PROXIMATE, PHYTOCHEMICAL AND PHENOLIC CONTENTS OF EXTRACTS OF *Cucumis sativus*

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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY, EDO STATE

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A PROJET WORK SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCES (B.SC) IN BIOCHEMITRY

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CERTIFICATION

This is to certify that this project report was presented by **EHIMWENMA OSASENAGA (LSC1705096)** of the department of Biochemistry, Faculty of Life sciences, University of Benin, Benin City, Edo state, Nigeria.

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DEDICATION

This project is dedicated to God almighty for his unending show of love and faithfulness.

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Class of 2021 (ADAPTOGENS) that persevered and still stood strong.

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ABSTRACT

Cucumis sativus (cucumber) is known to contain a variety of bioactive substances and phytochemicals. Some of these chemical elements have been connected to the plant's traditional therapeutic uses. The aim of this study was to evaluate the proximate, phytochemical and phenolic contents of aqueous and ethanol extracts of *Cucumis sativus*. The Proximate analysis results showed that the medicinal plant contained more Nitrogen-Free Substances (NFS) and protein, but low level of fibre ($p < 0.05$), phytochemical analysis showed that alkaloids, tannins, saponins, and other polyphenols were present in the plant, Phenols and saponins were present in high concentrations, while glycosides concentrations were low. The ethanol extract had significantly higher total phenol, but total flavonoid, flavonol and proanthocyanidin contents were significantly higher in the aqueous extract than in the ethanol extract ($p < 0.05$).

The results obtained in this study indicate that *C. sativus* is a reservoir of potentially useful chemical compounds which may serve as drugs and provide newer leads and clues for modern drug design. *C. sativus* is a good source of phenolic compounds and could be used as a natural constituent of food and medicines.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The use of plants in the maintenance of good health is well documented (Edeoga and Eriata, 2001). It has also been reported that the bases of many modern pharmaceuticals used today for the treatment of various ailments are plants and plant-based products (Kamba and Hassan, 2010). About 80 % of world population depends on plants-based medicine for their health care (WHO, 2001). Plants that could be utilized therapeutically or that act as building blocks for the manufacture of effective pharmaceuticals are known as medicinal plants (Abolaji et al., 2007). Only once a plant's biological activity has been noted by ethnobotanists or is proven by science can it qualify as a medicinal plant. Medicinal plants, since time immemorial have been used virtually in all cultures as a source of medicine. The use of medicinal plants is increasing worldwide, in view of the tremendous expansion of medicine and a growing interest in herbal treatments. Plants are used in medicine to maintain and augment health physically and mentally as well as to treat specific conditions and ailments. Medicinal plants are divine gifts to us from Mother Nature who has kept those remedies in the plant kingdom for mankind to use to fight diseases and cure themselves from ailments (Besong *et al.,* 2016).

Only a few of the 500 plants in Africa that are known to be good for medicine have been described or examined (Taylor *et al.,* 2001). Natural products from plants can be another potent source for the discovery of excellent activities such as blood booster, antioxidant, anti-ulcer, anticancer, antimicrobial amongst others. Scientific investigation into herbal medicine is crucial, according to the World Health Organization (WHO). Native medicinal plants are viewed as potential additions to the WHO's list of "essential medications" by many developing nations once their benefits have been scientifically demonstrated. The effectiveness with which man, using all the tools and technology at his disposal, develops and makes use of plants and plant products, in turn, determines our survival and continuous existence (Ayoka *et al.,* 2008). One of the emerging plants of interest is *Cucumis sativus*. *Cucumis sativus* is used in traditional medicine for the treatment of various ailments (Heidari *et al.,* 2012; Patil *et al.,* 2012).

1.1.2 Aim

To investigate the Proximate, phytochemical and phenolic contents of extract of *Cucumis sativus*

Objective of the Study

- To determine the quantitative and qualitative phytochemicals of *C.sativus*
- To determine the proximate analysis of *C.sativus*
- To determine the phenolic component of *C.sativus*

1.2 Literature Review

1.2.1 *Cucumis sativus*

There are around 118 genera and 825 species in the cucurbitaceae family. Approximately 5.6% of India's total vegetable production is made up of cucurbits, which are grown on 42.9 lakh hectares with a productivity of 10.52 t/ha, according to FAO estimates. Among the cucurbits, cucumber (*Cucumis sativus* L.) is precious vegetable crop grown for its immature fruits. After tomato and watermelon, cucumber is cultivated more broadly than any other vegetable and also very popular in kitchen garden (FAO, 2010). The fruits are used mainly as salad and pickles. In some places the seeds are used in a variety of ways for human consumption. Greek historians also have recorded the various uses of cucumber. Cucumber was domesticated about 3,000 years ago, and is indigenous to India (Whitaker and Davis, 1962; Jeffrey, 1980; Robinson and Decker-Walters, 1997). The cucumber plant is normally trailing or climbing in habits. The root is mainly superficial and extensive. Stem stout, 4-angled, hairy; climbing or trailing with simple tendrils. Leaves are triangular, ovate, 7 – 20 cm in length, base deeply cordate, apex acuminate; petiole 5 – 15 cm in length. Flowers are generally monoecious whereas, andromonoecious, gynoecious and tri-monoecious forms also exist. Petals are yellow, 3 – 4 cm in diameter. Staminate flowers are predominant (more numerous) in axillary clusters and pistillate flowers usually solitary, axillary, borne on short thick pedicels. Poor pollination is one of the main causes of fruit abortion, misshapen fruit or poor fruit set in cucumber. Pollination is usually by insects (bees). It is advocated to have one bee hive per acre. Some cucumber cultivars are able to set fruit without pollination as parthenocarpic fruits and are suitable for greenhouse production. Parthenocarpic fruits do not have seeds, even though; occasionally some seeds may be present. New cultivars contain a gene for parthenocarpy and do not require hormone spray for fruit set. Fruits are pendulous, variable in shape and size, flesh pale green, many seeded pepo. Seeds are flat, white in colour, 8 – 10 cm × 3 – 5 mm in size, and approximately 50 seeds are present in 1g. The cucumber on the basis of market are classified into two types: those that are eaten fresh (fresh or slicing market types) and those consumed as a processed product (processing or pickling types). The quality attributes may vary for pickling and slicing cucumber. Pickling type has smaller length/diameter ratios (L/D) than slicing cucumber and usually has lighter coloured skin with more pronounced wart (tubercles) at the immature stage. Whereas slicing cucumber are commonly called fresh market varieties. Fruits of this group are white spined and mostly had dark green exterior colour. Parthenocarpic cucumber is another group under slicing type, which is bred primarily for glasshouse production. The parthenocarpic variety/hybrid sets develop fruit without pollination. The fruits of parthenocarpic cucumber are seedless and non-bitter. The fruits have tender, smooth uniform, green coloured skin, usually in excess of 12 inch length. However, breeding for this kind of cucumber has received only limited attention in India. Generally imported seeds of parthenocarpic cucumber are used in India for protected condition (Jeffrey, 1980).

1.2.2 Origin, Evolution and Variation

The genus *Cucumis* is partitioned into two subgenera designated as *sativus* (2n = 2x = 14 and 24) and *melo* (2n = 2x = 24) that contain five cross-sterile species groups (Jeffrey, 1980). The subgenus *Cucumis* comprises three or four Sino-Himalayan species, including *C. sativus* (2n = 2x =14) and *C. hystrix* Chakr. (2n = 2x = 24). The *C. sativus* houses several botanical varieties including var. *sativus*, the cultivated cucumber, and the wild, free living var. *hardwickii* (R.) Alef. (Kirkbride, 1993). *Cucumis hystrix* Chakr. is a wild species originating in Asia (Kirkbride, 1993) which fruits possess a flavour typical of cultivated cucumber (Chen *et al*., 1994). Although the literature indicates that *C. hystrix* is 2n = 2x = 14 (Dane, 1991), no definitive cytogenetic analysis has been made of this species, and its taxonomic placement has been based solely on morphological characteristics (Kirkbride, 1993). Isozyme analysis of *C. hystrix*, *C. sativus* and *C. melo* led to the hypothesis that a triangular phylogenetic relationship exists among these species (Chen *et al*., 1995).

Cucumber is believed to have originated from India. Its Sanskrit equivalent name “Urvaruka” and “Ervaruka” as mentioned in the old treatises of India “Rigveda” and “Charaka Samhita” amply justifies its cultivation dates back to 3000 years. The wild species *Cucumis sativus* L. var.

hardwickii (R) Alex (Syn. *Cucumis hardwickii* Royle) found along the foothills of the Himalayas is a feral or progenitor form of the cultivated cucumber, *Cucumis sativus.*

1.2.3 Floral Biology and Crossability

According to Choudhury and Pathak (1959), the opening and closing of the staminate flowers in cucumber are mainly influenced by the sun-rise and sun-set, that is, by light and the time of the day. Anthesis is seen between 5.30 a.m. and 7.00 a.m. while, the dehiscence of anthers takes place from 4.30 a.m. to 5.00 a.m. Anther in all varieties dehisced between temperatures range of 20.5 – 21.5 °C. Pollen fertility increases up to noon and reduces greatly by afternoon (2.00 p.m.), and it negligible in the evening. The stigma become receptive 12 h before flower opening and continues to be till 6 – 7 h after. Stigmatic receptivity is of very short duration. Increased temperature causes early drying of stigmatic secretion. Different floral abnormalities like mixed inflorescence, hermaphroditism, fusion, dimorphic female flowers, reduction and increase in all the floral parts are observed.

Although cucumber is known as only a cultivated plant, a *Cucumis* form *Cucumis sativus* var. *hardwickii* R. (Alex.) with 2n = 2x = 14 crosses readily with cultivated cucumber. Isshiki *et al.* (1992) examined the Indian wild cucumber *Cucumis sativus* L. var. *hardwickii* Kitamura and 81 accessions of cultivated cucumber for six isozymes and concluded that Indian wild cucumber is a distant relative of cultivated cucumber. Wild African *Cucumis* species (mostly 2n = 2x = 24) are cross incompatible with cucumber, which are themselves cross-incompatible (Kroon *et al.*, 1979). Likewise, the wild, free living *C. hystrix* is only sparingly fertile with cucumber (Chen *et al.*, 1995; 1997a and b). This species is found only in the Yunnan Province of Southern China, and has unique genetic attributes that make its taxonomic determination complex. *Cucumis hardwickii* is a wild relative of *C. sativus* that grows in the foothills of Himalayan mountains and is used by native peoples of Northern India as a laxative (Deakin *et al*., 1971). This botanical variety is sympatric and cross compatible with *C. sativus* and possesses a multiple fruiting and branching habit that is not common in cucumber (Horst and Lower, 1978). The *C. hardwickii*, therefore, represents the extreme in vatiation in *C. sativus* germplasm (Dijkhuizen *et al.*, 1996), and, thus, has potential for increasing genetic diversity in commercial cucumber (Staub *et al.,* 1992).

1.2.4 Sex Expression

Gynoecious or monoecious are main sex type in cucumber and intensity of sex expression is important to commercial cucumber production since differences in sex type and flowering can affect harvest date and relative yield. The type and intensity of sex expression in cucumber is controlled and affected by genes as well as influenced by growing environment (Lower and Edwards, 1986; Tatlioglu, 1993; Staub and Bacher, 1997). Cucumber sex phenotypes are mainly monoecious (staminate and pistillate flowers) or gynoecious (pistillate flowers only), but androecious (staminate flowers only), hermaphroditic (perfect flowers), andromonoecious (staminate and perfect flowers), and tri-monoecious (staminate, perfect, and pistillate flowers) types also exist. Plants possessing pistillate and perfect flowers have also been observed and used in hybrid production (El-Shawaf and Baker, 1981). These sex types are determined by three major loci *F*, *M*, and *A* (Shifriss, 1961; Galun, 1961 and Kubicki, 1969). The *F* locus influences the degree of femaleness (*FF* > *Ff* > *ff*), while the *M* locus determines whether flowers are unisexual (*M*-) or bisexual (*mm*). The *A* locus conditions increased male tendency if a plant is homozygous recessive *aa* and *ff*. Interactions between these loci yield the basic sex types found in cucumber. While this three-gene model describes the basic regulation of sex types, a plants phenotype is also influenced by modifying genes and environmental factors (Serquen *et al.*, 1997a and b). The existence of sex modifying genes is supported by the observation that inbred gynoecious plants differ in their level of gynoecy and their capacity to confer femaleness in F1 hybrids (Kubicki, 1969; Zhang *et al.*, 1992). Monoecious plants also vary quantitatively in sex expression, ranging from predominately staminate to predominately pistillate. In fact, there are at least five genes that modify the expression of gynoecy in cucumber (Serquen *et al.*, 1997b; Fazio *et al.*, 2003a). Thus, hybrids between monoecious and gynoecious lines can show considerable variation in the frequency of female flowers depending upon the level of gynoecy in the parents (the *F* locus and the constitution of alleles at sex modifying loci). This variation in the level of gynoecy in gynoecious × gynoecious and gynoecious × monoecious hybrids remains a potential deficiency in many commercial cultivars. Hormonal factors are also responsible for controlling the sex expression in cucumber.

Genetic control and environmental variation of sex expression is mediated through changes in plant hormonal levels. Current theory holds that sex expression in cucumber is regulated by a balance between ethylene, auxins, abscisic acid (ABA) and gibberellins (GA) (Roy and Saran, 1990; Galun, 1959). While ethylene is considered the primary hormone affecting femaleness (Byers *et al.*, 1972), gibberellins regulate male sex expression (Atsmon *et al.*, 1968; Rudich *et al.*, 1972a and b). Ethylene mediates primordial changes to determine gynoecy where the enzyme ACC (1-aminocyclopropane-1-carboxylic acid) synthase plays a critical regulatory role. Trebitsh *et al.* (1997) isolated and mapped a partial sequence of the gene *CsACS1*, which co-segregates with *F* in cucumber. Another ACC synthase (*CsACS2*) gene was described by Kamachi *et al.* (1997 and 2000), and subsequently the gene for femaleness (dominant *F* allele) in cucumber was characterized and isolated (Mibus and Tatlioglu, 2004; Przybecki *et al.*, 2004).

1.2.5 Breeding Methods

Breeding methods are determined by the requirements associated with cucumber market classes (for example, pickling, fresh market). Breeding plans are driven by historically proven procedures and emerging technologies. Cucumber does not show inbreeding depression by inbreeding, although considerable heterosis has been noted by several scientists. Therefore, several procedures are applicable to cucumbers which are as follows:

1.2.5.1Mass Selection

Through mass selection, some desirable characters controlled by recessive genes like, bush habit, spinelessness and lack of bitterness could be obtained.

1.2.5.2Bulk Population Method

In cucumber, two selections *viz*. pickling and slicing with resistance to anthracnose and downy mildew have been developed from the crosses involving the anthracnose resistant introduction 'PI 197087' and downy mildew resistant lines.

1.2.5.3Backcross Breeding Method

Backcross breeding is used to transfer one qualitative (highly heritable) trait (for example, determinate character (de), downy mildew resistance (dm), nematode resistance (mj) into an otherwise superior inbred, which is referred to as the recurrent parent. Often six generations of selection and back crossing to the recurrent parent are required to recover the desired genotype (recurrent parent with the additional trait) and eliminate the undesirable traits inherited from the non-recurrent (donor) parent. Using this breeding approach, scab resistant lines have been evolved. Lines which combine high yielding with quality fruits have been developed though repeated back-crossing.

1.2.5.4Pedigree Method

This includes selection of single plant-segregates in segregating generations F2, F3, amongst others, derived from crosses between desirable parents.

1.2.5.5 Heterosis Breeding

Heterosis has been exploited for earliness, high yield and quality fruits of cucumber. Different hybrid seed production methods are available:

1.2.5.6 Protection of Flowers and Hand Pollination

In this widely employed hybrid seed production technique in cucumber, the pistillate and staminate flowers that are to be used in manual pollination of the following morning are identified in the afternoon prior to anthesis by the appearance of a slight touch of yellow at the apex of the corolla tube. Both the pistillate and staminate flowers are either covered with butter paper bags or prevented from opening by tying the tips of the corolla tube to protect the flowers from insect pollination. The following morning, pollens are transferred from the staminate flowers of the intended male parent to the stigma of the pistillate flowers of the intended female parent. In cucumber, usually anthesis occurs in the very early morning and stigma receptivity declines sharply after 7.00 a.m. so the pistillate flowers of the seed parent is pollinated with the pollen from the staminate flower of the intended male parent in the early morning hours within 7.00 a.m.

1.2.5.7 Removal of the Buds of Staminate Flowers and Open Pollination

In properly isolated hybrid seed production fields, one row of the intended male parent can be planted after every three rows of the intended female parent. Staminate flower buds are removed by hand regularly from the intended female parent.

1.2.5.8 Chemical Suppression of Staminate Flowers and Open Pollination

Monoecious sex expression can be regulated by exogenous application of ethrel, an ethylene-releasing chemical at 100 – 120 ppm in increasing the number of pistillate flowers when applied at three stages of plant development, that is, first true leaf, third true leaf and fifth true leaf (Singh and Chaudhury, 1998). Plants of the intended female parent generally produce only pistillate flowers for an extended period of 25 – 30 days after being treated with ethrel. Further application of ethrel would not be effective so development of later staminate flowers is stopped by cutting the growing point of the vine.

1.2.5.9 Use of Gynoecious Lines

Use of gynoecious lines is one of the most important methods of hybrid production of cucumbers. The gynoecious lines are maintained through spraying the line with gibberellic acid (15 ppm) or silver thiosulphate (6mM) which induces staminate flower production in gynoecious line. The first F1 processing cucumber developed from a gynoecious line (MSU 71305) is Spartan Dawn.

1.3.1 Agronomy of *Cucumis sativus*

1.3.1.2 Climate and Soil

Cucumber is essentially a warm season crop grown in subtropical and hot arid regions. Soil temperature for ideal germination is 27 °C. While for better pollination, fertilization and fruit setting, the temperature should be between 24° and 32 °C. Plants are very sensitive to low temperature and frost. Environmental factors play significant influence on sex expression in cucumber. High temperature and long days induced more male flower, while short days and low temperature promote femaleness. Besides, increased plant populations and low moisture also increase the induction of pistillate flowers. The intensity of light and time of day greatly influence anthesis more than temperature but temperature alone has greater influence on anther dehiscence and pollen fertility.

1.3.1.3 Land Preparation and Sowing

Cucumber can be grown well on deep sandy loam soil high in organic matter, well drained and slightly acidic in reaction. In heavy soil, plant grows slowly, and fruits are inferior in size and quality. It is well known that cucumber is fairly tolerant to acid soil, for its successful cultivation the pH of the soil ranged from 6.0 to 7.0. Acid tolerant limit of soil for cucumber is 5.0 pH (Saimbhi, 1993). Soil preparation is started 3 - 4 weeks before sowing, and the soil is thoroughly prepared since proper soil preparation significantly reduce the soil compactness and allow the roots to penetrate deeper into the soil. Cucumbers are grown both in summer and rainy season. For summer season, sowing is done during February - March and rainy season crop seeds are sown in June- July. In Western India, cucumber sowing extends from September to February. Under protected conditions, crop can be raised throughout the year. The seed rate of cucumber depends upon the sowing distance between hills and rows. The recommended seed rate is 2.5 to 3.0 kg/ha. Seed treatment is done with carbendazim at 2.5 g per kg of seeds. Cucumber seeds are sown at 2.0 - 2.5 m row to row and 80 cm plant-to-plant spacing. Optimum plant density is essential to harvest high yield and quality fruits (Dutta, 1966; Seshadri, 1986). The crop can be sown on shallow pit or flat bed or on mound.

1.3.1.4 Plant Nutrition

Cucumber is a quick growing crop and responds well to manuring and fertilization. It is recommended that 200 – 250 q/ha well decomposed FYM or compost must be added in the soil and mixed properly 20 – 25 days before sowing. Fertilizer requirements will vary depending on the soil type, fertility, previous crops, system of cultivation, cultural practices, method of fertilizer application and duration of crops, climate and yielding ability of varieties/hybrids. In general, band placement of fertilizer application is found most effective with low rate of fertilizer. Where drainage and quality of irrigation water is poor, and field is having high soluble salt concentration, a combination of band and broadcast placement can be adopted to provide adequate nutrient to plant and to lower the salinity injury. As a source of nitrogen urea can be used in small quantity to root zone during earthing up or during drip watering. Liquid manuring of urea promote growth and improve fruit quality than ammonium nitrate. Number and length of nodes, weight of fruits and total yield increase when solution culture of ammonium sulphate or calcium nitrate is applied at 300 ppm (Alan, 1984) but, when urea or NH_4^+ accounts for more than 50 % of N source, plant growth and fruit yield reduce drastically. More yield, earliness and improved fruit quality can be achieved if slow releasing fertilizers like KNO_3 + NH_4 NO_3 or NH_4 NO_3 + K_2SO_4 + $MgSO_4$ are applied in the form of perforated plastic tubes. Increase in yield can be obtained by spraying 3 % urea thrice at weekly interval starting after the first picking. Cucumber plants grown in cooler green house (14 to 29 °C) and receiving N at 2 and 4 g/plant produce a yield of 120 – 250 q/100m^2 compared with 70 – 106 q/100 m^2 in a warmer (19.5 to 40 °C) greenhouse. The quality of canned cucumber (freshness) before processing is better under application of N at 80 to 120 kg/ha. Method and time of application can be concluded as organic manure is mixed with soil at the time of filling the soil mixture in the pits. When cucumbers are sown in flat bed method, well-decomposed organic manure is applied during field preparation through broadcast. Whole amount of phosphorus and potassium along with half to three fourth dose of nitrogen is applied at the time of sowing or seedling transplanting. The remaining dose of nitrogen is top dressed in two splits, that is, 30 and 45 – 60 days after sowing. Dose of nitrogen may be reduced or omitted (particularly by the second split) if growth is luxuriant.

1.3.1.5 Weed and Water Management

Thinning of vines, protection of young seedlings and vines from frost is essential. One or two vines per hill is advisable. Weeds are major problem and 15 to 60 % reduction in yield has been observed due to weeds. Depending on the severity of weeds, total 2 – 3 weeding operations is required. Single application of Pendimethalin as pre-emergence at 3.3 litres mixed with 800 litres of water can be used in one hectare which checks the weed emergence up to 35 – 40 days from sowing. One hand weeding at about 35 – 40 days after sowing controls the weeds effectively. Cucumbers require a continuous supply of moisture during the growing period especially during flowering and fruiting period. Water and excess stress both can seriously reduce marketable and quality yields. Crop moisture requirement of cucumber range from 15 to 24 inches, depending on climate, soil type, plant populations and types of cultivars. On an average, cucumber need 1 inch of water each week, which may increase to 2 inches per week during hot and dry weather, or if plants are fruiting. Number of irrigation and quantity of water are based on the consumptive use of water by crop and relationship between the available moisture of root zone and yield potential of the crop. In arid areas, furrow irrigation is preferred to reduce evaporation losses, while in humid regions; overhead sprinkler is used. Light and frequent irrigations are preferable in comparison to heavy and delayed irrigations.

1.3.1.6 Harvest and Post-Harvest Management

Cucumbers are harvested at a range of developmental stages, depending on the intended use. The time from planting until the beginning of harvest generally ranges between 45 to 60 days, depending on the cultivar and growing conditions. Cucumber fruit is harvested at an immature stage; near full size but before the seeds are fully enlarged and become hard. The two main external indices of harvest maturity are fruit size and skin colour. The main internal indices of harvest maturity are seed development, locular jelly formation, and flesh texture. The principal index of harvest maturity is fruit size. The proper size depends on the use and the cultivar. Fresh market slicing cucumbers are at least 15 cm long and firm to the touch. Skin colour is another widely used index for assessing fruit maturity. The peel is uniform dark green colour when harvested. It also has a noticeable wax deposit on the surface. However, some cultivars may naturally produce a lighter green fruit and environmental conditions may also affect skin colour. Fruit are generally at their highest eating quality when the skin is uniformly green. The fruit is not allowed to turn yellow. Yellow fruits are over-matured. Fruit that are too mature have a tough leathery skin and are bitter in flavor. Old fruit left on the vine slow flowering and development of new fruit. A cross sectional slice obtained from the center of the fruit can be taken to assess internal fruit maturity. At proper harvest maturity, a jelly-like material is formed in the seed cavity. Seed development is also used to determine harvest maturity. Large, slightly yellow, or hard seeds are an indication of over-maturity and low fruit quality.

Harvesting is done during the coolest time of the day, preferably in the morning, after the leaves and fruit have completely dried. Harvesting when the plants are wet encourages the spread of foliar diseases. On the other hand, waiting until the heat of the afternoon to begin harvest results in slightly softer and more flaccid fruit. The fruits generally have their highest water content at this time. Harvested cucumbers are kept cool as possible. Cucumbers are harvested every other day for best yield and quality. The cucumber fruit grows rapidly to harvest size and picking the fruit as soon as they reach marketable size maintains the vitality and productive capacity of the plant. Cucumber fruits are carefully removed from the vine using one of two harvesting techniques. The first technique involves squeezing the stem attached to the vine between the thumbnail and forefinger, followed by pulling off the fruit from the vine. This leaves a jagged stem section which requires trimming with a pruning shear or knife. The second technique, which is preferable, is to use a small knife to severe the fruit stem from the vine at a point just above the shoulder of the fruit. Fruits which are pulled off the vine are often “plugged”. Plugging causes the very end of the fruit tissue to pull loose from the fruit. This is a quality defect, leaving an exposed crater of internal pulp tissue at the end of the fruit. The plugged area is an open wound which is highly susceptible to decay. Cucumbers after harvest are carefully put in a light-weight container, either made of wood, or plastic. The inside of the container may be lined with protective padding to prevent fruit scarring and abrasion. The container is kept well-ventilated and hold up to about 25 kg of fruit. It is not advisable to put cucumbers in plastic bags or containers where air is excluded for any period of time. Fruits kept in non-ventilated containers lose skin colour and firmness due to buildup of heat inside the container.

1.3.1.7 Seed Production

Typically, breeder’s seed of inbred lines is increased to produce enough seed for foundation and certified seed. Breeder’s and foundation seed of inbred lines is usually produced by hand-pollination under green house or cage isolation. Isolation blocks or screen cages are often employed for large scale seed production (inbred and hybrid).

1.3.2.1 Isolation

For seed production of cucumber, the isolation distance is kept at 1000 m in case of foundation seed and 500 m in case of certified seed. The field is isolated from related species or other members of the family which are cross compatible.

1.3.2.2Pollination

Cucumbers rely on insects (honeybees are ideal) for pollination. Each plant produces both male and female flowers. Varieties of the same species cross-pollinate with other varieties of the same species. Cucumber is relatively easy to hand-pollinate (although time-consuming) with the right materials and do remarkably well with hand-pollination. Where the number of wild bees is insufficient to ensure adequate pollination, beehives are introduced into the isolation block or cage. In large seed fields it is advisable to place beehives nearby, to increase pollination. Bumble bees (*Bombus impatiens*) have a great potential to serve as a supplemental pollinator for cucumbers (Stanghellini *et al*, 1998). Supplementary hand pollination is also beneficial.

1.3.2.3 Rouging

A thorough rouging is essential to produce quality seed in cucumber. The plant and fruit characteristics that aid in rouging the off-types include vine growth, leaf shape, colour, disease reaction, fruit size, colour and quality. The off type plants are removed as soon as noticed. The operation of rouging before flowering is very effective. All off type fruits borne by true to type plants are usually removed.

1.3.2.4Inspections

In order to ensure quality seed production, systematic and timely field inspections at different stages of plant growth is required. A minimum of three field inspections *viz*. before flowering, during flowering and fruiting and finally at fruit maturity are required to identify and remove the off type plants. The entire plant and not the individual fruit constitutes the basic unit in the rouging (Singh and Gill, 1987). Removal of off types even at this stage is helpful in preventing further genetic contamination and mechanical admixtures of the pure seeds.

1.3.2.5 Harvesting of Fruits for Seed

The seeds of cucumber are allowed to reach full maturity before harvesting. Cucumbers are mature when they have become extremely bloated and begin to soften. They change in color from green to yellow, orange or white.

1.3.2.6 Seed Extraction

After harvesting, the fruits are carefully cut open to remove the seeds along with the flesh (this is often done by hand). Seeds attached with the flesh are placed in a bucket with equal volume of water and allowed to ferment for 1 – 2 days depending on the atmospheric temperature with stirring twice daily. As the seed separates from the disintegrating pulp, it tends to sink to the bottom of the container. It is common that this mixture smells unpleasant and a layer of mold may form on the surface of the water. However, this does not damage the viable seeds if they are removed from the mixture promptly after the seed cases are separated from the seed. After fermentation, the seeds are then washed and dried in the sun to moisture content below seven percent, before storage.

CUCUMBER (*Cucumis sativus)*

1.4.1 Phytochemistry

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1991). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics and About 150 phytochemicals have been studied in detail (Meagher and Thomson, 1999).

In wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices (Mathai, 2000). Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans, legumes, and soy foods are common sources (Moorachian, 2000). Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds. Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions. Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals (American Cancer Society, 2000). These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases (Narasinga, 2003).

1.4.2 Phenolics

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom. The three most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols. Phenolic are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group. Phenol (C6H5OH) is considered the simplest class of this group of natural compounds. Phenolic compounds are a large and complex group of chemical constituents found in plants (Walton *et al.,* 2003). They are plant secondary metabolites, and they have an important role as defence compounds. phenolics exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protecting agents against Free radical-mediated disease processes. Flavonoids are the largest group of plant phenols and the most studied. Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids. Phenolic polymers, commonly known as tannins, are compounds of high molecular weight that are divided into two classes: hydrolyzable and condensed tannins (Dai and Mumper, 2010).

1.4.3 Flavonoids

Flavonoids are polyphenolic compounds that are ubiquitous in nature. More than 4,000 flavonoids have been recognized, many of which occur in vegetables, Fruits and beverages like tea, coffee and Fruits drinks. The flavonoids appear to have played a major role in successful medical treatments of ancient times, and their use has persisted up to now. Flavonoids are ubiquitous among vascular plants and occur as aglycones, glucosides and methylated derivatives. More than 4000 flavonoids have been described so far within the parts of plants normally consumed by humans and approximately 650 flavones and 1030 flavanols are known. Small amount of aglycones (i.e., flavonoids without attached sugar) are frequently present and occasionally represent a considerably important proportion of the total flavonoid compounds in the plant (Harborne, 1995).

1.4.4Tannins

From chemical point of view it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers (Harborne, 1999). It might be said that the tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids and minerals, etc. On the basis of their structural characteristics it is therefore possible to divide the tannins into four major groups: Gallotannins, ellagitannins, complex tannins, and condensed tannins (Schofield and Mbugua, 2001).

(1) Gallotannins are all those tannins in which galloyl units or their meta-depsidic derivatives are bound to diverse polyol-, catechin-, or triterpenoid units.

(2) Ellagitannins are those tannins in which at least two galloyl units are C–C coupled to each other, and do not contain a glycosidically linked catechin unit.

(3) Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit.

(4) Condensed tannins are all oligomeric and polymeric proanthocyanidins formed by linkage of C-4 of one catechin with C-8 or C-6 of the next monomeric catechin.

1.4.5 Alkaloids

Alkaloids are natural product that contains heterocyclic nitrogen atoms, are basic in character. The name of alkaloids derives from the “alkaline” and it was used to describe any nitrogen-containing base. Alkaloids are naturally synthesis by a large numbers of organisms, including animals, plants, bacteria and fungi. Some of the fires natural products to be isolated from medicinal plants were alkaloids when they first obtained from the plants materials in the early years of 19th century, it was found that they were nitrogen containing bases which formed salts with acid. Hence they were known as the vegetable alkalis or alkaloids and these alkaloids are used as the local anesthetic and stimulant as cocaine. Almost all the alkaloids have a bitter taste. The alkaloid quinine for example is one of the most bitter tasting substances known and is significantly bitter (1x10-5) at a molar concentration (Mueller-Harvey and McAllan, 1992). Alkaloids are so numerous and involve such a variety of molecular structure that their rational classification is difficult. However, the best approach to the problem is to group them into families, depending on the type of heterocyclic ring system present in the molecule. For historical reasons as also because of their structural complexities, the nomenclature of alkaloids has not been systematized. The names of individual members are generally derived fro*m* the name of the plant in which they occur, or from their characteristic physiological activity. The various classes of alkaloids according to the heterocyclic ring system they contain are listed below.

1.4.5.1 Pyrrolidine alkaloids

They contain pyrrolidine (tetrahydropyrrole) ring system. E.g Hygrine found in Erythroxylum coca leaves.

1.4.5.2 Pyridine alkaloids

They have piperidine (hexahydropyridine) ring system. E.g Coniine, piperine and isopelletierine.

1.4.5.3 Pyrrolidine-pyridine alkaloids

The heterocyclic ring system present in this alkaloids is Pyrrolidinepyridine.E.g Myosmine, Nicotine alkaloid found in tobacco (Nicotiana tabacum) plant.

1.4.5.4 Pyridine-piperidine alkaloids

This family of alkaloids contains a pyridine ring system join to a piperidine ring system the simplest member is Anabasine alkaloid isolated *From*poisonous Asiatic plant anabasis aphyllan.

1.4.5.5 Quinoline Alkaloids

These have the basic heterocyclic ring system quinolone E.g. Quinine occurs in the bark of cinchona tree.It has been used for centuries for treatment of malaria. Synthetic drugs such as primaquinine have largely replace quinine as an anti-malarial.

1.4.5.6 Isoquinoline alkaloids

They contain heterocyclic rig system isoquinoline. E.g. Opium alkaloids like narcotine, papaverine, morphine, codeine, and heroine.

1.4.6 Terpenoids

The terpenoids are a class of natural products which have been derived from five-carbon isoprene units. Most of the terpenoids have multi cyclic structures that differ from one another by their functional groups and basic carbon skeletons. These types of natural lipids can be found in every class of living things, and therefore considered as the largest group of natural products. Many of the terpenoids are commercially interesting because of their use as flavours and fragrances in foods and cosmetics examples menthol and sclareol or because they are important for the quality of agricultural products, such as the flavour of fruits and the fragrances of flowers like linalool (Elbein and Molyneux, 1999).

1.4.7 Terpenes

Are widespread in nature, mainly in plants as constituents of essential oils. Their building block is the hydrocarbon isoprene, CH2=C(CH3)-CH=CH2. Terpene hydrocarbons therefore have molecular formula (C5H8) n and they are classified according to the number of isoprene units (Langenheim, 1994).

1.4.7.1 Hemiterpenoids

Consist of a single isoprene unit. The only hemiterpene is the Isoprene itself, but oxygen-containing derivatives of isoprene such as isovaleric acid and prenol is classify as hemiterpenoids.

1.4.7.2 Monoterpenoids

Biochemical modifications of monoterpenes such as oxidation or rearrangement produce the related monoterpenoids. Monoterpenoids have two isoprene units. Monoterpenes may be of two types i.e linear (acyclic) or contain rings e.g. Geranyl pyrophosphate, Eucalyptol, Limonene, Citral, Camphor and Pinene.

1.4.7.3 Sesquiterpenes

Sesquiterpenes have three isoprene units e.g. Artemisinin, Bisabolol and Fernesol, oil of flowers, or as cyclic compounds, such as Eudesmol, found in Eucalyptus oil.

1.4.7.4 Diterpenes

It composed for four isoprene units. They derive from geranylgeranyl pyrophosphate. There are some examples of diterpenes such as cembrene, kahweol, taxadiene and cafestol. Retinol, retinal, and phytol are the biologically important compounds while using diterpenes as the base.

1.4.7.5 Triterpenes

It consists of six isoprene units e.g. Lanosterol and squalene found in wheat germ, and olives.

1.4.8 Saponins

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom They form a stable foam in aqueous solutions such as soap, hence the name "saponin". Chemically, saponins group include compounds that are glycosylated steroids, triterpenoids, and steroid alkaloids. Two main types of steroid aglycones are known, spirostan and furostan derivatives. The main triterpene aglycone is a derivative of oleanane. The carbohydrate part consists of one or more sugar moieties containing glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid glycosidically linked to a sapogenin (aglycone). Saponins that have that have a minimum of two sugars, one attached to the C-3 and one at C-22, are called bidesmoside saponins (Mamta *et al.,* 2013).

1.4.9 Proximate analysis

The proximate composition of foods and plants includes moisture, ash, lipid, protein and carbohydrate contents. These food and plant components may be of interest in the food industry and research for product development, quality control (QC) or regulatory purposes. Analyses used may be rapid methods for QC or more accurate but time-consuming official methods. Sample collection and preparation must be considered carefully to ensure analysis of a homogeneous and representative sample, and to obtain accurate results. Estimation methods of moisture content, ash value, crude lipid, total carbohydrates, starch, total free amino acids and total proteins are put together in a lucid manner.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Materials

- Micro pipette (Labline stock centre)
- Spectrophotometer (search Tech.721G)
- Distilled water
- Analytical weighing balance (OHAUS Corp pine , USA)
- Test tubes racks
- Test tubes (Pyrex , England)

2.2 Chemicals

- Methanol
- Gallic acid
- Folin-ciocalteau reagent
- 7.5 % sodium carbonate
- 2 % aluminium trichloride
- Quercetin
- 5 % sodium acetate
- HCL
- Ascorbic acid

2.2.1 Collection of Plant Material

The fruits of *Cucumis sativus* was obtained from a major market in Benin City and authenticated at the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.

2.2.2 Plant Preparation and Extraction

The fruits were washed and shade-dried at room temperature for a period of two weeks and crushed into a pulverized sample using clean mortar and pestle. Extract of the plant was obtained using cold maceration method as described in literature.

2.3 Proximate Analysis

Proximate Composition Analysis this refers to the determination of the major constituents of feed and it is used to assess if a feed is within its normal compositional parameters or somehow been adulterated. This method partitioned nutrients in feed into 6 components: water, ash, crude protein, ether extract, crude fibre and NFE.

Proximate analysis was carried out in the Central Laboratory, Faculty of Agriculture, University of Benin, Benin City Edo State. With little modification by (E. M. Isikhuemen and I. U. Efenudu et al, 2020)

2.3.1 Determination of Moisture Content

Moisture content of pulverized sample of *Cucumis sativus* was determined using the method of AOAC (2000). A porcelain crucible was dried at 105 °C for about 30 min after which it was cooled and weighed with the weight recorded as W_1 (g). The sample was added in piece meal to the crucible to obtain a weight recorded as W_2 (g). The crucible was then placed in the oven at 105 – 110 °C for 24 h after which the crucible was removed from the oven and cooled in a desiccator. The crucible was then weighed and placed back into the oven. The crucible was subjected to a cycle of weighing and drying until a constant weight C (g) was obtained.

$$\% \text{ Moisture} = \frac{Weight\ loss}{Weight\ of\ sample} \times 100$$

Weight loss = $[(W2 - W1) - (C - W2)]$, and weight of sample = $W2 - W1$

2.3.2 Determination of Ash Content

A porcelain crucible was weighed as W_1 (g). The sample was added to the crucible and the new weight was recorded as W_2 (g). The sample was first charred by gentle heating and then transferred to the muffle furnace operated at 500 – 600 °C using a tong. The crucible was left in the furnace for 3 h after which it was removed, cooled in a desiccator and weighed. Drying and weighing cycles were continued until a constant weight C (g) was obtained.

$$\% \text{ Ash} = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

$W\text{eight of ash} = \mathrm{C} - \mathrm{W1}$; and Weight of sample $= \mathrm{C} - \mathrm{W1}$

2.3.3 Determination of Crude Fibre Content

Crude fibre was determined according to the method of AOAC (2000). Portion of the pulverized sample (2 g) was weighed into a 250 mL beaker and 100 mL of 1.25 % sulphuric acid was added gently to the beaker. The mixture was boiled for 30 min while maintaining a constant volume. The mixture was then cooled and filtered using muslin cloth, and the residue was washed to neutrality using warm distilled water. Litmus paper was used to test the neutrality of the mixture. The washed residue was put back into another beaker and 100 mL of 1.25 % NaOH was added and boiled for 30 min while maintaining constant volume. The beaker was removed from the heater and the mixture filtered using muslin cloth after which it was thoroughly washed to neutrality with warm distilled water. The residue was finally rinsed with 25 mL of acetone, filtered and dried after each washing. The residue was transferred to a crucible of known weight,

W_1 (g), while the residue together with the crucible weighed W_2 (g). The crucible was put in a muffle furnace and the residue was ashed to constant weight (C) at 300 °C for 1 h.

$$\% \text{ Crude Fibre} = \frac{Weight\ of\ fibre}{Weight\ of sample} \times 100$$

Weight of Fibre = $W2 - C$, Weight of Sample = $W2 - W1$

2.3.4 Determination of Crude Fat Content (Soxhlet Extraction Method)

Crude fat was determined according to the method of AOAC (2000). The solvent apparatus consists of three units: extractor flask, extractor, and condenser. A thimble was dried and weighed as W_1 (g). The sample was then added to the extraction thimble and reweighed as W_2 (g). A pre-dried 500 mL extraction flask was filled up to two-third volume with petroleum ether. The extractor flask, the thimble and a condenser were then assembled into a soxhlet extraction apparatus. The heat source was adjusted so that the solvent boiled gently. The extraction was allowed to run for 6 h. The condenser was detached, and the thimble was removed from the soxhlet extractor. The thimble was dried to constant weight in an oven at 50 °C for 30 min, and its final weight W_3 (g) was recorded.

$$\% \text{ Crude Fat} = \frac{Weight\ of fat}{Weight\ of sample} \times 100$$

Weight of fat = $W3 - W2$, and weight of sample = $W2 - W1$

2.3.5 Determination of Crude Protein Content (Kjedahl Method)

Crude protein was determined according to the method of AOAC (2000). Kjedahl method involves three stages: digestion, distillation and titration. Wet digestion method was used. Portion of the pulverized sample (1 g) was weighed into 250 mL conical flask and placed in a fume cupboard. A pinch of mixed catalyst containing selenium dioxide, $CuSO_4$ and potassium sulphate (K_2SO_4) was added to the flask. Concentrated sulphuric acid (25 mL) was then added to the flask in the fume cupboard for 48 h at a temperature of 65 – 80 ºC until the solution was pale green. The flask was removed from the burner and allowed to cool. The solution was then diluted with distilled water. Boric acid (5 mL) was put in another conical flask (recovery flask), and 10 mL aliquot of the digest was taken and dispensed into a 500 mL kjedahl flask. Distilled water (30 mL) was added to the conical flask, and 15 mL of 40 % NaOH was then added down the side of the conical flask forming a layer underneath. The kjedahl flask was immediately stopped and mounted on a kjedahl distillation apparatus. The distillation flask was connected by a tube to the recovery flask containing boric acid. Heat was applied to the distillation flask to distil over ammonia into the boric acid in the recovery flask. A 50 mL burette was clamped to a retort stand and 25 mL of distillate was dispensed into a conical flask with methyl red added as indicator. A solution of 0.1 M HCl was poured into the 50 mL burette and titrated against the distillate solution to a pink endpoint. The nitrogen and crude protein contents were calculated as shown below:

$$\% \ Nitrogen = \frac{Va - Na}{W} \times \frac{14}{1000} \times 100$$

where,

Va = titre value

Na = Molarity of HCl

Mw = Atomic weight of nitrogen

W = weight of sample used

14/1000 = milliequivalent weight of nitrogen

$$\% \ Crude \ Protein = \% \ Nitrogen \times F$$

where F = conversion factor = 6.25

2.3.6 Determination of Nitrogen-Free Substances

Nitrogen -Free Substances (NFS) was determined according to the method of AOAC (2000) as shown

% NFS = 100 – (% Moisture Content + % Ash + % Crude Fat + % Crude Protein + Crude Fibre)

2.4 Phytochemical Analysis

Qualitative and quantitative phytochemical analyses was performed using standard procedures (Sofowora, 1993; Harborne, 1998; Trease and Evans, 2002). Portion of the pulverized plant material (5g) was boiled with 20 mL of distilled water gently on a water bath for 10 min. The mixture was allowed to cool and filtered. The resultant filtrate was used for the different tests.

2.4.1 Test for Alkaloids (Mayer's Test)

To 1 mL of filtrate, few drops of Mayer's reagent were added by the side of the test tube. Formation of white or creamy precipitate confirmed a positive result (Evans, 1997).

2.4.2 Test for Flavonoids

A given volume (5 mL) of 1 % ammonia solution was added to a portion of the aqueous filtrate followed by addition of concentrated H_2SO_4. Appearance of yellow colour which disappeared on standing indicated the presence of flavonoids (Sofowora, 1993).

2.4.3 Test for Tannins

A few drops of 0.1 % ferric chloride was added to 3 mL of the filtrate and observed for brownish green or a blue-black colouration (Sofowora, 1993).

2.4.4 Test for Glycosides (Keller-Killani Test)

Aqueous filtrate (5 mL) was mixed with 2 mL of glacial acetic acid containing a drop of ferric chloride solution. This was underlayed with 1 mL of concentrated sulphuric acid, and appearance of a brown ring at the interface was indicative of the presence of glycosides. Gradual formation of a violet ring below the brown ring, or a green ring in the acetic acid layer was also taken as a positive test (Sofowora, 1993).

2.4.5 Test for Saponins

A portion of the filtrate (10 mL) was diluted with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, and then observed for the formation of an emulsion (Sofowora, 1993).

2.4.6 Test for Steroids (Liebermann-Burchard's Test)

To 2 mL of the filtrate was added few drops of chloroform, 3 – 4 drops of acetic anhydride and a drop of concentrated H_2SO_4. The colour changed from violet to blue or green in some cases, indicating the presence of steroids (Finar, 1986).

2.4.7 Test for Carbohydrates (Molisch Test)

To 2 mL of the filtrate was added 2 drops of 10 % alcoholic solution of naphthol followed by 2 mL of concentrated H_2SO_4, gently poured along the side of the test tube at an angle of 45^o. Formation of a purple ring at the interface of the two liquid layers confirmed the presence of carbohydrates (Sofowora, 1993).

2.4.8 Test for Anthraquinones

Few drops of 2 % HCl were added to 0.5 mL of filtrate, and the formation of red precipitate confirmed the presence of anthraquinones.

2.4.9 Test for Xanthoprotein

To 2 mL of the filtrate was added few drops of concentrated nitric acid and 2 – 3 mL of ammonia. Appearance of a red precipitate confirmed the presence of xanthoprotein.

2.5 Determination of Phenolic compound in *Cucumis sativus*

2.5.1 Estimation of Total Phenolic Content (TPC)

Total phenolic content was determined according to the Folin and ciocalteau method as described by Cicco *et al* (2009). Varied concentration of gallic acid (0.2 – 1 mg/ml) were prepared in methanol. Then 0.5ml of the sample (1mg/ml) was mixed with 2.5ml of a ten-fold diluted Folin Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30min at room temperature, then absorbance was read 760nm. All determinations were performed in triplicates with gallic acid used as control.

2.5.2 Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined using the Ayoola *et al.* (2008). Briefly, 2ml of 2% $AlCl_3$ in ethanol was added to 2ml of extracts. A concentration of 1mg/ml of the extract prepared in methanol was used. Similar concentration of quercetin, the standard control were used. The absorbance was measured at 420nm after 1h of incubation at room temperature.

2.5.3 Determination of Total Flavonol Content

Flavonol content was determined according to the method described by Yermakov (1987). The quercetin calibration curve was prepared by mixing 2ml of varied concentration of standard quercertin solution (0.2- 1.0 mg/ml) with 2ml of 2% aluminium chloride and 6ml 0f 5% sodium acetate. The absorbance was read at 440nm after 2.5h of incubation at 20^{o}C.

2.5.4 Determination of Proanthocyanidin Content

The determination of proanthocyanidin was carried out according to the method of Sun *et al.* (1998). To 0.5ml of 1.0 mg/ml of each extract was added 1ml of 4% methanol solution and 0.75ml of concentrated hydrochloric acid. The mixture was left undisturbed for 15mins and the absorbance was read at 500nm. Ascorbic acid was used as standard.

2.6 Statistical Analysis

Data will be expressed as mean ± standard error of mean. All statistical analyses shall be performed with SPSS version 22. Values of $p < 0.05$ will be considered statistically significant.

CHAPTER THREE

3.1 Outcome of Phytochemical Evaluation of Aqueous and Ethanol Extracts of *Cucumis sativus*

The results of phytochemical analyses revealed the presence of alkaloids, tannins, saponins and other polyphenols (Table 3.1). Phenols and saponins were present in the highest concentration, while glycosides were present in the least amounts (Table 3.2).

Table 3.1: Phytochemicals Present in Aqueous and Ethanol Extracts of *Cucumis sativus*

Phytochemicals	Inference
Alkaloids	++
Tannins	+
Phenols	+++
Flavonoids	++
Saponins	+++
Steroids	+
Anthraquinones	++
Protein	+++
Glycosides	+
Carbohydrates	+
Fixed oil	+

+ = detected; ++ = moderately present; +++ = highly present

Table 3.2: Phytochemical Composition of Pulverized *Cucumis sativus*

Phytochemicals	% Composition
Alkaloids	6.13 ± 0.02
Tannins	2.60 ± 0.15
Phenols	11.79 ± 0.73
Flavonoids	3.75 ± 0.28
Saponins	8.50 ± 0.37
Glycosides	2.13 ± 0.01
Anthraquinone	2.87 ± 0.12

Data are percentage composition of phytochemicals, and are expressed as mean ± SEM (n = 3).

3.2 Results of Proximate Analysis of *Cucumis sativus*

Results of proximate analysis showed that the medicinal plant contained more Nitrogen-Free Substances and protein, but low level of fibre ($p < 0.05$; Table 3.3).

Table 3.3: Proximate Composition of Pulverized *Cucumis sativus*

Parameters	% Composition
Moisture (Fresh sample)	96.33 ± 0.27
Moisture (Dried sample)	7.69 ± 0.34
Dry matter	92.31 ± 0.34
Ash	3.89 ± 0.22
Fibre	3.00 ± 0.24
Fat	7.91 ± 0.24
Crude protein	24.25 ± 0.80
Nitrogen-Free Substances	53.59 ± 1.04

Data are percentage proximate composition and are expressed as mean ± SEM (n = 3), on “Dry Weight (DW)” basis.

3.3 Phenolic Content of *C. sativus*

The ethanol extract had significantly higher total phenol, but total flavonoid, flavonol and proanthocyanidin contents were significantly higher in the aqueous extract than in the ethanol extract ($p < 0.05$; Table 3.4).

Table 3.4: Phenolic Content of *C. sativus*

Extracts	Total Phenol (mg GAE/g of extract)	Total Flavonoid (mg QE/g of extract)	Total Flavonol (mg QE/g of extract)	Proanthocyanidin (mg AAE/g of extract)
Aqueous	554.30 ± 141.45	713.30 ± 79.65	720.00 ± 43.59	366.70 ± 15.28
Ethanol	854.00 ± 76.00	381.00 ± 120.00	104.70 ± 44.20	206.70 ± 6.67

Data for phenolic composition of aqueous and ethanol extracts of *C. sativus,* and are expressed as mean ± SEM (n = 3).

CHAPTER FOUR
4.0 DISCUSSION AND CONCLUSION

4.1 Discussion

The use of plants for the treatment of diseases remains the oldest and most popular form of healthcare practice (Chikezie *et al.,* 2015). Herbal medicine involves the use of plant parts without isolating specific phytochemicals (Chikezie *et al.,* 2015). The current efforts of modern medicine include detailed analysis of phytochemical constituents of plant materials. Factors such as species, geographical location, method of extraction and type of solvent used for extraction, determine the levels of phytochemicals in a particular plant (Ojezele and Agunbiade, 2013). Secondary plant metabolites can be isolated, characterized and refined to produce drugs (Ojezele and Agunbiade, 2013).

Plants produce phytochemicals as part of their normal metabolic activities which they use for defense against predators. Phytochemicals are bioactive non-nutrient plant compounds present in fruits, vegetables, grains and other plant foods (Muller, 1998). Their ingestion has been linked to reductions in the risk of major chronic diseases. The different compounds are classified according to common structural features as carotenoids, phenolics, alkaloids and nitrogen containing and organosulfur compounds. Phenolics, flavonoids and phytoestrogens are of particular interest because of their potential effects as antioxidants, anti-estrogenic, anti-inflammatory, immunomodulatory, cardioprotective and anticarcinogenic compounds.

In the present study, qualitative and quantitative phytochemical screening as well as proximate analysis were performed on *C. sativus*. Studies suggest that phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low density lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity (Mathai, 2000). The physiological properties of relatively few phytochemicals are well understood (Mathai, 2000). Phytochemicals have been promoted for the prevention and treatment of diabetes mellitus, high blood pressure, and muscular degeneration (Mathai, 2000). Results of this study have shown that *C sativus* is rich in important phytochemicals. Phytochemical screening showed that the medicinal plant contains alkaloids, tannins, saponins and other polyphenols. Studies have shown that environmental factors and time of collections affect the type and quantity of secondary metabolites in a particular part of a plant. The specific activity of this plant may be attributed to the presence of quinones, terpenoids, steroids and phenolic compounds which need further investigation. Saponins are known to reduce blood cholesterol by preventing its reabsorption and may also be a potent inhibitor of hydroxyl methylglutaryl CoA (HMG-CoA) reductase: an enzyme that catalyzes the conversion of HMG-CoA to mevalonate an early and rate limiting step in cholesterol biosynthesis (Osagie and Eka, 1998). Medicinal agents containing tannins have been shown to possess antidiabetic properties (Iwu, 1983). Saponins, flavonoids, quercetin and ferulic acid synergistically reduce blood glucose level via correction of defective insulin secretion and peripheral insulin resistance (Mahesh, 2004). The presence of alkaloids in *C. sativus* could make it effective against cardiovascular diseases (Tan and Reinhold-Hurek, 2003). Alkaloids are known to possess pharmacological activities such as antihypertensive, antiarrhythmic and anticancer effects. A number of alkaloids are used as drugs and the best known is quinine used as an antimalarial (Cordell, 1983). Steroids and cardiac glycosides are presently used for the treatment of cardiac failure. These agents increase the force of contraction in a failing heart by increasing the interaction of actin and myosin filament of cardiac sarcomere, thereby increasing calcium concentration in the vicinity of the contractile protein during systole (Prohp and Onoagbe, 2012).

Fruits, vegetables and seeds are the main sources of antioxidants in the diet. Oxidative stress results in the damage of biopolymers such as nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates (Sun *et al.,* 1998; Mogana *et al.,* 2013). Bioactive metabolites in plants contribute to their medicinal effects (Akinpelu *et al.,* 2018). Antioxidants of nutritional origin play key roles in complementing *in vivo* antioxidant enzymes and molecules in the fight against free radicals. Phenols and flavonoids represent phytochemicals whose relative abundance in plant extracts has been linked to antioxidant effect (Ayoola *et al.,* 2008; Padmanabhan and Jangle, 2012). Phenolics possess diverse biological activities, such as antiulcer, anti-inflammatory, antioxidant, antitumor and antidepressant properties (Saxena *et al.,* 2013). Phenolic compounds are antioxidant agents which act as free radical terminators. The antioxidant potential of phenols is believed to be conferred on them by their hydroxyl group (-OH), which is bonded directly to an aromatic hydrocarbon (phenyl) ring. This makes them donate electrons easily to electron-seeking free radicals, thus down-regulating their menace in living cells (Ghasemi *et al.,* 2009). Studies have revealed a direct relationship between total phenol content and antioxidant effect in different plants. High phenolic content-containing plant materials have high radical scavenging capacities (Hegazy and Ibrahium, 2012).

Flavonoids possess potent and appreciable antioxidant, anti-inflammatory and anticancer effects Adetutu *et al.,* 2015; Oyedapo *et al.,* 2015).

Proanthocyanidins are a class of polyphenols found in a variety of plants. Chemically, they are oligomeric flavonoids. Many are oligomers of catechin and epicatechin and their gallic acid esters. More complex polyphenols, having the same polymeric building block, form the group of tannins. Plant proanthocyanidins are involved in induced defense mechanisms against plant pathogens and predators. They possess vasodilatory, anti-carcinogenic, antiallergic, anti-inflammatory, antibacterial, cardioprotective, immunostimulating, antiviral and estrogenic effects (Yıldırım *et al.,* 2015).

In this study, the ethanol extract had significantly higher total phenol, but total flavonoid, flavonol and proanthocyanidin contents were significantly higher in the aqueous extract than in the ethanol extract. The results indicate that both extracts have good antioxidant property.

4.2 Conclusion

The results obtained in this study indicate that *C. sativus* is a reservoir of potentially useful chemical compounds which may serve as drugs and provide newer leads and clues for modern drug design. The plant is a good source of phenolic compounds and could be used as a natural constituent of food and medicines.

REFERENCES

Abolaji, O.A., Adebayo, A.A., Odesanmi O.S. (2007). Nutritional qualities of three medicinal plant parts (Xylopia aethiopica, Blighia sapida and parinari polyandra) commonly used by pregnant women in the western part of Nigeria. *Park J. nutrition* **6**: 665-668.

Adetutu, A., Olorunnisola, O.S. and Owoade, O.A. (2015). Nutritive values and antioxidant activity of Citrullus lanatus fruit extract. *Food and Nutrition Sciences*. 6 (11): 1056.

Alan, R. (1984). Doga Bilim Dergisi. *Tarim Ve Orman ilik*. **8:** 258–291.

AOAC (2000). Official Methods of Analysis. 17th Edition,The Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Akinpelu, B.A., Godwin, A., Aderogba, M.A., Makinde, A.M., Azeez, S.O. and Oziegbe, M. (2018). Evaluation of anti-inflammatory and genotoxicity potentials of the fractions of Archidium ohioense (Schimp. ex Mull) extract. *Ife Journal of Science*. 20 (3): 487 - 496.

Arumuganathan, K. and Earle, E.D. (1991). Nuclear DNA content of some important plant species. *Plant Molecular Biology Reporter*. **9:** 211–215.

Atsmon, D., Lang, A. and Light, E.N. (1968). Contents and recovery of gibberellins in monoecious and gynoecious cucumber plants. *Plant Physiology*. **43:** 806–810.

Ayoola, G.A., Folawewo, A.D., Adesegun, S.A., Abioro, O.O., Adepoju-Bello, A.A. and Coker, H.A. (2008). Phytochemical and antioxidant screening of some plants of Apocynaceae from South West Nigeria. *African Journal of Plant Science*. 2 (10): 124 - 128.

Ayoka, A.O., Akomolafe, R.O., Akinsomisoye, O.S and Ukponmwan, O.E. (2008). Medicinal and economic value of Spondias mombin. *Afr. J. Biomed. Res.* **11**: 129-136.

Besong, E.E., Balogun, M.E., Djobissie, S.F.A., Obu, D.C and Obimma, J.N. (2016). Review Medicinal and Economic Value of Dialium Guineense. *Afr. J. Biomed. Res.* **19:** 163-170.

Bohnr, M.K. and Kocipai, A. (1994). Flavonoids composition and uses. Smith Sonian Inst. Press, Washiton, D. C. Pp 106 – 109.

Bradeen, J.M., Staub, J.E., Wyse, C., Antonise, R. and Peleman, J. (2001). Towards an expanded and integrated linkage map of cucumber (*Cucumis sativus* L.). *Genome*. **44:** 111–119.

Burger, Y., Katzire, N., Tzuri, G., Portnoy, V., Saar, U., Shriber, S., Perl-Treves, R. and Cohen, R. (2003). Variation in the response of melon genotypes to *Fusarium oxysporum* f. sp. *Melonis* race 1 determined by inoculation test and molecular markers. *Plant Pathology*. **52:** 204–211.

Byers, R.E., Baker, L.R., Sell, H.M, Herner, R.C. and Dilley, D.R. (1972). Female flower induction on androecious cucumber, *Cucumis sativus* L. *Journal of American Society of Horticultural Science*. **98:** 197–199.

Capoor, S.P. and Verma, L.M. (1948). A mosaic disease of *Lagenaria vulgaris* in the Bombay province. *Current Science*. **17:** 274–275.

Chen, J.F., Isshiki, S. Tashiro, Y. and Miyazaki, S. (1995). Studies on a wild cucumber from China (*Cucumis hystrix* Chakr.).1. Genetic distances between C. *hystrix* and two cultivated *Cucumis* species (*C. sativus* L. and *C. melo* L.) based on isozyme analysis. *Journal of the Japanese Society for Horticultural Science*. **64 (2):** 264–265.

Chen, J.F., Staub, J.E., Tashiro, Y., Isshiki, S. and Miyazaki, S. (1997a). Successful interspecific hybridization between *Cucumis sativus* L. and *Cucumis hystrix* Chakr. *Euphytica*. **96:** 413–419.

Chen, J.F., Zhang, Sh.L. and Zhang, X.G. (1994). The xishuangbanna gourd (*Cucumis sativus* var. xishuangbannesis Qi et Yuan), a traditionally cultivated plant of the Hanai people. Xishuangbanna, Yunnan, China. *Report of the Cucurbit Genetics Cooperative*. **17:** 18–20.

Chikezie, P.C., Ojiako, O.A. and Nwufo, K.C. (2015). Overview of Anti-Diabetic Medicinal Plants: The Nigerian research experiment. *J. Diabetes Metab.* 6: 546 - 570.

Choudhury, B. and Pathak, S.C. (1959). Studies on floral biology in cucumber (*Cucumis sativus .L) Indian Journal of Horticulture. 18: 212–221.*

Cicco, N., Lanorte, M.T., Paraggio, M., Viggiano, M. and Lattanzio, V. (2009). A reproducible, rapid and inexpensive Folin-Ciocalteu micromethod in determining phenolics of plant methanol extracts. *Microchemical Journal*. 91: 107 – 110.

Cordell, G.A. (1983). The Chemistry of Heterocyclic compounds: Indoles, Part 4. The monoterpenoid Indole Alkaloids. Vol 25.

Cramer, C.S. and Wehner, T.C. (1998). Fruit yield and yield component means and correlations of four slicing cucumber populations improved through six to ten cycles of recurrent selection*. Journal of American Society of Horticultural Science*. 123: 388–39

Dane, F. (1991). Cyotgenetics in genus *Cucumis*. *In*: *Chromosome engineering in plants: Genetics, Breeding and Evolution* Part B (*Eds*. Tsuchiya, T. and Gupta, P.K.), Elsevier, Amsterdam. Pp. 201–214.

Deakin, J.R., Bohn, G.W. and Whitaker, T.W. (1971). Interspecific hybridization in *Cucumis*. *Economic Botany*. **25:** 195–211

Dijkhuizen, A., Kennard, W.C., Havey, M.J. and Staub, J.E. (1996). RFLP variability and genetic relationships in cultivated cucumber. *Euphytica*. **90:** 79–89.

Dolan M.E (1997): Inhibition of DNA repair as a means of increasing the antitumor activity of DNA active agents. *Advanced Drug Delivery Reviews*. **26**: 105–118.

Dutta, S. (1966). *Horticulture in Eastern Region of India*. Ministry of Food and Agriculture, New Delhi. Fanourakis, N.E. and Simon, P.W. (1987). Analysis of genetic linkage in cucumber. *Journal of Heredity.* **78:** 238–242.

Edeoga, H.O. and Eriata, D.O. (2001). Alkaloid, tannin and saponin components of some Nigerian medicinal plants. *Journal of Medicinal and Aromatic plant science*, **23**: 244249.

El-Olemy, M.M., Farid, J.A. and Abdel-Fattah, A.A. (1994). Experimental Phytochemistry. A Laboratory Manual. (King Saud University Press, KSA). Pp. 8 - 9.

Evans, W.C. (1997): Trease and Evans Pharmacology. 7. Fixed oils and fat (Kokate, 1999): By Spots test. 14th edn. Harcourt Brace and company. Asia. Pvt. Ltd. 8. Gum and mucilage (Whistler and BeMiller, 1993). Singapore.

Fazio, G., Chung, S.M. and Staub, J.E. (2003a). Comparative analysis of response to phenotypic and marker-assisted selection for multiple lateral branching in cucumber (*Cucumis sativus* L.). *Theoretical and Applied Genetics*. **107:** 875–883.

Fazio, G., Staub, J.E. and Stevens, M.R. (2003b). Genetic mapping and QTL analysis of horticultural traits in cucumber (*Cucumis sativus* L.) using recombinant inbred lines. *Theoretical and Applied Genetics*. **107:** 864–874.

Finar, I.L. (1986). Organic Chemistry: The Fundamental Principles Volume 1. Longman publishing company, London

Franco, J., Crossa, J., Ribaut, J.M., Betran, J., Warburton, M.L. and Khairallah, M. (2001). A method for combining molecular markers and phenotypic attributes for classifying plant genotypes. *Theoretical and Applied Genetics.* **103:** 944–952.

Galun, E. (1959). Effects of gibberellic acid and naphthalene acetic acid on sex expression and some morphological characters in the cucumber plant. *Phyton.* **13:** 1–8.

Galun, E. (1961). Study of the inheritance of sex expression in the cucumber-the interaction of major genes with modifying genetic and non-genetic factors. *Genetica*. **32:** 134–163.

Ghasemi, K., Ghasemi, Y. and Ebrahimzadeh, M.A. (2009). Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pakistan Journal of Pharmaceutical Science.* 22 (3): 277 – 281.

Gill, H.S, Singh, J.P. and Pachuri, D.C. (1973). “Pusa Sanyog” out yields other cucumber. *Indian Horticulture.* **18:** 11.

Harborne, J.B. (1998). Textbook of Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 5th Edition, Chapman and Hall Ltd, London. Pp. 21 - 72.

Hegazy, A.E. and Ibrahium, M.I. (2012). Antioxidant activities of orange peel extracts. *World Applied Sciences Journal.* 18 (5): 684 - 688.

Horejsi, T. and Staub, J.E. (1999). Genetic variation in cucumber (*Cucumis sativus* L.) as assessed by random amplified polymorphic DNA. *Genetic Resources and Crop Evolution*. **46:** 337–350.

Horejsi, T., Staub, J.E. and Thomas, C.E. (2000). Linkage of random amplified polymorphic DNA markers to downy mildew resistance in cucumber (*Cucumis sativus* L.). *Euphytica*. **115:** 105–113.

Horst, E.K. and Lower, R.L. (1978). *Cucumis hardwickii*, a source of germplasm for the cucumber breeder. *Report of the Cucurbit Genetics Cooperative.* **1:** 5.

Icraf Agroforestry database (2002). View crop: Dialium guineese Food and Agriculture *Organisation of the UN*. Copy Right 2002-2007.

Isshiki, S., Okubo, H. and Fujeda, K. (1992). Isozyme variation in cucumber (*Cucumis sativus* L.). *Journal of the Japanese Society for Horticultural Science*. **61:** 595–601.

Iwu, M.M. (1983). Hypoglycemic properties of *Bridelia furruginear* leaves. *Fitoterapia*. 54: 243 - 248.

Jeffrey, C. (1980). A review of the Cucurbitaceae. *Botanical Journal of Linnean Society*. **81:** 233–247.

Kamachi, S., Mizusawa, H., Mazuura, S. and Sakai, S. (2000). Expression of two 1-aminocyclopropane-1-carboxylate synthase genes, CS-ACS1 and CS-ASC2, correlated with sex phenotypes in *Cucumis* plants (*Cucumis sativus* L.). *Plant Biotechnology.* **17:** 69–74.

Kamba, A.S. and Hassan, L.G. (2010). Phytochemical screening and antimicrobial activities of Euphorbia balasamifera leaves, stem and root against some pathogenic microorganisms. *Africa Journal Pharmaceutical Science* **(1)**, 5764.

Kaur, J. and Jhooty, J.S. (1986). Pathological specialization in *Sphaerotheca fuliginea* causing powdery mildew of cucurbits. *Indian Phytopathology.* **39:** 297–299.

Kennard, W.C. and Havey, M.J. (1995). Quantitative trait analysis of fruit quality in cucumber: QTL detection, confirmation, and comparison with mating design variation. *Theoretical and Applied Genetics*. **91:** 53–61.

Kennard, W.C., Poetter, K., Dijkhuizen, A., Meglic, V., Staub, J.E. and Havey, M.J. (1994). Linkages among RFLP, RAPD, isozyme, disease resistance, and morphological markers in narrow and wide crosses of cucumber. *Theoretical and Applied Genetics.* **89:** 42–48.

Kirkbride, J.H.Jr. (1993). *Biosystematic monograph of the genus Cucumis* (Cucurbitaceae). Parkway Publishers, Boone, North Carolina, USA.

Knerr, L.D. and Staub, J.E. (1992). Inheritance and linkage relationships of isozyme loci in cucumber (*Cucumis sativus* L.). *Theoretical and Applied Genetics*. **84:** 217–224.

Kokate, C.K. (1999). Phytochemical Methods. *Phytotherapy*, 78: 126 - 129.

Kroon, G.H., Custer, J.B.M., Kho, Y.O. and den Nijs, A.M.P. (1979). Interspecific hybridization in *Cucumis* L. I. Need for genetic variation, biosystematic relations and possibilities to overcome crossing barriers. *Euphytica*. **28:** 723–728.

Kubicki, B. (1969). Investigations on sex determination in cucumber (*Cucumis sativus* L.). *Genetica Polonica*. **10:** 3–143.

Mahesh, T. (2004). Quercetin alleviates stress in streptozotocin- induced diabetic rats. *Phytotherapy Research*. 18 (2): 123 – 127.

Mathai, K. (2000). Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump. Pp. 274 - 275.

Meglic, V. and Staub, J.E. (1996). Inheritance and linkage relationships of allozyme and morphological loci in cucumber (*Cucumis sativus* L.). *Theoretical and Applied Genetics*. **92:** 865–872.

Mibus, H. and Tatlioglu, T. (2004). Molecular characterization and isolation of the *F/f* gene for femaleness in cucumber (*Cucumis sativus* L.). *Theoretical and Applied Genetics*. **109:** 1669–1676.

Michelmore, R.W., Paran, I. and Kesseli, R.V. (1991). Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of National Academy of Science.* **88:** 9828–9832.

Mogana, R., Teng-Jin, K. and Wiart, C. (2013). Anti-inflammatory, anticholinesterase, and antioxidant potential of scopoletin isolated from Canarium patentinervium Miq.(Burseraceae Kunth). Evidence-based complementary and alternative medicine.

More, T.A. and Budgujar, C.D. (2002). Isolation of parthenocarpic tropical gynoecious lines in cucumber (*Cucumis sativus* L.). *Acta Horticulturae*. **588:** 255–260.

Muller, J.L. (1998). Love potions and the ointment of witches: Historical aspects of the nightshade alkaloids. *Clinical toxicology*. 36: 619 - 627.

Munger, H.M. and Robinson, R.W. (1991). Nomenclature of *Cucumis melo* L. *Report of the Cucurbit Genetics Cooperative*. **14:** 43–44.

Ngugi, M. P., Njagi, J. M., Kibiti, C. M. and Miriti, P. M. (2012). Diabetes Management. *Asian Journal of Biochemical and Pharmaceutical Research*. **2** (2) 2012 . pp 375-381

Nwosu M.O. (2000). Plant resources used by traditional women as herbal medicine and cosmetics in southwest Nigeria, *Arzte fur Natur Fahr*. **41**:11.

Obadoni. B. and Ochuko. O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some home state plants in Edo and Delta State of Nigeria. *Glo. J.Pur.A.Sc.* 81: 203 – 208.

Ojezele, M.O. and Agunbiade, S. (2013). Phytochemical Constituents and Medicinal Properties of Different Extracts of *Anacardium occidentale* and Psidium guavaja. *Asian journal of Biomedical and Pharmaceutical Science. 3* (16), 20 - 23.

Okafor, J.C (1975). The place of the wild (uncultivated) fruit and vegetable in Nigeria diet. *Proceedings of National seminar on fruits and vegetables Ibadan, Nigeria*. 262-299

Osagie, A.U. and Eka, O.U. (1998). Mineral elements in plant foods. In: Nutritional quality of Plant foods. Ambik press, Benin City, Edo State, Nigeria. Pp. 8, 14, 43 and 86.

Oyedapo, O.O., Makinde, A.M., Ilesanmi, G.M., Abimbola, E.O., Akinwunmi, K.F. and Akinpelu, B.A. (2015). Biological activities (anti-inflammatory and anti-oxidant) of fractions and methanolic extract of Philonotis hastate (Duby Wijk and MargaDant). *African Journal of Traditional, Complementary and Alternative Medicines*. 12 (4): 50 - 55.

Pandey, K.K. and Pandey P.K. (2003). *Disease of Vegetable Crops and Integrated Management.* Technical Bulletin No. 16, Indian Institute of Vegetable Research, Varanasi.

Pandey, S., Ansari, W.A., Mishra, V.K. Singh, A.K. and Singh, M. (2013). Genetic diversity in Indian cucumber based on microsatellite and morphological markers. *Biochemical Systematics and Ecology.* **51:** 19–27.

Pangalo, K.J. (1929). Critical review of the main literature on the taxonomy, geography and origin of cultivated and partially wild melons. *Trudy po Prikladnoj Botanike*. **23:** 397–442.

Patil, K., Kandhare, A. and Bhise, D. (2012). Effect of aqueous extract of *Cucumis sativus* Linn. fruit in ulcerative colitis in laboratory animals. *Asian Pacific Journal of Tropical Biomedicine.* 2012: S962 - S969.

Peterson, C.E. and Anhder, L.D. (1960). Induction of staminate flowers on gynoecious cucumbers with gibberellic acid. *Science.* **131:** 1673–1674.

Prohp, T.P. and Onoagbe, I.O. (2012). Acute toxicity and dose response studies of aqueous and ethanol extracts of *Triplochiton scleroxylon* k. Schum, (Sterculiaceae). *International Journal of Applied Biology and Pharmaceutical Technology.* 3 (1): 400 – 409.

Przybecki, Z., Kowalczyk, M.E., Witkowicz, J., Filipecki, M. and Siedlecka, E. (2004). Polymorphom of sexually different cucumber (*Cucumis sativus* L.) NIL. *Cellular and Molecular Biology Letters.* **9:** 919–933.

Rai, A.B., Lognathan, M., Halder, J., Venkatravanappa, V. and Naik, P.S. (2014). Ecofriendly approaches for sustainable management of vegetable pests. IIVR Technical Bulletin No. 53, IIVR, Varanasi. Pp. 104.

Rao, A.L.N. and Verma, A. (1974). Transmission studies with green mottle mosaic virus. *Phytopathologische Zeitschrift*. **109:** 325–335.

Robinson, R.W. and Decker-Walters, D. (1997). *Cucurbits*. CAB International, Wallingford, England. Pp. 226.

Roy, R.P. and Saran, S. (1990). Sex expression in the Cucurbitaceae, *In*: *Biology and Utilization of the Cucurbitaceae* (*Eds*. Robinson, R.W. and Jeffery, C.), Cornell University Press. Ithaca, USA. Pp. 251–268.

Rudich, J., Halevy, A.H. and Kedar, N. (1972b). The level of phytohormones in monoecious and gynoecious cucumbers as affected by photoperiod and ethephon. *Plant Physiology*, **50:** 585–590.

Saimbhi, M.S. (1993). Agro techniques for cucurbits. *In*: Advances in Horticulture, Volume 5, Vegetable crops (*Eds*. Chadha, K.L. and Kalloo, G.), Malhotra Publishing House, New Delhi. Pp. 401–432.

Satpathy, S., and Rai, S. (2002). Luring ability of indigenous food baits for fruit fly (*Bactrocera cucurbitae* Coq.). *Journal of Entomological Research*. **26:** 249–252.

Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A. (2013). Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry*. 1 (6).

Serquen, F.C., Bacher, J. and Staub, J.E. (1997a). Genetic analysis of yield components in cucumber (*Cucumis sativus* L.) at low plant density. *Journal of American Society of Horticultural Science*. **122:** 522–528.

Serquen, F.C., Bacher, J. and Staub, J.E. (1997b). Mapping and QTL analysis of a narrow cross in cucumber (*Cucumis sativus* L.) using random amplified polymorphic DNA markers. *Molecular Breeding*. **3:** 257–268.

Seshadri, V.S. (1986). Cucurbits. *In*: *Vegetable crops in India* (*Eds*. Bose, T.K. and Som, M.G.), Naya Prokash, Calcutta. Pp. 128–176.

Shifriss, O. (1961). Sex control in cucumbers. *Journal of Heredity*. **52:** 5–12.

Singh, R.K. and Choudhury, B. (1998). Differential response of chemical on sex modification of genera of cucurbits. *Indian Journal of Horticulture*. **45:** 84–89.

Singh, H. and Gill, K.S. (1987). *Procedures for breeder and foundation seed production in vegetable crops*. Punjab Agricultural University, Ludhiana. Pp. 78.

Sofowora, A.O. (1993). *Medicinal Plants and Traditional Medicine in Africa*. 2nd ed. University of Ife Press. Pp 320.

Sohi, H.S. and Maholay, M.N. (1974). Studies on mycoflora of watermelon seed. *Indian Journal of Mycology and Plant Pathology*. **14:** 1–34.

Sohi, H.S. (1984). Present status of our knowledge of important fungal diseases of selected vegetables in India and future needs: Presidential address. *Indian Journal of Mycology and Plant Pathology.* **4:** 25–28.

Stanghellini, M.S., Ambrose, J.T. and Schultheis, J.R. (1998). Using commercial bumble bee colonies as backup pollinators for honey bees to produce cucumbers and watermelons. *Hort. Technology.* **8**: 590–594.

Staub, J.E. and Bacher, J. (1997). Cucumber as a processed vegetable. *In*: *Processing Vegetables: Science and Technology* (*Eds*. Smith, D.S., Ash, J.N., Nip, W. and Hui, Y.H.), Technomic Publishing Co., Inc. Lancaster, PA. Pp. 129–193.

Staub, J.E. and Meglic, V. (1993). Molecular genetic markers and their legal relevance for cultivar discrimination: A case study in cucumber. *Horticultural Technology*. **3:** 291–300.

Staub, J.E., Knerr, L.D. and Hopen, H.J. (1992). Effects of plant diversity and herbicides on cucumber productivity. *Journal of the American Society for Horticultural Science*. **117:** 48–53.

Sun, B., Ricardo-da-Silva, J.M. and Spranger, I. (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. Journal of agricultural and food chemistry. 46 (10): 4267 - 4274.

Tan, Z. and Reinhold-Hurek, B. (2003). Dechlorosoma suillum is a later subjective synonym of *Azospira oryzae*. *Int J Syst Evol Microbio*l. 53 (4): 1139 – 1142.

Tatlioglu, T. (1993). Cucumber *Cucumis sativus* L. *In*: Genetic Improvement of Vegetable Crops (*Eds.* Kalloo, G. and Bergh, B.O.), Pergamon Press Ltd., Oxford, New York. Pp. 197–234.

Taylor, J.L.S., Rabe, T., Mcgaw, L.J., Lager, A.k., Van Staden, J. (2001). Toward the scientific validation of traditional medicinal plants. *Plant Growth Regulation* **34**:23-37.

Trebitsh, T., Staub, J.E. and O'Neill, S.D. (1997). Identification of a 1-aminocyclopropane-1-carboxylic acid synthase gene linked to the female (*F*) locus that enhances female sex expression in cucumber. *Plant Physiology*. **113:** 987–995.

Vakalounakis, D.J. (1992). Heart leaf, a recessive leaf shape marker in cucumber: Linkage with disease resistance and other traits. *Journal of Heredity*. **83:** 217–221.

Van-Buren, J.P. and Robinson, W.B. (1981). Formation of complexes between protein and tannic acid. *Journal of Agricultural Food Chemistry*. 17: 772 – 777.

Vani, S. and Verma, A. (1988). Viral disease problem in muskmelon. *Proceedings of the National Symposium on Plant Virus Problems in India*, IARI, New Delhi.

Vasudeva, R.S., Raychaudhuri, S.P. and Singh, J. (1950). A new strain of *Cucumis* virus of melon. *Indian Phytopathology*. **2:** 180–185.

Wang, Y.H., Thomas, C.E. and Dean, R.A. (2000). Genetic mapping of a *Fusarium* wilt resistance gene (*Fom-2*) in melon (*Cucumi melo* L.). *Molecular Breeding*. **6:** 379–389.

Yermakov, A.I., Arasimov, V.V. and Yarosh, N.P. (1987). Methods of biochemical analysis of plants. Leningrad: Agropromizdat (in Russian). Pp. 55 - 60.

Yıldırım, S., Topaloglu, N., Tekin, M., Kucuk, A., Erdem, H. and Erbaş, M. (2015). Protective role of Proanthocyanidin in experimental ovarian torsion. *Medical Journal of the Islamic Republic of Iran.* 29: 185.

APPENDICES

APPENDIX 1

ANOVA SUMMARY FOR PROXIMATE ANALYSIS

Descriptive								
VAR00002								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	96.3333	.47258	.27285	95.1594	97.5073	95.80	96.70
2	3	7.6867	.58227	.33617	6.2402	9.1331	7.26	8.35
3	3	92.3133	.58227	.33617	90.8669	93.7598	91.65	92.74
4	3	3.8933	.38397	.22169	2.9395	4.8472	3.45	4.12
5	3	7.9133	.41885	.24182	6.8729	8.9538	7.43	8.17
6	3	3.0000	.41328	.23861	1.9734	4.0266	2.54	3.34
7	3	24.2500	1.39194	.80364	20.7922	27.7078	22.75	25.50
8	3	53.5900	1.79268	1.03500	49.1367	58.0433	51.52	54.63
Total	24	36.1225	37.83263	7.72255	20.1472	52.0978	2.54	96.70

Multiple Comparisons							
Dependent Variable:VAR00002							
	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	88.64667*	.73859	.000	87.0809	90.2124
		3	4.02000*	.73859	.000	2.4542	5.5858
		4	92.44000*	.73859	.000	90.8742	94.0058
		5	88.42000*	.73859	.000	86.8542	89.9858
		6	93.33333*	.73859	.000	91.7676	94.8991
		7	72.08333*	.73859	.000	70.5176	73.6491
		8	42.74333*	.73859	.000	41.1776	44.3091
	2	1	-88.64667*	.73859	.000	-90.2124	-87.0809
		3	-84.62667*	.73859	.000	-86.1924	-83.0609
		4	3.79333*	.73859	.000	2.2276	5.3591
		5	-.22667	.73859	.763	-1.7924	1.3391
		6	4.68667*	.73859	.000	3.1209	6.2524
		7	-16.56333*	.73859	.000	-18.1291	-14.9976
		8	-45.90333*	.73859	.000	-47.4691	-44.3376
	3	1	-4.02000*	.73859	.000	-5.5858	-2.4542
		2	84.62667*	.73859	.000	83.0609	86.1924
		4	88.42000*	.73859	.000	86.8542	89.9858
		5	84.40000*	.73859	.000	82.8342	85.9658
		6	89.31333*	.73859	.000	87.7476	90.8791
		7	68.06333*	.73859	.000	66.4976	69.6291
		8	38.72333*	.73859	.000	37.1576	40.2891
	4	1	-92.44000*	.73859	.000	-94.0058	-90.8742
		2	-3.79333*	.73859	.000	-5.3591	-2.2276
		3	-88.42000*	.73859	.000	-89.9858	-86.8542
		5	-4.02000*	.73859	.000	-5.5858	-2.4542
		6	.89333	.73859	.244	-.6724	2.4591
		7	-20.35667*	.73859	.000	-21.9224	-18.7909
		8	-49.69667*	.73859	.000	-51.2624	-48.1309
	5	1	-88.42000*	.73859	.000	-89.9858	-86.8542
		2	.22667	.73859	.763	-1.3391	1.7924
		3	-84.40000*	.73859	.000	-85.9658	-82.8342
		4	4.02000*	.73859	.000	2.4542	5.5858
		6	4.91333*	.73859	.000	3.3476	6.4791

		7	-16.33667*	.73859	.000	-17.9024	-14.7709
		8	-45.67667*	.73859	.000	-47.2424	-44.1109
	6	1	-93.33333*	.73859	.000	-94.8991	-91.7676
		2	-4.68667*	.73859	.000	-6.2524	-3.1209
		3	-89.31333*	.73859	.000	-90.8791	-87.7476
		4	-.89333	.73859	.244	-2.4591	.6724
		5	-4.91333*	.73859	.000	-6.4791	-3.3476
		7	-21.25000*	.73859	.000	-22.8158	-19.6842
		8	-50.59000*	.73859	.000	-52.1558	-49.0242
	7	1	-72.08333*	.73859	.000	-73.6491	-70.5176
		2	16.56333*	.73859	.000	14.9976	18.1291
		3	-68.06333*	.73859	.000	-69.6291	-66.4976
		4	20.35667*	.73859	.000	18.7909	21.9224
		5	16.33667*	.73859	.000	14.7709	17.9024
		6	21.25000*	.73859	.000	19.6842	22.8158
		8	-29.34000*	.73859	.000	-30.9058	-27.7742
	8	1	-42.74333*	.73859	.000	-44.3091	-41.1776
		2	45.90333*	.73859	.000	44.3376	47.4691
		3	-38.72333*	.73859	.000	-40.2891	-37.1576
		4	49.69667*	.73859	.000	48.1309	51.2624
		5	45.67667*	.73859	.000	44.1109	47.2424
		6	50.59000*	.73859	.000	49.0242	52.1558
		7	29.34000*	.73859	.000	27.7742	30.9058

*. The mean difference is significant at the 0.05 level.

APPENDIX 2

ANOVA SUMMARY FOR QUANTITATIVE PHYTOCHEMICAL SCREENING

Descriptive								
VAR00002								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	6.1333	.03215	.01856	6.0535	6.2132	6.11	6.17
2	3	2.6000	.21213	.15000	.6941	4.5059	2.45	2.75
3	3	11.7900	1.25575	.72501	8.6706	14.9094	10.34	12.52
4	3	3.7500	.49000	.28290	2.5328	4.9672	3.26	4.24
5	3	8.5033	.63799	.36834	6.9185	10.0882	8.13	9.24
6	3	2.1267	.02517	.01453	2.0642	2.1892	2.10	2.15
7	3	2.8700	.20809	.12014	2.3531	3.3869	2.74	3.11
Total	20	5.5360	3.49744	.78205	3.8991	7.1729	2.10	12.52

Multiple Comparisons							
Dependent Variable:VAR00002							
	(I) VAR 00001	(J) VAR 00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	3.53333*	.54202	.000	2.3624	4.7043
		3	-5.65667*	.48479	.000	-6.7040	-4.6093
		4	2.38333*	.48479	.000	1.3360	3.4307
		5	-2.37000*	.48479	.000	-3.4173	-1.3227
		6	4.00667*	.48479	.000	2.9593	5.0540
		7	3.26333*	.48479	.000	2.2160	4.3107
	2	1	-3.53333*	.54202	.000	-4.7043	-2.3624

		3	-9.19000*	.54202	.000	-10.3610	-8.0190
		4	-1.15000	.54202	.054	-2.3210	.0210
		5	-5.90333*	.54202	.000	-7.0743	-4.7324
		6	.47333	.54202	.398	-.6976	1.6443
		7	-.27000	.54202	.627	-1.4410	.9010
	3	1	5.65667*	.48479	.000	4.6093	6.7040
		2	9.19000*	.54202	.000	8.0190	10.3610
		4	8.04000*	.48479	.000	6.9927	9.0873
		5	3.28667*	.48479	.000	2.2393	4.3340
		6	9.66333*	.48479	.000	8.6160	10.7107
		7	8.92000*	.48479	.000	7.8727	9.9673
	4	1	-2.38333*	.48479	.000	-3.4307	-1.3360
		2	1.15000	.54202	.054	-.0210	2.3210
		3	-8.04000*	.48479	.000	-9.0873	-6.9927
		5	-4.75333*	.48479	.000	-5.8007	-3.7060
		6	1.62333*	.48479	.005	.5760	2.6707
		7	.88000	.48479	.093	-.1673	1.9273
	5	1	2.37000*	.48479	.000	1.3227	3.4173
		2	5.90333*	.54202	.000	4.7324	7.0743
		3	-3.28667*	.48479	.000	-4.3340	-2.2393
		4	4.75333*	.48479	.000	3.7060	5.8007
		6	6.37667*	.48479	.000	5.3293	7.4240
		7	5.63333*	.48479	.000	4.5860	6.6807
	6	1	-4.00667*	.48479	.000	-5.0540	-2.9593
		2	-.47333	.54202	.398	-1.6443	.6976
		3	-9.66333*	.48479	.000	-10.7107	-8.6160
		4	-1.62333*	.48479	.005	-2.6707	-.5760
		5	-6.37667*	.48479	.000	-7.4240	-5.3293
		7	-.74333	.48479	.149	-1.7907	.3040
	7	1	-3.26333*	.48479	.000	-4.3107	-2.2160
		2	.27000	.54202	.627	-.9010	1.4410
		3	-8.92000*	.48479	.000	-9.9673	-7.8727
		4	-.88000	.48479	.093	-1.9273	.1673
		5	-5.63333*	.48479	.000	-6.6807	-4.5860
		6	.74333	.48479	.149	-.3040	1.7907

*. The mean difference is significant at the 0.05 level.

APPENDIX 3

SUMMARY OF ANOVA FOR PHENOLIC CONTENT

Descriptive								
VAR00002								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	.5543	.24500	.14145	-.0543	1.1630	.31	.80
2	2	.8540	.10748	.07600	-.1117	1.8197	.78	.93
3	3	.7133	.13796	.07965	.3706	1.0560	.61	.87
4	3	.3810	.20785	.12000	-.1353	.8973	.20	.61
5	3	.7200	.07550	.04359	.5325	.9075	.65	.80
6	3	.1047	.07656	.04420	-.0855	.2949	.03	.19
7	3	.3667	.01528	.00882	.3287	.4046	.35	.38
8	3	.2067	.01155	.00667	.1780	.2354	.20	.22
Total	23	.4717	.27182	.05668	.3541	.5892	.03	.93

Table 1: Phenolic Content of the Extracts as Extrapolated from the Calibration Curve

Conc of Extract	**(mg/mL)**	**Absorbance**	
Aqueous	**Ethanol**	**Aqueous**	**Ethanol**
0.310	0.934	0.127	0.868
0.799	0.778	0.316	0.808
0.553	1.001	0.221	0.794
0.5543 ± 0.14145	**0.8540 ± 0.0760**		

Table 2: Total Flavonoid Content of the Extracts as Extrapolated from the Standard Calibration Curve

Conc of extract (mg/mL)		Absorbance	
Aqueous	Ethanol	Aqueous	Ethanol
0.608	0.656	1.625	1.209
0.335	0.873	1.698	1.117
0.198	0.608	1.409	1.071
0.7133 ± 0.0797	**0.3810 ± 0.1200**		

Table 3: Total Flavonol Content of the Extracts as Extrapolated from the Standard Calibration Curve

Conc of Extract (mg/mL)		Absorbance at 440 nm	
Aqueous	Ethanol	Aqueous	Ethanol
0.796	0.034	0.972	1.016
0.706	0.186	0.942	0.467
0.650	0.094	0.923	0.336
0.7200 ± 0.0436	**0.1047 ± 0.0442**		

Table 4: Proanthocyanidin Content of the Extracts as Extrapolated from the Standard Calibration Curve

Conc of Extract (mg/mL)		Absorbance at 500 nm	
Aqueous	Ethanol	Aqueous	Ethanol
0.354	0.224	1.696	0.432
0.382	0.204	1.753	0.392
0.376	0.202	1.740	0.388
0.3667 ± 0.0153	**0.2067 ± 0.0067**		

Multiple Comparisons							
Dependent Variable:VAR00002							
	(I) VAR 00001	(J) VAR 00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1	2	-.29967*	.12471	.030	-.5655	-.0339
		3	-.15900	.11154	.174	-.3967	.0787
		4	.17333	.11154	.141	-.0644	.4111
		5	-.16567	.11154	.158	-.4034	.0721
		6	.44967*	.11154	.001	.2119	.6874
		7	.18767	.11154	.113	-.0501	.4254
		8	.34767*	.11154	.007	.1099	.5854
	2	1	.29967*	.12471	.030	.0339	.5655
		3	.14067	.12471	.277	-.1251	.4065
		4	.47300*	.12471	.002	.2072	.7388
		5	.13400	.12471	.300	-.1318	.3998
		6	.74933*	.12471	.000	.4835	1.0151
		7	.48733*	.12471	.001	.2215	.7531
		8	.64733*	.12471	.000	.3815	.9131
	3	1	.15900	.11154	.174	-.0787	.3967
		2	-.14067	.12471	.277	-.4065	.1251
		4	.33233*	.11154	.009	.0946	.5701
		5	-.00667	.11154	.953	-.2444	.2311
		6	.60867*	.11154	.000	.3709	.8464
		7	.34667*	.11154	.007	.1089	.5844
		8	.50667*	.11154	.000	.2689	.7444
	4	1	-.17333	.11154	.141	-.4111	.0644
		2	-.47300*	.12471	.002	-.7388	-.2072
		3	-.33233*	.11154	.009	-.5701	-.0946
		5	-.33900*	.11154	.008	-.5767	-.1013
		6	.27633*	.11154	.026	.0386	.5141
		7	.01433	.11154	.899	-.2234	.2521
		8	.17433	.11154	.139	-.0634	.4121
	5	1	.16567	.11154	.158	-.0721	.4034
		2	-.13400	.12471	.300	-.3998	.1318

		3	.00667	.11154	.953	-.2311	.2444
		4	.33900*	.11154	.008	.1013	.5767
		6	.61533*	.11154	.000	.3776	.8531
		7	.35333*	.11154	.006	.1156	.5911
		8	.51333*	.11154	.000	.2756	.7511
	6	1	-.44967*	.11154	.001	-.6874	-.2119
		2	-.74933*	.12471	.000	-1.0151	-.4835
		3	-.60867*	.11154	.000	-.8464	-.3709
		4	-.27633*	.11154	.026	-.5141	-.0386
		5	-.61533*	.11154	.000	-.8531	-.3776
		7	-.26200*	.11154	.033	-.4997	-.0243
		8	-.10200	.11154	.375	-.3397	.1357
	7	1	-.18767	.11154	.113	-.4254	.0501
		2	-.48733*	.12471	.001	-.7531	-.2215
		3	-.34667*	.11154	.007	-.5844	-.1089
		4	-.01433	.11154	.899	-.2521	.2234
		5	-.35333*	.11154	.006	-.5911	-.1156
		6	.26200*	.11154	.033	.0243	.4997
		8	.16000	.11154	.172	-.0777	.3977
	8	1	-.34767*	.11154	.007	-.5854	-.1099
		2	-.64733*	.12471	.000	-.9131	-.3815
		3	-.50667*	.11154	.000	-.7444	-.2689
		4	-.17433	.11154	.139	-.4121	.0634
		5	-.51333*	.11154	.000	-.7511	-.2756
		6	.10200	.11154	.375	-.1357	.3397
		7	-.16000	.11154	.172	-.3977	.0777

*. The mean difference is significant at the 0.05 level.