 96 |

As per table-5, out of the 14 housewives who consume green leafy vegetables for not more than 3 days in a week, 12 have pallor, and among the 82 housewives who consume green leafy vegetables for more than 3 days in a week, 56 do not have pallor. Here, the p-value for chi-square test is, $p2 = 1.3598 \text{ X } 10^{-4} < 0.05$. So, there is a significant relation between the frequency of consumption of green leafy vegetables and the presence of pallor, among the housewives, and more is the frequency of consumption, less is the chance of occurrence of pallor.

Table-6 shows the association between physiological condition and presence of pallor, among the housewives.

*Table 6:* Relation between physiological condition and presence of pallor, among the housewives

| Physiological condition | Presence of pallor | No presence of pallor | Total |
|---|---|---|---|
| Pregnant/lactating housewife | 26 | 7 | 33 |
| Non-pregnant and non-lactating housewife | 12 | 51 | 63 |
| Total | 38 | 58 | 96 |

According to table-6, out of the 33 pregnant/lactating housewives, 26 have pallor, and among the 63 non-pregnant and non-lactating housewives, 51 do not have pallor. Here, the p-value for chi-square test is, $p2 = 1.3142 \text{ X } 10^{-8} < 0.05$. Hence, there is a significant association between the physiological condition and the presence of pallor, among the housewives, and the chance of occurrence of pallor is more among the pregnant/lactating housewives than among the non-pregnant and non-lactating ones.

Thus, the results of the binomial tests and the chi-square tests indicate that:

- A significant number of housewives of the concerned slum of the Kidderpore region, are not

suffering from malnutrition, so far as the three indicators viz., BMI, presence of angular stomatitis, and presence of pallor, are concerned; besides, a significant number of housewives are also not predisposed to malnutrition, as indicated by the predisposing factor viz., frequency of consumption of green leafy vegetables. This is probably because, Kidderpore, being a port area, offers various jobs with not-very-low salaries to the slum-dwellers.

- There is a significant association between the frequency of consumption of green leafy vegetables and the presence of pallor, among the housewives, and the chance of the occurrence of pallor lessens with the increase in the frequency of consumption.
- There is a significant relation between the physiological condition and the occurrence of pallor, among the housewives, and the pregnant/lactating housewives are more susceptible to the occurrence of pallor than the non-pregnant and the non-lactating ones. This is most probably because, the pregnant/lactating housewives of the concerned slum are not getting sufficient nutrition to meet their higher nutritional needs. Therefore, though a significant number of housewives of that slum are not suffering from malnutrition, this is not the case for most of the pregnant/lactating women among them.

## IV. Conclusions

This study shows that a significant number of housewives of the concerned slum of the Kidderpore region, are not suffering from malnutrition, so far as the three indicators viz., BMI, presence of angular stomatitis, and presence of pallor, are concerned, and that a significant number of housewives are also not predisposed to malnutrition, as indicated by the predisposing factor viz., frequency of consumption of green leafy vegetables. This is an interesting result because, in India, the slum-dwelling people are generally considered to be belonging to the BPL category, and the housewives of BPL families normally suffer from malnutrition. A possible explanation for this anomalous outcome is that, since Kidderpore is a port area, a considerable number of slum-dwellers of this region are employed with not-very-low salaries. However, to ascertain this theory, a thorough study on the occupations and incomes of the people of the concerned slum, should be undertaken. Also, other indicators associated with the nutritional status of the housewives of that slum, should be investigated. Besides, nutritional studies should be conducted on the housewives living in other nearby slums (if any) of this region, to see whether similar results are obtained.

This work also shows that there is a significant association between the frequency of consumption of green leafy vegetables and the presence of pallor, among the housewives, and that the chance of the occurrence of pallor lessens with the increase in the frequency of consumption. This is an expected result.

Another outcome of the study is that there is a significant relation between the physiological condition and the occurrence of pallor, among the housewives, and that the pregnant/lactating housewives are more susceptible to the occurrence of pallor than the non-pregnant and the non-lactating ones. This shows that the nutritional levels of the housewives of the concerned slum are not sufficient to meet the higher nutritional needs of most of the pregnant/lactating women among them. To address this problem, the government can take appropriate measures to provide financial assistance to the pregnant/lactating housewives of BPL families, and to educate the members (both male and female) of BPL families regarding the higher nutritional needs of the pregnant/lactating women.

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- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

***Structure and Format of Manuscript***

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

## Format Structure

***It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.***

All manuscripts submitted to Global Journals should include:

**Title**

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

**Author details**

The full postal address of any related author(s) must be specified.

**Abstract**

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

**Keywords**

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning 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 a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

**Numerical Methods**

Numerical methods used should be transparent and, where appropriate, supported by references.

**Abbreviations**

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

**Formulas and equations**

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

**Tables, Figures, and Figure Legends**

Tables: Tables should be 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. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

**Figures**

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

## Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). 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.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

## Tips for writing a good quality Medical Research Paper

***1. Choosing the topic:*** In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

***2. Think like evaluators:*** If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. 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.

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

***4. Use of computer is recommended:*** As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

***5. Use the internet for help:*** An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

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

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

***8. Make every effort:*** Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

***9. Produce good diagrams of your own:*** Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

***10. Use proper verb tense:*** Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

***11. Pick a good study spot:*** Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

***12. Know what you know:*** Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

***13. Use good grammar:*** Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

***14. 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 for your topic. You may also maintain your arguments with records.

***15. Never start at the last minute:*** Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

***17. Never copy others' work:*** Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

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

***19. Refresh your mind after intervals:*** Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

***20. Think technically:*** Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

***21. Adding unnecessary information:*** Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

***22. Report concluded results:*** Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

***23. Upon 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 for 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 of 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 criteria peer reviewers will use for grading the final paper.

**Final points:**

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

*The introduction:* This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

**The discussion section:**

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to 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 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 avoid:*

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

**Title page:**

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

**Abstract:** This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite 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 approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

*Reason for writing the article—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 for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

**Approach:**

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity 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 of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

*The following approach can create a valuable beginning:*

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets 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 for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

**Procedures (methods and materials):**

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate 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 to give the least amount of information that would permit another capable scientist to replicate 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 section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show 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:**

*Materials may be reported in part of a section or else they may be recognized along with your measures.*

**Methods:**

- Report the method and not the 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—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

**Approach:**

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

**What to keep away from:**

- 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.

**Results:**

The principle of a results segment is to present and demonstrate your conclusion. Create this part as 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. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

**Content:**

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give 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 manuscript.

**What to stay away from:**

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

**Approach:**

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

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

**Figures and tables:**

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

**Discussion:**

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an 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 implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully 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 the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

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 an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of 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 other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

## The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

*Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection*.

*Segment draft and final research paper:* You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

*Written material:* You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

## Criterion for Grading a Research Paper (Compilation) by Global Journals

**Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.**

| 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<br>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 |

# INDEX

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# Global Journal of Medical Research

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