Full Length Research Paper

Nutritional and chemical evaluation of *Momordica* charantia

Bakare R. I.*, Magbagbeola O. A., Akinwande A. I. and Okunowo O. W.

Department of Biochemistry, College of Medicine, University of Lagos. Idi-Araba, Lagos State, Nigeria.

Accepted 25 August 2010

Momordica charantia is used for some medicinal purposes in traditional medicine. The nutritional and chemical compositions of M. charantia were investigated using standard analytical methods. The proximate composition showed the percentage moisture, ash, crude lipid, crude fibre, crude protein and carbohydrate content of the plant materials. The calorific values for leaf, fruit and seed were 213.26, 241.66 and 176.61 Kcal/100 g respectively. The elemental analysis of M. charantia leaf revealed the presence of potassium (413 ppm), sodium (2200 ppm), calcium (20510 ppm) as well as zinc (120 ppm). Other elements found present in the leaf include magnesium, iron, manganese and copper. Vitamin A (β-carotene) (0.03 ppm), vitamin E (α-tocopherol) (800 ppm), folic acid (20600 ppm), cyanocobalamin (5355 ppm) and ascorbic acid (66000 ppm) were present. Trace amount of some other vitamins such as niacin (B_3), pyridoxine (B_6) cholecalciferol (Vitamin D) and phylloquinone (Vitamin K) were also found present in the methanolic and pet-ether leaf extract of M. charantia. Phytochemicals like alkaloids, tannins, flavonoids, saponins and glycosides were also found present. The study indicates the presence of nutritional and chemical components that are beneficial in addition to the purported numerous medicinal values of the plant.

Key words: Atomic absorption spectrophotometry, *Momordica charantia*, nutrition, phytochemicals, vitamins.

INTRODUCTION

Nutrition is the science of food, the nutrients and other substances there in, their action, interaction and balance is related to health and diseases (Clamp, 2007). Nutrients are grouped into six classes namely carbohydrate, fats (lipids), proteins, vitamins, mineral and water. The general functions of these nutrients include fuel (energy) expressed in Kcal, building materials for body structures and regulation and control of body processes (Akinwande, 1999). Over the years, medicinal plants have been recognized to be of great importance to the health of individuals and communities. In many developing countries, herbal medicines are assuming greater importance in primary health care and their international trade has increased though many of these herbal products in circulation are unregistered by national regulatory bodies (WHO, 1996). A great variety of plants

are used for mechanical treatment, either the dried plant or a specific part of it (root, leaves, fruit, flower and seed) is formulated into suitable preparations. In recent past, an increasing research evidence is getting accumulated which clearly indicate the positive role of traditional medicinal plants in the prevention or control of some metabolic disorders like diabetes, heart diseases and certain types of cancers (Zhang, 1996).

Momordica charantia (bitter melon) is a tropical and subtropical vine of the family cucurbitaceous widely grown in India, south Asia, China, Africa and the Caribbean. Bitter melon as fondly called has been implicated experimentally to achieve a positive sugar regulatory effect by suppressing the neural response to sweet taste stimuli and also keep the body functions operating normally. Other use of the plant include to expel intestinal gas, for tumors, wound treatment, rheumatism, malaria, vaginal discharge and the seeds are used to induce abortion (Sofowora, 2006; Taylor, 2005). A tea preparation from the leaf is used for

^{*}Corresponding author: E-mail: rasheedatbakare1@yahoo.com.

diabetes. In Nigeria, Ghana and India peninsula, the root of the plant is used as an abortifacient together with the fruit as well as an ingredient in aphrodisiac preparation (Sofowora, 2006). The young fruits and shoots are reported to serve as supplementary or emergency food in some part of West Africa, and as an effective emanagogue to facilitate child birth in Ivory Coast (Burkil, 1985). All plant species contain poisonous, medicinal and nutritional compounds. Many of these traditional plants are used with no attention paid to their nutritional values. Thus, this work is aim to exploit the nutritional composition and chemical profile of *M. charantia*.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves, seeds and fruits of *M. charantia* were harvested between June and August, 2005, from a home garden at Amuwo-Odofin LGA of Lagos State, Nigeria. The plant was identified and authenticated by Prof. J.D. Olowokudejo of the Department of Botany and Microbiology, University of Lagos, Nigeria. A voucher specimen was deposited in the herbarium. The plant material was sorted out to eliminate all extraneous materials. The leaves were dried in a hot air oven (SD 93114624, Gallenkamp, United Kingdom) for five days at 40°C and were further processed and kept until required (Odetola and Akojenu, 2000).

Proximate analysis

The leaf of *M. charantia* is consumed as a vegetable in some parts of the world, this necessitate the investigation of the proximate composition of the leaves in comparism with the fruits and seeds of the plant. However, other analyses were carried out on the leaves due to its dual usage as vegetable and medicinal purposes. The proximate analysis was carried out according to the procedure of Association of Official Analytical Chemist (AOAC, 2000). The AOAC Official methods for analyzing the various parameters are as listed. Crude protein 955.04 (2.4.03), crude fibre 962.09 (4.6.01), moisture 934.01 (4.1.03), ash 942.05 (4.1.10), crude fat 920.39 (4.5.01), and carbohydrate by difference. The calorific values of the samples were also estimated (Asibey-Berko and Tayie, 1999).

Digestion of leaves of M. charantia and mineral analysis

The sample of the of *M. charantia* were washed with the deionised water to remove dust particles. The sample was dried and ground to fine powder. A weighed portion of the ground sample of leaves was placed in a pre-cleaned silica crucible and heated on flame for about 10 min to remove moisture and volatile matter. The crucible was heated in a muffle furnace at 600°C for about 4 h to convert the sample into ash. The ashed sample was dissolved in conc. HNO₃ (12 mL). Then the total volume was made to 100 mL with distilled-deionised water. The content was then filtered, and the filtrate was used for the mineral analysis (Ahmed and Chaudhary, 2009).

Standard solutions of the minerals (sodium, potassium, calcium, magnesium, manganese, iron, zinc and copper) to be analyzed were prepared. The atomic absorption spectrophotometer (Analyst 200, Perkin Elmer Inc., United State) was set with power on for 10 min to stabilize. The standard metal solutions were injected to

calibrate the AAS using acetylene as the carrier gas. An aliquot of the mineral solution obtained from the plant digest was injected and the concentration was obtained from the AAS for each mineral element (Okunowo and Ogunkanmi, 2010). The experiments were done in triplicate and the results were averaged.

Vitamin composition

The vitamin composition of both the dried *M. charantia* leaves and the leaf extracts (aqueous, methanolic and pet-ether) were determined by chemical methods and high precision analytical method (HPLC), respectively. The concentrations of vitamins A, E, C, B₁₂ in the dried leaves were determined by the procedures of association of official analytical chemist (AOAC, 2000) and the folic acid as described by Pearson (1985). While the vitamins present in the leaf extracts were analysed by high performance liquid chromatography (HPLC) (Upchurchill, 2007). The water soluble vitamins were analyzed using APS-1 Reverse Phase C18 (100 x 4.6 mm) and the fat soluble vitamins by Zorbax Eclipse XDB Reverse Phase C18 (150 x 4.6 mm) (Upchurchill, 2007).

Phytochemical screening

The dried leaf sample (100 g) of M. charantia was extracted in 300 ml methanol using a Soxhlet apparatus as described by Akueshi et al. (2002). To obtain the aqueous extract, M. charantia leaves were dried in an oven (SD 93114624, Gallenkamp, United Kingdom) at 40°C and milled to powder. This was extracted in boiling water for 30 min and filtered with a glass wool. The filtrate was concentrated in a lyophilizer. The dried powder was stored at 4°C (Akueshi et al., 2002; Oben et al., 2006). The petroleum ether extract was also obtained using Soxhlet apparatus (Mallikharjuna et al., 2007). Phytochemical screening of the aqueous and methanolic extracts of M. charantia leaf was carried out. The phytochemical screening for the presence of alkaloids, tannins, flavonoids, saponins, and anthraquinones were carried out according to the methods of Sofowora (2006), Harbone (1991), Trease and Evans (2002) and Edeoga et al. (2005). Also, for glycosides (cyanogenic and cardiac), phylobatannins, reducing compounds, steroids and terpenoids, the methods of Sofowora (2006), Harbone (1991), and Trease and Evans (1996) were used.

Statistical analysis

Results are presented as mean \pm SEM and the difference between the data sets was analyzed using students T-test, with (P<0.05) considered significant.

RESULTS

The result obtained in this study shows the proximate composition, vitamin content and the phytochemicals in M. charantia. The leaf and fruit contain considerable amount of carbohydrate. This was significantly (P<0.05) higher than the amount present in the seed (Table 1). The percentages of moisture, total ash, crude fat and crude fibre were also low in the seed. The most abundant mineral present in the leaf of M. charantia was calcium. This was significantly (P<0.05) greater than the amount of

Table 1. Proximate composition of *M. charantia* leaf, fruit and seed.

Parameter (%)	Leaf	Fruit	Seed
Moisture (wet wt)	17.97 ± 1.00a	10.74 ± 2.29b	20.69 ± 5.85c
Total ash (dry wt)	15.42 ± 2.08b	$7.36 \pm 0.52a$	9.73 ± 2.34a
Crude fat (DW)	$3.68 \pm 0.68a$	6.11 ± 0.42b	11.50 ± 1.77c
Crude fibre (DW)	3.31 ± 1.25a	13.60 ± 1.13b	29.60 ± 1.25c
Crude protein (DW)	27.46 ± 1.60a	27.88 ± 3.75a	$19.50 \pm 0.73b$
Carbohydrate (DW)	$32.34 \pm 0.24b$	$34.31 \pm 0.30b$	9.18 ± 0.86a
Caloric value kcal/100 g	213.26	241.66	176.61

Results are mean of 3 determinations \pm SEM. Means with same superscript down the row are not significant (P>0.05), DW = Dry weight.

Table 2. Mineral composition of *M. charantia* leaf.

Element	ppm	
Calcium	20510.00 ± 5.77	
Magnesium	255.00 ± 0.69	
Sodium	2200.00 ± 1.15	
Potassium	413.00 ± 1.45	
Iron	98.00 ± 0.23	
Zinc	120.00 ± 1.15	
Manganese	156.00 ± 0.33	
Copper	32.00 ± 1.85	

Results are mean of 3 determinations ± SEM.

Table 3. Vitamin content of Momordica charantia dried leaf.

Vitamin	ppm	
A	0.03 ± 0.003	
E	800.00 ± 14.14	
С	66000.00 ± 141.42	
B ₁₂	5355.00 ±7.10	
Folic acid	20600.00 ± 42.43	

Results are mean of 3 determinations ± SD.

sodium, potassium, manganese, zinc, magnesium, iron and copper present (Table 2). The dried leaves of M. charantia contain small amount of vitamin A, E, C, B_{12} and folic acid (Table 3). While trace amount of vitamin B_3 , B_6 , A, D and K were found present in the methanolic and pet-ether leaf extract of the plant (Table 4).

Phytochemical screening of the aqueous and methanolic leaf extracts indicated the presence of alkaloids, tannins, saponins, cardiac glycosides and steroids (Table 5).

DISCUSSION

Nutrients are necessary for life and good health; these may be found in a number of different foods. The general

functions of nutrients include fuel (energy) expressed in kcal, building materials for body structures and regulation and control of body processes. The proximate analysis shows that the leaf and fruit of M. charantia are good sources of carbohydrate and protein; these may serve as source of energy and nutrients for the body metabolic activities in addition to its medicinal properties. The carbohydrates and proteins present in the plant may be a conglomerate of bioactive sugars, glycoproteins or proteins which gives the plant its medicinal potency against certain diseases. Some plants are known to contain certain sugars which are biologically active against some diseases (Srivastava et al., 1989; Hokputsa et al., 2004). Also, some plant proteins such as trichosanthin (isolated from tubers of Trichosanthes β-trichosanthin (isolated from tubers of kirilowii). Trichosanthes cucumeroides), α - and β -momorcharins (isolated from seeds of M. charantia), momorcochin (isolated from tubers of Momordica cochinchinensis), luffaculin (isolated from seeds of Luffa acutangula) and luffin-a and luffin-b (isolated from seeds of Luffa cylindrica) have been reported to exhibit abortifacient, antitumor, ribosome inactivating and immunomodulatory properties (Tsao et al., 1990; Ng et al., 1992). Trichosanthin manifests anti-human immunodeficiency virus activity (Ng et al., 1992).

The total ash content of the plant materials are low indicating low total mineral elements in the plant materials. However, these values are comparable to values reported for some Nigerian leafy vegetables (Akindahunsi and Salawu, 2005). The elements such as calcium, magnesium, potassium, zinc, iron, manganese and sodium found in reasonable amount in the leaf are nutritionally and biochemically important for proper body function. For instance, calcium is known to play a significant role in muscle contraction, bone and teeth formation and blood clotting (Ahmed and Chaudhary, 2009; Heaney, 2009; Peters and Martini, 2010). Some of these minerals such as magnesium and zinc are needed as cofactor in enzyme catalysis in the body (Ahmed and Chaudhary, 2009). Sodium and potassium which are present in the intracellular and extracellular fluid helps to

Vitamin	Aqueous extract	Methanolic extract	Pet-ether extract
Vit B ₃	ND	0.08	ND
Vit B ₆	ND	98.38	ND
Vit C	115	ND	0.08
Vit A	ND	ND	11.25
Vit D	ND	ND	14.25
Vit E	ND	ND	ND
Vit K	0.06	ND	5.00

Table 4. Vitamin content (ppm) of *M. charantia* leaf extracts.

ND - Not detected.

maintain electrolyte balance and membrane fluidity (Ahmed and Chaudhary, 2009). Iron is known to be a component of some metalloenzymes, myoglobin and heamoglobin (Ahmed and Chaudhary, 2009), which is needed in the transport of oxygen and carbon dioxide during respiration or cellular metabolism. This heamoglobin (containing iron) also serve as buffer to regulate changes in blood pH (Kamshilov and Zaprudnova, 2009).

It is known that inorganic mineral elements such as potassium, calcium and zinc play important roles in the maintenance of normal glucose-tolerance and in the release of insulin from beta cells of islets of Langerhans (Choudhary and Bandyopadhyan, 1999). Zinc present in the plant is beneficial to prevention and treatment of diarrhoeal episode, it is also involves in normal functioning of immune system. Iron is an essential trace element for haemoglobin formation and normal functioning of the central nervous system (Adeyeye and Otokiti, 1999).

The crude fat content in *M. charantia* fruit and seed were higher than in the leaf and also higher than the value reported for Amaranthus hybridus (4.65%) (Akubugwo et al., 2007). The crude fat may add to the caloric value extractable from the plant for metabolic activities. The study also shows that the plant contain small amount of fiber, this could be beneficial when consumed. Dietary fibre is important for lowering blood cholesterol and blood sugar. It is known to reduce the risk of diseases such as obesity, diabetes, breast cancer, hypertension and gastrointestinal disorder (Saldanha, 1995). Vitamins A, E, C, B₁₂ and folic acid were found present in the plant. Some of these vitamins such as vitamin B₁₂ found majorly in animal sources function as of coenzymes methylcobalamin deoxyadenosylcobalamin used in new cell synthesis (Robert et al., 2003). It helps to maintain nerve cell function and the deficiency leads to pernicious anaemia (Robert et al., 2003). In addition to the antioxidant property of vitamin C and E, vitamin C strengthen the body immunity against infections, helps in collagen and thyroxin synthesis and enhance iron absorption (Robert et al., 2003) while vitamin E play a role in immune function, cell growth, reproduction and DNA repair (Robert et al., 2003; Stryer, 1995, Traber and Packer, 1995). Vitamin A is a component of the visual pigments in the retina; regulates gene expression and cell differentiation. It is an antioxidant. The deficiency may lead to night blindness, xerophthalmia; keratinization of skin (Robert et al., 2003; Stryer, 1995). Folic acid is a hematopoetic vitamin and when deficit in the body leads to anaemia (Robert et al., 2003; Stryer, 1995). Vitamins are a diverse group of organic molecules required in very small quantities in the diet for health, growth, and survival (Stenesh, 1975). The absence of a vitamin from the diet or an inadequate intake results in characteristic deficiency signs and, ultimately, death (Stenesh, 1975; Smith et al., 1997).

The presence of secondary metabolites such as alkaloids, saponins, tannins, glycosides and cardiac glycosides in the leaf M. charantia may contribute to its medicinal value. Some of these compounds are well documented to exhibit hypoglyceamic activity in animals (Akhtar et al., 1981; Singh, 1986; Ng et al., 1986). Saponins inhibit Na+ efflux leading to higher Na+ concentration in cells, thereby activating a Na+-Ca2+ antiport (Schneider and Wolfing, 2004). This effect produces elevated cytosolic Ca2+ which strengthens the contraction of the heart muscle and thereby reducing congestive heart failure (Schneider and Wolfing, 2004). The protective and metabolic role of alkaloids in animals has been documented (Edeoga and Eriata, 2001). The plant is used by the orthodox doctors in some parts of Nigeria to cure diarrhea, further work will therefore include the investigation of the anti-diarrhoeal effects of the leaf extract of plant in rats.

Conclusion

The result obtained in this study showed that *M. charantia* contains appreciable amount of nutrients, vitamins and minerals and these can contribute to the nutrient and energy requirement of man when the plant is taken for curative purposes in certain disease conditions.

REFERENCES

- Adeyeye E, Otokiti MKO (1999). Proximate composition and some nutritional valuable minerals of two varieties of *Capsicum annum* (Bell and cherry peppers). Discov. Innov., 11: 75-81.
- Ahmed D, Chaudhary MA (2009). Medicinal and nutritional aspects of various trace metals determined in *Ajuga bracteosa*. J. Appl. Sci. Res., 5(7): 864-869.
- Akhtar MS, Athar MA, Yaqub M (1981). Effect of Momordica charantia on blood glucose level of normal and alloxan-diabetic rabbits. Planta Med., 42: 205-212.
- Akindahunsi AA, Salawu SO (2005). Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. Afr. J. Biotechnol., 4: 497-501.
- Akinwande AI (1999). Body requirements: In Nutritional Biochemistry. University of Lagos Press, Nigeria, pp. 11-13.
- Akubugwo IE, Obasi NA, Chinyere GC, Ugbogu AE (2007). Nutritional and chemical value of *Amaranthus hydrides*. Leaves from Akikpo, Nigeria. Afr. J. Biotechnol. 6(24): 2833-2839.
- Akueshi CO, Kadiri CO, Akueshi EU, Agina SE, Ngurukwem C (2002). Anti-microbial potential of *Hyptis suaveolens poit* (Lamiaceae). Nig. J. Bot., 15: 37-41.
- Asibey-Berko E, Taiye FAK (1999). Proximate analysis of some underutilized Ghananian vegetables. Ghana J. Sci., 39: 91-92.
- Association of Official Analytical Chemists (AOAC) (2000). Official Methods of Analysis of Association of Analytical Chemists international, 17th ed. Horwitz, W. (ed). Vol I and II. AOAC International Publs, Maryland USA. Ch. 45: 12 20.
- Burkil HM (1985). Useful plants of West Tropical Africa. 2nd ed. Kew Royal Bot. Garden, pp. 597-599.
- Choudhary KA, Bandyopadhyay NG (1999). Preliminary studies on the inorganic constituents of some indigenous hyperglycaemic herbs on oral glucose tolerance test. J. Ethnopharmacol., 64: 179-184.
- Clamp B (2007). Overview of nutrition: Module Ohlone College CFS 109, Nutrition.
- Edeoga HO, Eriata DO (2001). Alkaloid, tannin and saponin contents of some Nigerian medicinal plants. J. Med. Aromatic Plant Sci., 23: 344-349.
- Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4(7): 685-688.
- Harbone JB (1991). Bioactive constituents of plant. In: Phytochemical methods. A guide to modern techniques of plant analysis (2nd ed.). Chapman and Hall Plubl. Co. London, pp. 46-288.
- Heaney RP (2009). Dairy and bone health. J. Am. Coll. Nutr., 28(Suppl. 1): 82S-90S.
- Hokputsa S, Harding SE, Inngjerdingen K, Jumel K, Michaelsen TE, Heinze T, Koschella A, Paulsen BS (2004). Bioactive polysaccharides from the stems of the Thai medicinal plant *Acanthus ebracteatus*: Their chemical and physical features. Carbohydr. Res., 339(4): 753-762.
- Kamshilov IM, Zaprudnova RA (2009). Interspecies differences of hemoglobin buffer properties and of ion environment in some freshwater fish. J. Evolut. Biochem. Physiol., 45(2): 242–244.
- Mallikharjuna PM, Rajanna LN, Seetharam YN, Sharanabasappa GK (2007). Phytochemical studies of *Strychnos potatorum* L.f- A Medicinal plant. e-J. Chem., 4(4): 510- 518.
- Ng TB, Chan WY, Yeung HW (1992). Proteins with abortifacient, ribosome inactivating, immunomodulatory, antitumor and anti-AIDS activities from Cucurbitaceae plants. General Pharmacology: The Vascular Syst., 23(4): 575-590.

- Ng TB, Li WW, Yeung HV (1986). Insulin-like Molecules in *Momordica charantia* seeds. J. Ethnopharmacol., 15: 107-117.
- Oben JE, Assi SE, Agbor GB, Musoro DF (2006). Effect of *Eremomastax speciosa* on experimental diarrhoea. Afr. J. Trad. Complementary Alternative Med., 3(1): 95-100.
- Odetola AA, Akojenu SM (2000). Anti-diarrhoeal and gastrointestinal potentials of the aqueous extract of *Phyllanthus amarus* (Euphorabiaceae). Afr. J. Med. Sci., 29: 119-122.
- Okunowo WO, Ogunkanmi LA (2010). Phytoremediation potential of some heavy metals by water hyacinth. Int. J. Biol. Chem. Sci., 4(2): 347-353.
- Pearson D (1985). Pearson's composition and analysis of food technology. Roland. S. Kirkland ed. (9th ed.). Longman Scientific Publ. pp. 314, 650-657.
- Peters BSE, Martini LA (2010). Nutritional aspects of the prevention and treatment of osteoporosis. Arq. Bras. Endocrinol. Metab., 54(2): 179-185
- Robert KM, Daryl KG, Peter AM, Victor WR (2003). Harper's Illustrated Biochemistry. In Benders and Mayes (eds) Vitamins and Minerals, Lange Medical Books/McGraw-Hill, Medical Publishing Division, New York, pp. 481-497.
- Saldanha LG (1995). Fiber in the diet of United States children results of National Surveys. Pediat. 96: 994-996.
- Schneider G, Wolfing J (2004). Synthetic cardenolides and related compounds. Current Organic Chem., 8: 14.
- Singh YN (1986). Traditional Medicines in Fji Indians. J. Ethnopharmacol., 15: 57-58.
- Smith AD, Datta SP, Smith GH, Campbell PN, Bentley R, McKenzie HA (1997). Oxford Dictionary of Biochemistry and Molecular Biology. Oxford University Press, Oxford, New York, p. 683.
- Sofowora A (2006). Medicinal Plants and Traditional Medicine in Africa. 2nd Ed., Spectrum Books Ltd., Ibadan, Nigeria. pp. 151-153, 209-214
- Srivastava R, Kulshreshtha DK (1989). Bioactive polysaccharides from plants. Phytochem., 28(11): 2877-2883.
- Stenesh J (1975) Dictionary of biochemistry and molecular biology. A Wiley-Interscience publication. New York, p. 512.
- Stryer L (1995). Metabolism: Basic Concepts and Design. In: Biochemistry 4th Ed. Freeman W.H and Company, New York.
- Taylor L (2005). Bitter melon: Herbal properties and actions. In: The healing power of rainforest herbs. Raintree Nutrition Inc. Square one Publ. Inc. New York, pp. 1-5.
- Traber MG, Packer L (1995). Vitamin E: Beyond antioxidant function. Am. J. Clin. Nutr., 62: 15010-15095.
- Trease GE, Evans WC (2002). Pharmacognosy. 15th ed. Saunders, London. pp. 53-336.
- Tsao SW, Ng TB, Yeung HW (1990). Toxicities of trichosanthin and alpha-momorcharin, abortifacient proteins from Chinese medicinal plants, on cultured tumor cell lines. Toxicol., 28(10): 1183-1192.
- Upchurchill (2007). Upchurchill Scientific Co: High performance liquid chromatography www.upchuch.com.
- World Health Organisation (WHO) (1996). Traditional Medicine fact sheet N134. WHO, Geneva.
- Zhang X (1996). Traditional Medicine and World Health. The Magazine of WHO, 49th year, 2: 4-5.