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Full Length Research Article

Evaluation Of Vitamin Composition and Anti-Inflammatory Properties of Cucumber (*Cucumis Sativus*) Peels.

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ABSTRACT

Inadequate vitamins in humans can cause significant impairment in cellular and immune functions, as well as trigger inflammatory responses. Boosting immunity with vitamins helps in prevention and treatment of many diseases. There is a need to search for diets rich in vitamins which can boost immunity. This study explored vitamin composition and anti-inflammatory properties of cucumber peels using standard methods. The *in-vitro* anti-inflammatory effects were measured on the ability of the ethanol extract of cucumber peels to inhibit proteinase activities, albumin denaturation, and stabilize erythrocyte membrane, using blood samples from laboratory rat, while Aspirin and Diclophenac Sodium served as reference drugs. Vitamins found in cucumber peels were A (0.35 ± 0.03 mg/g), β-carotene (0.86±0.04mg/g), B_1 (1.14±0.38mg/g), B_2 (0.24±0.02mg/g), B_3 (0.71±0.03mg/g), B_6 (1.04±0.06mg/g), B_9 (0.66±0.01mg/g), C (1.58±0.01mg/g), D (1.11±0.01mg/g), E (0.54±0.01mg/g), and K (0.78±0.01mg/g). The extracts inhibited proteinase activity, albumin denaturation, and stabilization of erythrocyte membrane in a concentration-dependent manner, and recorded maximum activities of 19.14% anti-proteinase, 26.78% inhibition of albumin denaturation, 12.92% inhibition of heat-induced haemolysis, and 26.90% inhibition of hypotonicity-induced haemolysis at the highest concentration of 500µg/ml. These results indicated that cucumber peels are good sources of vitamins and possess anti-inflammatory properties.

Keywords: Cucumber, vitamins, anti-inflammation, membrane stabilization, immunity.

INTRODUCTION

Fruits and vegetables are important sources of nutrients that synergistically contribute to the health and nutritional benefits of foods. Epidemiological studies (Hu, 2003; Ikram *et al*., 2009) have

reported positive association between fruit and vegetable intake and reduced cardiovascular diseases and certain cancers. Vitamins are essential nutrients that cannot be synthesized in sufficient amounts to meet bodily needs; and must be obtained from either dietary or synthetic sources (Jane *et al.*, 1983). Functions of vitamins are generally of a catalytic or regulatory nature, facilitating or controlling vital biochemical processes in the cell. Absence of vitamin from the diet or improper absorption by the body can lead to a specific deficiency or disease condition Deficiency of certain vitamins such as vitamin D has been implicated in types 1 and 2 diabetes mellitus, rheumatoid arthritis, cardiovascular disease, osteoporosis, multiple sclera.sis, depression, irritable bowel disease, asthma, colorectal, lung and breast cancers, upper respiratory tract infections, tuberculosis (TB), and HIV/AIDS progression and mortality (Finklea *et al*., 2011; Fleet *et al*., 2012; Meems *et al*., 2011).The most recognized functions of the vitamin D metabolic and signaling system relate to its classical effects on musculoskeletal health (Haussler *et al*., 2012). There has been an exponential increase in studies of non-classical, extra-skeletal actions of vitamin D (Christakos *et al*., 2013). The potential role of vitamin D as an endogenous regulator of both innate and adaptive immunity has garnered considerable attention because of the apparent prevalence of vitamin D deficiency in communities where coronavirus disease 2019 (COVID-19) infection and disease severity are equally pronounced (Bishop *et al*., 2021). Innovative investigation into the role of vitamins in physiology and pathophysiology and newer concepts of biological actions of vitamins are being explored.

There is exciting potential development in the analgesic and anti-inflammatory properties of vitamins. Inflammation response elicited by the vascular tissues of the body to injurious stimulant is a complex process, which is frequently associated with pain and leads to increase of vascular permeability, increase of protein denaturation and membrane alteration (Gunathilake *et al*., 2018). Pain and inflammation involve a complex array of biochemical processes such as enzyme activation, inflammatory mediator release and extravasation of fluid, cell migration, tissue damage and repair (Medzhitov, 2008). Despite the availability of drugs and the usage in treatment, side effects of analgesic and anti-inflammatory agents which include gastrointestinal upset, gastric ulcer, bleeding, and liver damage are of major concern in clinical practice.

The management of vitamin deficiency and inflammation-related diseases with medicinal plants seems like a Herculean task but exploring the benefits of cucumber (*Cucumis sativus*) fruit in that regard deserves consideration. Cucumber belongs to the gourd family "cucurbitaceae" which includes important crops such as melon, watermelon and squash (Virek *et al*., 2017). There are several varieties of cucumber, but the edible cucumber is classified under two groups, the slicing cucumber and pickling cucumbers (Eifediyi *et al*., 2010). The plant has large leaves that form a canopy over the fruit. The fruit of the cucumber is roughly cylindrical, elongated with tapered ends, and may be up to 60 cm (24 inches) long and 10 cm (3.9 inches) in diameter - with an enclosed seed and developing from flowers - and can be classified botanically as an accessory fruit (Huang *et al*., 2009). There is increased consumption of cucumber fruits possibly because of their perceived high content of fibre which is important for the digestive system as

well as the seeds which are highly nourishing (Gogte, 2000). So, it is expedient to explore the potentials of cucumber peels for therapeutic use. In this study, the vitamin composition and the anti-inflammatory effects of cucumber peels were studied their potential value in boosting immunity.

MATERIALS AND METHODS

Collection, processing and extraction of cucumber peels: Cucumber fruits procured from the major market in Enugu were authenticated at the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology. After washing thoroughly with clean water, the green cucumber fruits were peeled off with sharp knife, and air dried for two weeks before the dried sample was pulverized with an electric blender. One hundred grams (100g) of the powered sample was weighed out and kept under a cool dry condition prior to extraction. Five grams of the ground cucumber peel was thereafter soaked in 50ml of 70% ethanol and later placed in HY-4A multipurpose Oscillator for one hour. After one hour of shaking, the mixture was allowed to stand for 24h at room temperature before it was filtered through whatman paper No. 4. The filtrate was evaporated at 78^{o}C using a water bath (Techmel and Techmel, 420. USA), and the dried residue was weighed and reconstituted in 70% ethanol at a concentration of 10mg/ml and stored at 4^{o}C in a refrigerator until further analysis.

Vitamin composition of cucumber peels: The vitamin composition of cucumber peels was determined using the method of AOAC (2005).

Inhibition of albumin denaturation: The inhibition of albumin denaturation was studied according to the method of Leelaprakash and Mohan (2010), with minor modifications. The reaction mixture consists of test extracts and 1% aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted using a small amount of 1N HCl. The cucumber peel extracts were incubated at 37^{o}C for 20 minutes; then heated to 51^{o}C for 20 minutes. After cooling the samples, the turbidity was measured at 660nm (UV-Visible Spectrophotometer Axiom 722). Aspirin was used as standard. The Percentage inhibition of protein denaturation was calculated as:

Percentage inhibition = (Abs Control –Abs Sample) x 100/ Abs control. Where Abs is the absorbance.

Anti-proteinase action: The test was performed according to the modified method of Oyedepo and Femurewa (1995). The reaction mixture (2ml) contained 0.06mg trypsin, 1ml 20mMTrisHCl buffer (pH 7.4) and 1ml of cucumber peel extract of different concentrations (100, 200, 300, 400 and 500 µg/ml). The mixture was incubated at 37^{o}C for 5 minutes and then 1ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 minutes; 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210nm against buffer as blank. Diclophenac Sodium was used as standard. The percentage inhibition of proteinase activity was calculated as:

Percentage inhibition = (Abs control –Abs sample) x 100/ Abs control.

Membrane stabilization: This was carried out according to the method of Leelaprakash and Mohan (2010).

Preparation of Red Blood cells (RBCs) suspension: Blood samples were taken from healthy Wistar Albino rat that has not taken Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) for 2 weeks prior to the experiment and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 minutes and were washed three times with equal volume of normal saline. The volume of blood was measured and re-constituted as 10% v/v suspension with normal saline.

Heat-induced haemolysis: This was done according to the method of Shinde *et al*. (1995). The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (100 - 500µg/ml) and 1ml of 10% RBCs suspension. Instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56ºC for 30 minutes at the end of incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 minutes, and the absorbance of the supernatants was taken at 560nm. The Percentage inhibition of Haemolysis was calculated as:

Percentage inhibition = (Abs control –Abs sample) x 100/ Abs control

Hypotonicity-induced haemolysis: This was done according to the method of Azeem *et al*. (2010). Different concentration of extract (100-500µg/ml), reference sample, and control were separately mixed with 1ml of phosphate buffer, 2ml of hyposaline, and 0.5ml of HRBC suspension. Diclofenac sodium was used as a standard drug. All the assay mixtures were incubated at 37^0c for 30min and centrifuged at 3000rpm. The supernatant liquid was decanted, and the haemoglobin content was estimated by a spectrophotometer at 560nm. The percentage hemolysis was estimated by assuming the haemolysis produced in the control as 100%. Percentage protection = 100- (OD sample/OD control) x 100.

RESULTS

Vitamin Composition of Cucumber peels: Vitamin composition of cucumber peels are shown in Table 1. Vitamins C was the highest in concentration (1.58 ± 0.01 mg/g) while vitamin B2 was the least (0.24 ± 0.02 mg/g)

Table 1: Vitamin Composition of Cucumber peels

Vitamins	Concentration (mg/g)
Vitamin A	0.35 ± 0.03
B-carotene	0.86 ± 0.04
Vitamin B1	1.14 ± 0.38
Vitamin B2	0.24 ±0.02
Vitamin B3	0.71 ± 0.03
Vitamin B6	1.04 ± 0.06
Vitamin B9	0.66 ± 0.01
Vitamin C	1.58 ± 0.01
Vitamin D	1.11 ± 0.01
Vitamin E	0.54 ± 0.01

Vitamin K	0.78 ± 0.01

Inhibition of albumin denaturation (Anti-inflammatory effect): The ability of the extract to inhibit albumin denaturation is shown in Figure 1. The percent inhibition of both extract and standard increased with the concentration but the standard exhibited higher inhibition percentage. The inhibitory concentration at 50% activity (IC_{50}) of the extract was 260µg/ml while that of the standard was 44µg/ml.

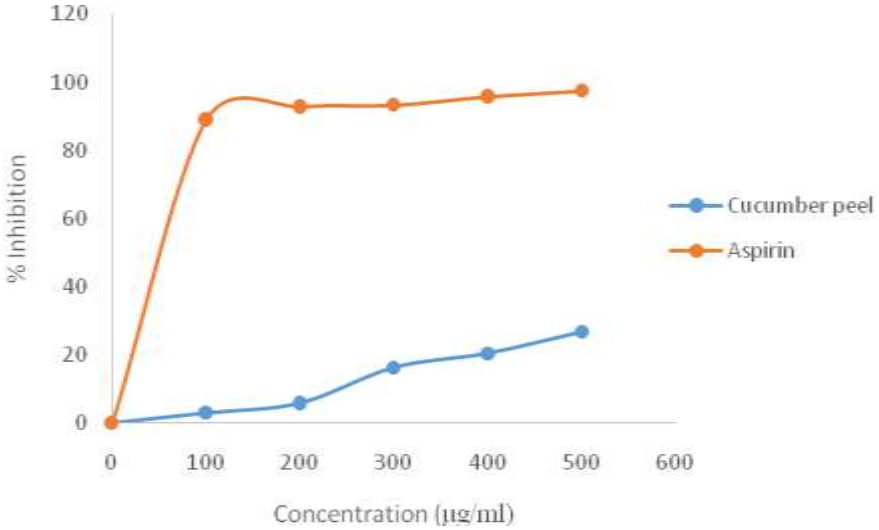

Figure 1: Inhibition of albumin denaturation of ethanol extract of cucumber peels and the standard.

Anti-proteinase activity: The anti-proteinase activity of the extract with that of the standard is presented in Figure 2. Maximum activity (19.14%) of the extract was observed at 500µg/ml comparable with the standard (25.90%). The IC50 of the extract was 290µg while that of the standard was 220µg.

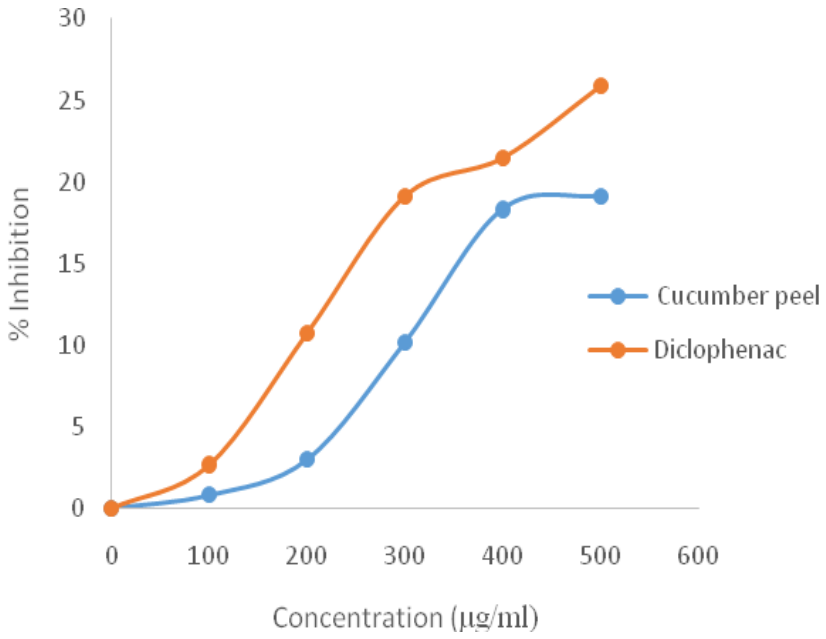

Figure 2: Anti-proteinase activity of ethanol extract of cucumber peels and the standard.

Membrane stabilization ability: Heat-induced and hypotonicity-induced haemolysis of rat erythrocyte were used to assess membrane stabilization ability of the extract and compared with the standard.

Inhibition of heat-induced haemolysis: The *in-vitro* membrane stabilization ability of ethanol extract of cucumber peels and that of the standard on rat erythrocyte exposed to heat are shown in Figure 3. The extract displayed monophasic mode of protection on the erythrocytes (i.e., the extract protected redblood cells from heat-induced Lysis) at all the concentrations tested in dose-dependent manner. The inhibition of haemolysis in erythrocytes by the extract at concentrations of 100, 200, 300, 400 and 500 mg/ml were 1.54, 2.77, 5.17, 11.69 and 12.92% respectively. However, the standard exhibited higher activities of 5.78, 17.16, 25.71, 91.39 and 92.50% respectively, at the same concentrations. The IC_{50} of the extract was 330µg/ml while that of the standard was 328µg/ml.

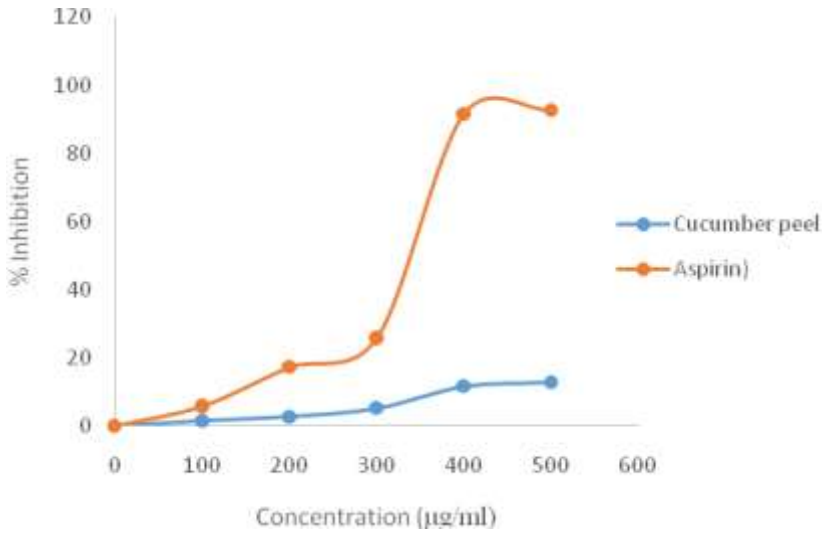

Figure 3: Inhibition of heat-induced haemolysis by ethanol extract of cucumber peels and the standard.

Inhibition of hypotonicity-induced haemolysis: The *in-vitro* membrane stabilization ability of ethanol extract of cucumber peels and that of the standard on rat erythrocyte exposed to hypotonic solution are shown in Figure 4. The extract also displayed monophasic mode of protection on the erythrocytes with percentage inhibition activities of 12.17, 21.74, 26.90 at concentrations of 200, 300, 400 µg/ml respectively. There was no activity at 100µg/ml and no further increase in activity at 500µg/ml. The standard exhibited percentage inhibition activities of 43.48, 65.21, 80.00, 84.35 and 86.09% at the concentrations of 100, 200, 300, 400, and 500µg/ml respectively. The IC_{50} of the extract was 200µg/ml while that of the standard was 100µg/ml.

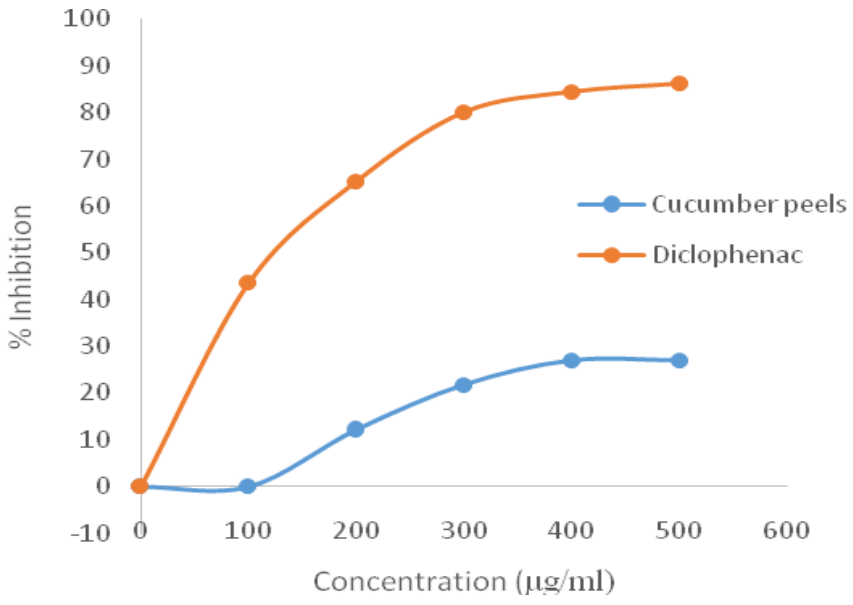

Figure 4: Inhibition of hypotonicity-induced haemolysis by ethanol extract of cucumber peels and the standard.

DISCUSSION

From this study, cucumber peels revealed the presence of various vitamins in different concentrations. Vitamins are classified into two groups based on solubility: water-soluble vitamins and fat-soluble vitamins. The water-soluble vitamins are the B vitamins and vitamin C while the fat-soluble vitamins are vitamins A, D, E and K. The water-soluble vitamins determined were vitamins B1, B2, B3, B6, B9 and C. Their composition was found to be 1.14 ± 0.38, 0.24 ±0.02, 0.71 ± 0.03, 1.04 ± 0.06, 0.66 ± 0.01 and 1.58 ± 0.01 mg/g respectively (Table 1). The B vitamins are well known for their participation in biochemical reactions where they serve as co-enzymes in different metabolic processes. Apart from this basic function, they also play roles in different areas. The immune system is influenced by vitamin B complex, and insufficiency can cause significant impairment in cellular and immune function and trigger an inflammatory response (Babar *et al*., 2021). A lack of vitamin B can seriously alter the function of a cell and immune system that leads to hyperhomocysteinemia inflammation (Babar *et al.*, 2021). Vitamin B helps to reduce inflammation, strengthens respiratory functioning, preserves endothelial homogenity, inhibits hypercoagulation, activate innate and adaptive immune responses properly, and can decrease hospitalisation for long periods of time (Zhang and Liu, 2020).

Notably, are the impact of Vitamins B_1 and B_6. Vitamin B_1 has an impact on anti-inflammatory characteristics, cytochrome C release, mitochondrial membranes, oxidative stress-induced, NF-kappaβ and protein kinases and P38-MAPK. Deficiency of vitamin B1 leads to over expression of proinflammation cytokines like TNF, IL-1, IL-6, and arachidonic acid products, nervous system malfunction, T-cycle infiltration, neuroinflammation, expression CD40 by the microglia and CD40L, causing the loss of astrocytes, beriberi, CL2 chemokine over expression (Spinas *et al*., 2015). Vitamin B6, in addition to its role as a cofactor for many enzymes involved in macromolecular metabolism, exerts a protective effect against chronic diseases such as cardiovascular diseases (CVD) and diabetes by suppressing inflammation, inflammasomes, oxidative stress and carbonyl stress (Zhang and Liu, 2020). Vitamin B-6 played an important role during the last decades in the mechanism for inflammatory and antioxidant activities (Bilski *et al*., 2000). It has been shown from studies that the concentrations of plasma pyridoxal 59-phosphate (PLP), the biologically active form of vitamin B-6), were lowered by inflammation over the idea that low vitamin B-6 status increasesthe risk of inflammation or inflammatory diseases (Ohta and Foote, 2002; Taysi, 2005). Also, PLP may interact with peroxy radicals and sequester free radicals and, through its group of hydroxyls, prevent lipid peroxidation on the pyridine ring (Ohta and Foote, 2002). The PLP plays the role as a coenzyme in the manufacturing, throughout inflammation, of cytokines as well as other multipeptide intermediaries (Friso *et al.*, 2001). Therefore, insufficient vitamin B-6 may diminish its antioxidant potential directly or interfere with inflammatory reactions (Taysi, 2005).

Vitamin C plays a basic role in prevention of scurvy and as an antioxidant. Its immunostimulant and anti-inflammatory roles are well-known (Sorice *et al*., 2014).

The fat-soluble vitamins were also present in high to moderate amounts, vitamin D being the highest in concentration (1.112 ± 0.01 mg/g). Vitamin D plays an important role in the development and maintenance of the skeleton, as well as bone and cartilage metabolism, and its deficiency is implicated in the pathological process of osteoarthritis (Zheng *et al.*, 2018). Prominent among fat soluble vitamins in the proper function of the immune system are vitamins A and E because they also have antioxidant properties. Vitamin E enhances chondrocyte growth and exhibits an anti-inflammatory activity, as well as plays an important role in the prevention of cartilage degeneration (Zheng *et al.*, 2018). Vitamin A has several functions in the body. Between the many functions of vitamin, A, particularly important is its role in lymphocyte function and antibody response to infections (Riccioni *et al.*, 2003). An adequate supply of vitamin A is needed for the normal development of many types of blood cells, including lymphocytes. Networks of cytokines that influence immuneresponses may also be altered during vitamin Adeficiency, along withantibody responses to antigensmay be modified (Semba, 1998; Stephensen *et al.*, 2002). Therefore, the presence of these vitamins suggests that cucumber peels could have anti-inflammatory effects.

The extract in this study exhibited varying anti-inflammatory activities which were dose dependent. From the anti-proteinase assay, the extract inhibited proteinase activity with maximum inhibition of 19.14% at 500 µg/ml and comparable with the standard (25.90%). Proteinases are implicated in tissue damage during inflammatory reactions. They abundantly exist in lysosomal granules of neutrophils. Proteinase inhibitors provide a significant level of protection (Govindappa *et al.*, 2011). Therefore, the ethanol extract of cucumber peels was able to inhibit the activity of these proteinases. Protein denaturation results in loss of biological properties of protein molecules. Proteins could be denatured through application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Protein denaturation has been correlated with the formation of inflammatory disorders like rheumatoid arthritis, diabetes and cancer. Therefore, the ability of a substance to prevent the protein denaturation may also help to prevent the inflammatory disorders (Sangeetha and Vidhya, 2016). The extract of cucumber peels inhibited protein denaturation in a concentration-dependent manner exhibiting maximum inhibition of 26.78% at 500µg/ml (Fig 1). This ability could be linked to the vitamin content of the cucumber peels. Vitamins are strong antioxidants and so their activities could prevent denaturation of protein which results from oxidative stress. From studies it was shown that vitamin E inhibited oxidative stress-induced denaturation of nerve terminal proteins involved in neurotransmission (Kaneai *et al*., 2012).

Membrane stabilization is a process of maintaining the integrityof biological membranes such as erythrocyte and lysosomal membranes against osmotic and heat-induced lysis (Sadique *et al.*, 1989). In this study, stabilization of erythrocyte membranes exposed to hypotonic, and heat induced lysis was employed. When red blood cells are placed in hypotonic solution in which osmolarity is diminished, the gain in red blood cellwater is both instantaneous and quantitative (Anosike *et al*., 2019). This phenomenon is put into practical use in the red blood cell osmotic fragility test, which determines the release of haemoglobin from red blood cellsin hypotonic sodium chloride (NaCl) solution. In this study, the membrane stabilization effect of various

concentrations of the ethanol extract of cucumber peels on rat erythrocyte exposed to hypotonic and heat-induced lysis was determined. The mode of response was concentration-dependent for both the extract and the standard drugs. However, the standard drug was more effective. The extract exhibited membrane stabilization activities of 12.17- 26.90% at concentration range of 200 to 500 µg/ml for inhibition of hypotonicity-induced lysis, and 1.54 - 12.92% at concentration range of 100 -500 µg/ml for heat-induced lysis while the standard drug exhibited membranestabilization activities of 43.48 - 86.09% for hypotonicity-induced lysis and 5.78-92.50% for heat-induced lysis at concentration range of 100 -500 µg/ml. From reports, it has been shown that oxidative damage of erythrocyte membrane is the primary cause of reduced capacity of the red blood cells to withstand mechanical and osmotic stress (Chikezie *et al.*, 2010). Antioxidant phytochemicals such as flavonoids and tannins have been reported to prevent oxidative damage. The mode of action of the extracts with membrane stabilization potentials could be attributed to their binding to the erythrocyte membranes with subsequent alteration of the surfacecharges of the cells (Anosike *et al*., 2019). This may prevent physical interaction with aggregating agents or promote dispersal by mutual repulsion of like charges which are involved in the haemolysis of red blood cells.

Reports have shown that certain saponins and flavonoids exert profound stabilizing effect on lysosomal membrane both *in vivo* and *in vitro*, while tannins and saponins possess ability to bind cations, thereby stabilizing erythrocyte membranes and other biological macro molecules (Middleton, 1996; El-Shabrany *et al*., 1997). Vitamin E has also been shown to possess membrane stabilizing ability (Wang and Quinn, 1999). The presence of these phytochemicals suggests that the extract contains constituents that protected the erythrocytes membranes effectively. Studies have shown that flavonoids contribute significantly to the anti-inflammatory activities of many plants. Previous studies have shown that cucumber peels are rich in flavonoids, alkaloids, saponins and steroids (Sheila *et al*., 2018). Therefore, the presence of these bioactives in the peels may contribute to its anti-inflammatory activity.

CONCLUSION

Our study showed the presence of different vitamins in cucumber peels with vitamin C being the most prominent and the anti-inflammatory effects of the ethanol extract. On the basis of analysis on vitamin compositions of cucumber peels, cucumber peels are good sources of essential vitamins. The adequate vitamin contents of cucumber peels have potentials to meet the vitamin requirement of the body especially vitamins needed in metabolism and proper functioning of the body. The extract of cucumber peels also showed various anti-inflammatory abilities. This study suggests that cucumber peels could be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases such as cancer, neurological disorder, aging and inflammation. The active constituents need to be isolated for better results.

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