African Journal of Pharmacology and Therapeutics Vol. 3 No. 3 Pages 99-104, 2014
Open Access to full text available at http://journals.uonbi.ac.ke/ajpt

Research Article

Comparison of variously prepared *Momordica charantia* aqueous leaf extracts on the isolated mammalian jejunum

Jacob W. Adegu [a,*], Anne Muriithi [a], and Frederick Bukachi [a]

[a] *Department of Medical Physiology, School of Medicine, University of Nairobi, Kenya*

* **Corresponding author:** Department of Medical Physiology, School of Medicine, University of Nairobi, P.O. Box 4327-00100, Nairobi, Kenya; **Tel**: +254-73-8867725; **Email**: jacobadegu@yahoo.com

Background: *Momodica charantia* is a highly valued herb in tropical and subtropical region for its numerous medicinal advantages. These include: management of bacterial infections and diabetes. In spite of wide consumption of its aqueous leaf extract stored and prepared in various conditions, their pharmacological activities on the jejuna are yet to be documented. The potential of differently prepared extracts having varied effects on the jejuna focused the researcher's attention.

Objectives: To demonstrate the effects of *Momordica charantia* aqueous leaf extract prepared and stored under various conditions on the isolated mammalian jejuna.

Methodology: Six healthy rabbits were included. Each rabbit was sacrificed and the jejuna mounted on the Langerndorff apparatus. Baseline rate and force of contraction were recorded, after which each of the various aqueous leaf extract was administered in increasing doses and changes in rate and force of contraction noted. Paired T-test and repeated measures ANOVA were used to test for statistical significance. P values less than 0.05 indicated statistical significance.

Results: There was a significant ($P<0.05$) dose depended increase in jejuna rate and force of contraction with the administration of the various extract preparations. The increase noted was highest and lowest after the administration of fresh boiled extract and fresh aqueous leaf extract respectively.

Discussion: *Momordica charantia* aqueous leaf extract stored under different conditions have a significant ($P<0.05$) dose related effect on jejuna rate and force of contraction. The increase is dose depended and irreversible.

Keywords: *Momordica charantia*, various extract, jejuna.

Received: August, 2014
Published: September, 2014

1. Introduction

Mormodica charantia is a herb belonging to the Cucurbitaceae family. It is commonly found in Africa, Asia and the Carribean region. It is one of the herbs widely explored because of its medicinal value and to some extent, spiritual value as well. The parts of the plant consumed are leaf, roots and fruits. Different phenotypes exist. These include the Chinese and the Indian phenotypes. The parts of the plant consumed are leaves, roots and seeds that are either consumed when still fresh or after drying.

Various medicinal properties have been attributed to *Momordica charantia*. The most known is blood glucose lowering property especially the fruit. This is thought to be via increasing insulin sensitivity as well as insulin levels (Chaturvedi, 2011; Blum et al, 2011). *Mormodica charantia* has also been documented to have anti-viral properties against Human Immunodeficiency Virus

A KeSoBAP Publication ©2014. All rights reserved. **ISSN 2303-9841**

(HIV) (Nerurkar et al, 2006). Tea prepared from *Momordica charantia* leaves is also used in the management of diabetes. (Bakare et al, 2010). Other properties attributed to *Mormodica charantia* include antimalarial, anti-hypertensive (Sook, 2006), anthelminthic, especially against *Caenorhabditis elegans* (Nadine et al, 2005) anti-dysmenorrhea and anticancer (Ray et al, 2010; Hiroyuki et al, 2004; Puriet al, 2009) that is extensively investigated.

Despite its wide medicinal value, *Mormodica charantia* aqueous leaf extract is contraindicated in pregnancy because it has been reported to stimulate uterine smooth muscle contraction leading to abortion. (Rae 2011; Nadine et al, 2005). Consequently, it is used as an abortifacient by some communities e.g. in the Philippines. (Rae 2011).

Mormodica charantia leaf contains a wide range of biologically active components. These primarily comprise momordicin I, momordicinII, and cucurbitacin B. (Majekodunmi et al., 1990) that have been associated with purgative and cytotoxic properties. Charantin, a steroid saponin with insulin like effect, is the main anti diabetic agent in *Mormodica charantia* plant. (Ernest et al, 2011). *Mormodica charantia* leaf also contains bioactive glycosides including momordin, charantosides, glycosides, momordicosides, goyaglycosides and other terpenoid compounds that include momordicin-28, momordicinin, momordicilin, momordenol, and momordol. (Sabira et al, 1997; Kimura et al, 2005). The terpenoids are also responsible for the anti-diabetic, anti-cancer, anti-obesity and anti-HIV properties that are attributed to *Momordica charantia.* (Sook et al, 2009). Momorcharin and momordicin (cytotoxic proteins) are also present.

There are several methods by which different *Momordica charantia* extracts are prepared. These include methanolic extract and aqueous extract, of which the latter is more common. A large population that consumes the extract prepare large volumes and some kept for use the following day. In some communities, the excess solution is left covered in the open while in some it is refrigerated. Some populations boil the leaves while others use hot water infusion.

Regardless of the numerous uses of *Mormodica charantia* aqueous leaf extract stored under various conditions, few studies have been carried out to investigate and compare their effect on the smooth muscle rate and force of contraction.

2. Materials and Methods

2.1 Sample Collection and Preparation

Fresh leaves of *Momordica charantia* were procured from the local market and authenticated by the Department of Botany, University Of Nairobi. The leaves were assigned voucher number: JA2012/01. A voucher specimen was deposited in the herbarium.

Preparation of fresh *Momordica charantia* aqueous leaf extract (sample 1)

The leaves were air dried under the shade for three days. The dried leaves were wrapped in water proof paper bags and stored for 2 weeks until the time of extraction (Bakare et al, 2011). Extraction of the dried leaves was done by hot infusion using 20 ml of hot water for every 1 g of leaf powder. The extract was allowed to cool before filtering.

Preparation of Refrigerated *Momordica charantia* aqueous leaf extract (sample 2)

This was prepared by storing fresh *Momordica charantia* aqueous leaf extract in the fridge overnight and used the following day.

Preparation of 24hr standing *Momordica charantia* aqueous leaf extract (sample 3)

This was prepared by leaving fresh *Momordica charantia* aqueous leaf extract to stand overnight and used the following day.

Preparation of boiled *Momordica charantia* aqueous leaf extract (sample 4)

This was prepared by boiling dried *Momordica charantia* leaves for 5 minutes in water, in the ratio of 20ml of water for every 1 gm. of leaf powder. The aqueous leaf extract was then filtered using Whattman filter paper number 1.

2.2 Animal Handling

New Zealand White rabbits were procured locally from Tony Rabbits and Guinea Pigs Farm (Kenya). They were housed in a clean environment in the animal house, Department of Medical Physiology. Standard laboratory conditions of humidity 50 ±15% and temperature of 25 ±2^{0}C, 12h/12h light-dark cycle was maintained. They had free access to food and water. The procedures and experiments were performed according to the guidelines stated by the Federation of European Laboratory Animal Science Associations (FELASA) (Adegu et al, 2014).

2.3 Preparation of the physiological salt solution

Tyrode's solution was prepared using standard procedures. The composition of Tyrode's solution was (g/L): Sodium chloride (8.0); Sodium bicarbonate (1.0); Glucose (1.0); Potassium chloride (0.2); Calcium chloride (0.2); Sodium dihydrogen phosphate (0.05); and Magnesium chloride (0.1). During mixing, calcium chloride was dissolved separately in distilled water and added last to avoid precipitation. The salts were manufactured by Muby Chemicals (India).

2.4 Investigating effects of boiled aqueous leaf extract on isolated jejuna

Each rabbit was sacrificed by cervical dislocation. The jejunum was removed and mounted on a Langendorff apparatus. Baseline jejuna rate and force of contraction was then recorded.

Starting with the least volume, 0.2 ml (1 mg equivalent of dried leaf powder) of the fresh extract was

A KeSoBAP Publication ©2014. All rights reserved. **ISSN 2303-9841**

administered by infusion into the organ bath and the effects recorded. The volume of the extract was successfully increased in 0.2 ml increments to a maximum of 1.0 ml.

This was repeated 6 times. Thereafter, sample 2, 3 and 4 were each administered to a different isolated mammalian jejuna and changes in rate and force of contraction taken.

Prior to addition of new volumes of the extract, the organ bath was completely drained, rinsed at least twice using Tyrode's solution followed by recording of baseline readings. This was repeated 6 times. Acetylcholine (0.01 IU) was used as the positive control.

2.5 Data and statistical analysis

Rate and strength of contraction was determined using the Langendorff apparatus. Data generated from the study was analyzed in terms of frequency and force of contraction. Frequency referred to the number of contractions per unit time while force referred to height of amplitude (in mm).

The changes in rate and force of contraction were analyzed and expressed as Mean and Standard Error of Mean. Statistical analysis to find out whether there was significant change in rate and force of contraction after administration of the various dosages was done using Analysis of Variance (ANOVA) and unpaired t test. Data analysis was done using Statistical Package for Social Sciences (SPSS version 17.0).

Changes in rate and force of contraction after administration of the various extracts was compared with baseline readings using unpaired sample t test with the p value set at $p<0.05$.

2.6 Ethical considerations

The animals that were used in the study were handled with care as the welfare of the laboratory animals is important in influencing results. Moreover, the standard operating procedures (SOP) of the Department of Medical Physiology animal laboratory was adhered to. In addition to FELASA guidelines, the 3R principles (reduction, refinement and replacement) were adhered to.

3. Results

Table 1 shows changes in rate and force of jejuna contraction after administration of various doses and extracts.

There was a significant increase in both the force and rate with each increase in dosage. However, there was a larger increase in force as compared to rate of contraction. The increase in rate and force of contraction was directly proportional to the increase in the extract's dose. This increase was statistically significant ($P < 0.05$) for both force and rate. The largest magnitude of increase in rate and force of contraction was elicited by the boiled *Momordica charantia* aqueous leaf extract and least by the fresh extract (**Figure 2** and **3**).

The effects of the extracts were however irreversible after wash out.

The average percentage change in force of contraction after administration of 0.2 ml of the fresh boiled extract was highest for boiled extract and least for the fresh extract. The gradient of changes in rate and force of contraction after administration of the various extracts was largest in boiled extract and least in the refrigerated extract.

Table 1: Rate and force of contraction of the jejuna after administration of various concentrations of aqueous extract of *Momordica charantia* (N=6)

Extract Conc. (mg/ml)	Force of contraction (mm) (mean±SEM)					Frequency of contraction (per min) (mean±SEM)				
	Baseline	Sample 1	Sample 2	Sample 3	Sample 4	Baseline	Sample 1	Sample 2	Sample 3	Sample 4
0.2	2.24	4±0.71	6±1.11	8±1.19	11±1.16	0.52	1.63±0.51	2.89±1.78	3.57±1.98	4.77±0.43
0.4	2.76	8±1.37	11±1.95	15±1.87	17±1.23	0.36	2.56±0.81	3.32±1.68	4.25±1.12	5.22±1.57
0.6	2.51	13±1.89	16±1.41	19±0.08	22±0.46	0.51	3.36±1.27	4.50±0.65	5.52±0.73	6.26±1.84
0.8	2.96	17±1.16	21±1.82	26±1.43	31±1.71	1.19	4.19±1.62	5.56±1.76	6.62±0.01	7.51±0.61
1.0	2.05	19±1.20	25±1.39	29±1.31	36±1.30	1.41	5.66±1.13	6.62±1.96	7.23±1.83	8.84±1.62
P Value		0.025	0.018	0.011	0.10		0.006	0.002	0.001	0.001

A KeSoBAP Publication ©2014. All rights reserved.
ISSN 2303-9841

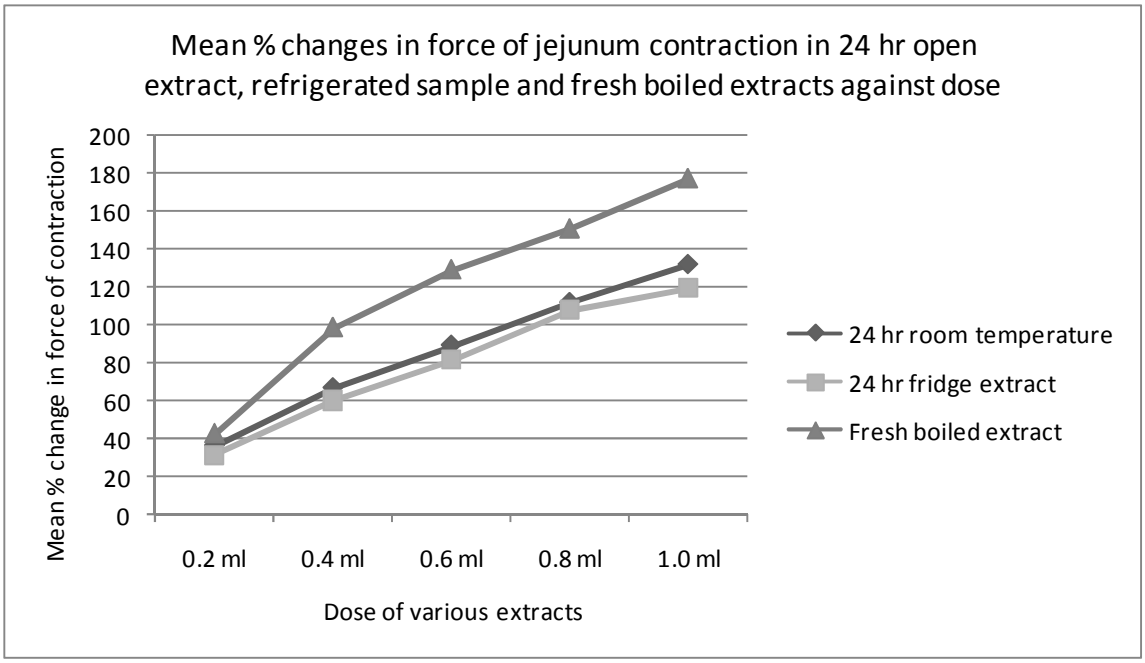

Figure 1: Mean percentage changes in force of jejuna contraction after administration of 3 different samples

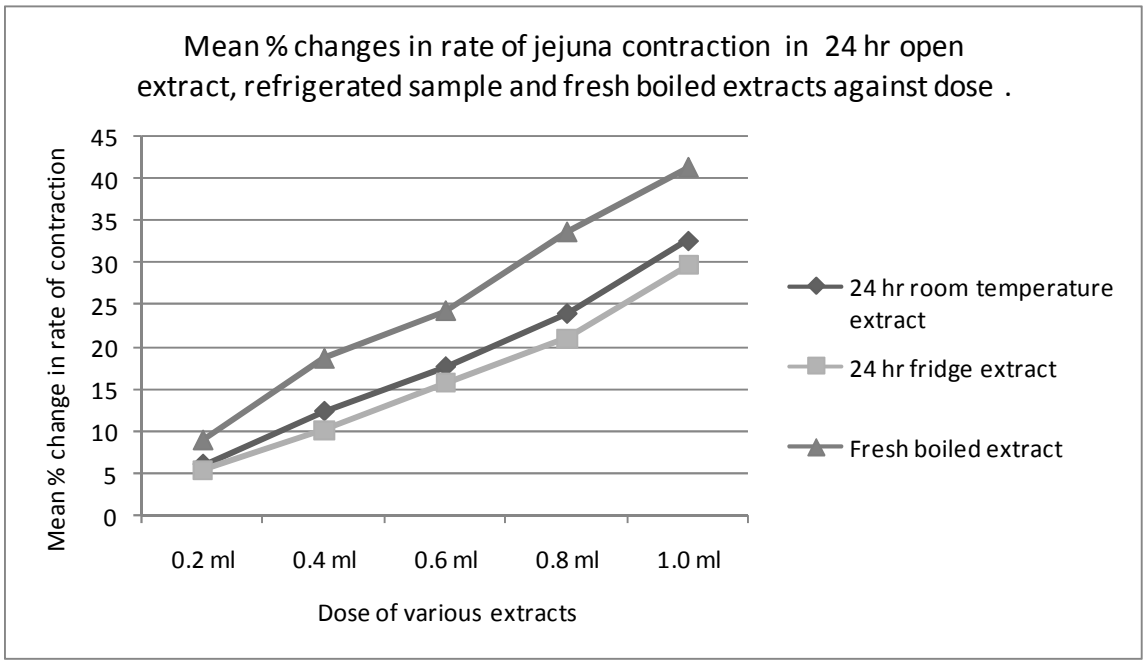

Figure 2: Mean percentage changes in jejuna rate of contraction after administration of 3 different extracts

4 Discussion

There was a pattern of increase in rate and force of contraction after the administration of the various doses of all the fresh, 24hr standing sample, refrigerated and fresh boiled extract. The gradient of change rate and force of contraction after administration of the various doses of the fresh extract increased as the doses were increased.

The increase in gradient as the increase in doses of the extract was administered is because the higher the dose, the higher the fraction of receptors bound per unit time. The result is a bigger change in contractility per unit time. The effects of the extract were however irreversible after wash out. The irreversibility of the effects indicates that the extract binds permanently to receptors involved.

A KeSoBAP Publication ©2014. All rights reserved. **ISSN 2303-9841**

The magnitude of change in rate and force of contraction for equal amount of dose administered was highest after administration of the boiled extract followed by 24 hr. open sample and least in the refrigerated sample. This was postulated to be most probably due to method of preparation and storage. Boiling most probably increased the concentration of the active ingredients. Boiling also could enhance the extraction of the active phytochemicals (agonists). This increases its potency. For the 24hr extract that was left in the open, it probably underwent degradation and other chemical changes that could have slightly enhanced its potency against the fresh aqueous extract. The degradation process could have been precipitated by the microorganisms present in the air. The 24 hr. fridge extract was less potent than the 24 hr. room temperature extract because the lower temperatures in the fridge slowed down the degradation process of the active ingredients into more toxic compounds. The significant differences in gradients showing change of contractility after the administration of same dose of boiled, 24 hr. room temperature and 24 hr. fridge extract is a further indication that the boiled extract is the most highly potent than the open and fridge extracts respectively.

5. Conclusion

The present study shows *Momordica charantia* aqueous leaf extract stored in various conditions and or prepared differently have an effect on jejuna rate and force of contraction. It has further demonstrated that the extract increases both jejuna force and rate of contraction. The increase is dose depended and is further supported by the tests of significance. It also determined that boiled *Momordica charantia* aqueous leaf extract has the largest effect on jejuna rate and force of contraction.

The present study did not seek to establish toxic levels of the various extract doses but recommends further studies to establish toxic levels be undertaken. The researcher also recommends that for a targeted effect, the prescriber of the extract should indicate dosages based on the method of extract preparation and storage, as they exhibit different potencies.

Conflict of Interest declaration

The authors declare no conflict of interest

Acknowledgements

The authors greatly acknowledge D. Wafula and W. Njeri for their technical assistance.

References

Adegu WJ, Anne M and Frederick B (2014). Effect of *Momordica charantia* aqueous leaf extract on the isolated mammalian heart. *Afr. J. Pharmacol. Ther*. **3:** 62-66.

Antonio ML, Maria VC, Villarruz, Cecilia A, Jimeno, Mark AU, Javelosa, Joel C, Rhida B and Gwyneth GB (2007). The effect of *Momordica charantia* capsule preparation on glycemic control in Type 2 Diabetes Mellitus needs further studies. *J. Clin. Epi.* **6**: 554-559.

Anura P, Jayasooriya, Masanobu S, Chizuko Y, Mikio K, Kyosuke Y and Nobuhiro F (2000). Effects of *Momordica charantia* powder on serum glucose levels and various lipid parameters in rats fed with cholesterol-free and cholesterol-enriched diets. *J. Ethnopharmacol.***72**: 331-336.

Asli S and Alaattin SN (2007). Antioxidant and chemoprotective properties of *Momordica charantia* L. (bitter melon) fruit extract. *Afr. J. Biotech.* **6:** 273-277.

Boston healing landscape project: https://www.bu.edu/bhlp/Clinical/cross-cultural/herbal_index/herbs/Momordica%20Charantia.html. (Accessed 20 May 2014).

Bakare RI, Magbagbeola OA, Akinwande AI, Okunowo OW and Green M(2011). Antidiarrhoreal activity of aqueous leaf extract of *Momordica charantia* in rats. *J. of Pharmacogn. Phytother.* **3:**1-7.

Bakare RI, Magbagbeola OA, Akinwande AI and Okunowo OW (2010). Nutritional and chemical evaluation of *Momordica charantia. J. Med. Plants Res*. **4:** 2189-2193.

Basch E, Gabardi S and Ulbricht C (2003). Bitter melon (*Momordica charantia*): a review of efficacy and safety. *Am. J. Health Sys. Pharmacol.* **60:** 356-359.

Clinton W (2003). Smooth muscle contraction and relaxation. *J. Adv. Physiol. Ed.* **27:** 201-206.

Ernest A, Jaipaul S, Emmanuel C, Gunasekar M and Huba K (2011). Medicinal Chemistry of the Anti-Diabetic Effects of *Momordica charantia*: Active Constituents and Modes of Actions. *The Open Medicinal Chemistry J.***5:**70-77.

Faraj OA, Duncan M, Fatma KA and Anne M (2012). Inhibitory effect of *Mangifera indica* on gastrointestinal motility. *Med. Chem. Drug Disc.* **2:** 9-16.

Francesca B, Francesco C, Raffaele C, Valeria A, Gabriella A, Rocco L and Angelo AI (2006). Effect of Boswelliaserrata on intestinal motility in rodents:inhibition of diarrhea without constipation. *Br. J. Pharmacol.* **148:** 553-560.

Grover JK and Yadav SP (2004). Pharmacological actions and potential uses of *Momordica charantia*: a review. *J. Ethnopharmacol*. **93:**123-132.

Hiroyuki K, Yumiko Y, Rikako S, Masashi H, Kazuo M and Takuji T (2004). Dietary seed oil rich in conjugated linolenic acid from bitter melon inhibits azoxymethane-induced rat colon carcinogenesis through elevation of colonic PPAR γ expression and alteration of lipid composition. *Int. J. Cancer.***110:** 896-901.

Julius EO, Sheila E, Assi G, Agbor A and David FM (2006). Effect of *eremomastax speciosa* on experimental diarrhea. *Afr. J. Trad. Complement. Alt. Med.* **1:** 95-100.

Kimura, Yumiko, Akihisa, Toshihiro, Yuasa, Noriko, Ukiya, Motohiko, Suzuki, Takashi, Toriyama, Masahar, Motohashi, Shigeyasu, Tokuda and Harukuni (2005). Cucurbitane-type

triterpenoids from the fruit of *Momordica charantia*. *J. Nat. Prod.* **68:** 807-809.

Liu Y, Ali Z and Khan I(2008). A. Cucurbitane-type triterpene glycosides from the fruits of *Momordica charantia. Planta Med.* **74:**1291-1294.

Majekodunmi O, Fatope, Yoshio T, Hiroyasu Y, Hikaru O and Tatsuo Y (1990). New cucurbitane triterpenoids from *Momordica charantia. J. Nat. Prod.* **53:**1491-1497.

Medicinal herbs and common uses: http://www.motherearthnews.com/homesteading-and-livestock/medicinal-herbs-common-uses.aspx#axzz38SP4rSS5. (Accessed 20 May 2014).

Medicinal plants. http://www.infoplease.com/dk/science/encyclopedia/medicinal-plants.html. (Accessed 20 May 2014).

Nadine B, Gbeassor M and Akpagana K (2005). Ethnomedicinal uses of *Momordica charantia* (Cucurbitaceae)in Togo and relation to its phytochemistry and biological activity. *J. Ethnopharmacol.* **96:** 49-55.

Nakamura S, Murakami T, Nakamura J, Kobayashi H, Matsuda H, and Yoshikawa M (2006). Structures of new cucurbitane-type triterpenes and glycosides, karavilagenins and karavilosides, from the dried fruit of *Momordica charantia* L. in Sri Lanka. *Chem. Pharm. Bull*. **54:**1545-1550.

Pratibha VN, Yun KL, Ellen HL, Steven L, Laurel P, Jennifer F and Vivek, RN (2009). Lipid lowering effects of *Momordica charantia* (Bitter Melon) in HIV-1-protease inhibitor-treated human hepatoma cells, HepG2.*British. J. Pharmacol.* **148:**1156–1164.

Pulok KM, Kuntal M, Kakali M and Peter JH (2006). Leads from Indian medicinal plants with hypoglycemic potentials. *J. Ethnopharmacol.* **106:**1-28.

Rae U (2011). The side effects of *Momordica charantia*. http://www.livestrong.com/article/392687-the-side-effects-of-momordica-charantia/. Last accessed: (Accessed May 21, 2012).

Ratna B, RayAR, Robert S and Pratibha N (2010).Bitter Melon (*Momordica charantia*) extract inhibits breast cancer cell proliferation by modulating cell cycle regulatory genes and promotes apoptosis. *Cancer Res.* **70:**1925-1931.

Rojas A, Hernandez L and Pereda RM (1992). Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. *J. Ethnopharmacol.* **35:** 275 -283.

Sabira B, Mansoor A, Bina S, Siddiqui AK, Zafar S and Saify MA (1997). Triterpenes, a sterol, and a monocyclic alcohol from *Momordica charantia. J. of Phytochem.* **44:**1313-1320.

Sook, YL, Seok, HE, Yong KK, Nam and Sang UP (2009). Cucurbitane-type triterpenoids in *Momordica charantia* Linn. *J. Med. Plants Res*. **3:**1264-1269.

Spencer NJ, Walsh M and Smith TK (2000). Purinergic and cholinergic neuro-neuronal transmission underlying reflexes activated by mucosal stimulation in the guinea-pig small intestine. *J. Physiol.* **522:**321-331.

Spencer NJ, Smith CB. and Smith TK (2001). Role of muscle tone in peristalsis in guinea-pig small intestine. *J. Physiol*. **530:** 295-306.

Stevens RJ, Publicover NG and Smith TK (2000). Propagation and neural regulation of calcium waves in longitudinal and circular muscle layers of guinea-pig small intestine. *Gastroenterol.* **118:** 892-904.

Subbroto KS, Ezazul H, Dipa, I, Matiar R, Rezuanul I, Anzana P and Shahedur R (2012). Comparative study between the effect of *Momordica charantia* (wild and hybrid variety) on hypoglycemic and hypolipidemic activity of alloxan induced type 2 diabetic long-Evans rats. *J. Diabetes Mell.* **2:**131-137.

Suhail A, Amjad AK and Qayyum H (2005). Potential of immobilized bitter gourd (*Momordica charantia*) peroxidases in the decolorization and removal of textile dyes from polluted wastewater and dyeing effluent. *Chemosphere* 3: 291-301.

Tan SP, Vuong QV, Stathopoulos CE, Parks SE and Roach PD (2014). Optimized aqueous extraction of saponins from bitter melon for production of a saponin-enriched bitter melon powder. *J. Food Sci.* **79:**72-81.

Yao M, Sen L, Shuangfeng L,Xiang F, Gangrui L, and Yanfa M (2014). A Novel Method for Simultaneous Production of Two Ribosome-Inactivating Proteins, α-MMC and MAP30, from *Momordica charantia L. PLoS One.*9: 91-98.

A KeSoBAP Publication ©2014. All rights reserved. **ISSN 2303-9841**