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## Antimicrobial property of *Momordica charantica* incorporated biscuits

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### Abstract

*Momordica charantia* is an important commercial cucurbit belonging to the family cucurbitaceae, genus *Momordica*. The fruits are bitter in taste. The bitter principle in bitter gourd is monordicine, an alkaloid, which is different from cucurbitacins present in other cucurbits. Ripe fruits are rich in vitamin-A. Among all cucurbits vegetable bitter gourds contains the maximum amount of minerals and vitamins. Biscuits are oven- baked food items with greater nutritive value than plain bread of equal weight. It is also the most desirable snack for both youth and elderly people due to their low manufacturing cost, more convenience, long shelf-life and ability to serve as vehicle for important nutrients. Thus biscuit has been chosen as a means for delivering the constituents of *Momordica charantica* with the objectives of formulating and assessing the antimicrobial properties of *momordica charantica* incorporated biscuits. The phytochemical screening of the *momordica charantica* powder and biscuits showed that they are a good source of steroid, flavonoid, alkaloid, saponin, tannin and cardiac glucosides. As per the antimicrobial studies, the presence of various bioactive compounds in biscuits possessed antibacterial and antifungal property which was proven from their zone of inhibition in the culture strains.

**Keywords:** *Momordica charantia*, flavonoid, alkaloid, saponin, tannin

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### 1. Introduction

Cucurbitaceae is a plant family, also known as gourd family. *Momordica charantia* is an important commercial cucurbit belonging to the family cucurbitaceae, genus *Momordica*. The fruits are bitter in taste. The bitter principle in bitter gourd is monordicine, an alkaloid, which is different from cucurbitacins present in other cucurbits (www.etd.uasd.edu). *Momordica charantica* has several uses. The fruits are used as vegetables in many ways and

quite commonly used in cooked, fried and stuffed forms. The fruits are also pickled, canned and dehydrated. Ripe fruits are rich in vitamin-A. Among all cucurbits vegetable bitter gourds contains the maximum amount of minerals and vitamins (Islam *et al.*, 2010). The fruits are cooling, digestive, laxative and antipyretic. The fruit powder is used for healing wounds, leprosy and malignant ulcers, useful in snakebites. Biscuits are oven-baked food items with greater nutritive value than plain bread of equal weight (www.ethioembassy.org). It is also the most desirable snack for both youth and elderly people due to their low manufacturing cost, more convenience, long shelf-life and ability to serve as vehicle for important nutrients (Akubor, 2003). Thus biscuit has been chosen as a vehicle for delivering the constituents in *Momordica charantica* with the objectives of formulation of *Momordica charantica* powders incorporated biscuits and study the antimicrobial properties of *momordica charantia* powder and the biscuits.

## 2. Materials and Method

### Preparation of *Momordica charantica* incorporated biscuits

The matured green *Momordica charantica* were obtained from the local market, cleaned and cut into small pieces (P1) and a portion was made into paste (P2) for variations in processing. Both the samples were oven dried at 50°C about 24 hours until it reaches a constant weight (Asan *et al.*, 2013). The dried sample was then pulverized into fine homogenous powder. Biscuits preparation was done using the standard (S1) procedure of Thangam E Philip (2003) with slight modification required for the study. *Momordica charantia* P1 and P2 samples were incorporated replacing refined wheat flour at levels of 2, 4 and 6 percent in preparation of biscuits.

### Sensory evaluation of *momordica charantica* biscuits

The developed biscuits were subjected to sensory evaluation. The quality factors such as appearance, colour, flavour, crispiness, doneness, brittleness, taste and overall acceptability were allotted a maximum score of 9 each. The scoring scale was: 1 ( Dislike extremely), 2 ( Dislike very much), 3(Dislike moderately), 4(Dislike slightly), 5 ( Neither dislike nor acceptable), 6 ( slightly acceptable), 7 ( Moderately acceptable), 8 ( Highly acceptable) and 9 (Extremely acceptable) (Amerine *et al.*, 1965).

### Physiochemical properties of *momordica charantica* biscuits

Physiochemical properties such as moisture, pH, Ash, Acid number, saponification number, peroxide value and total sugar were analyzed in the standard biscuits and highly acceptable P1 and P2 biscuits using standard analytical procedures.

### Screening of photochemical

#### Aqueous extraction

Aqueous extraction was done as per the procedure of Santhi *et al.*, (2011) with slight modification as follows; 10g of air dried powder was added to distilled water and boiled on slow heat for 2 hours. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 minutes. The supernatant was collected at an interval of every 2 hours and was pooled together, concentrated to make the final volume one- fourth of the original volume. It was then autoclaved at 121°C and at 15 Lbs pressure and stored at 4°C.

#### Solvent extraction

10 g of air dried powder was taken in 100 ml of ethanol, chloroform, diethyl ether and ethyl acetate. Plugged with cotton wool and then kept in rotary shaker at 190-200 rpm for 24 hours. After 24 hours the supernatant was collected and the solvents were evaporated to make the final volume one- fourth of original volume and stored at 4°C in air tight containers.

#### Flavonoids

To 1 ml of aqueous extract was added 1 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.

#### Terpenoids (Salkowski test)

5ml of extract was mixed with 2ml of chloroform and carefully added conc. H<sub>2</sub>SO<sub>4</sub> (3ml) to form a layer. A reddish brown coloration at the interface shows positive results for the presence of terpenoids.

#### Cardiac glycosides (Keller-Killiani test)

Crude extract 2ml was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl<sub>3</sub>. The mixture was then poured into another test tube containing 2ml of Concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring at the inter phase indicated a deoxy sugar characteristic of cardiac glycosides. A violet ring might appear below ring whereas the acetic acid layer, greenish ring might form just gradually throughout thin layer.

#### Tannins

5 ml of extract was added to few drops of 1 % lead acetate. A yellow precipitate indicated the presence of tannins.

#### Steroids (Liebermann Burchard reaction)

2ml of acetic anhydride was added to 0.5 g of each extract with 2ml of H<sub>2</sub>SO<sub>4</sub>. The colour change from violet to blue or green in some samples indicated the presence of steroids.

#### Saponins

Foam test was conducted by diluting the extract with 20 ml of distilled water agitated in graduated cylinder 0.1 cm layer of foam was formed and the result was recorded.

**Alkaloids:** Extracts (2ml) were dissolved individually in 1 % dilute hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

**Mayer's Test:** Filtrates were treated with few drops of Mayer's reagent ( potassium mercuric iodide). Formation of a yellow cream precipitate indicated the presence of Alkaloids.

**Wager's Test:** Filtrates were treated with wayer's reagent ((iodine in potassium iodide). Formation of brown/ precipitate indicate the presence of alkaloids.

**Hagner's Test:** A few ml of extract was treated with Hagner reagent (saturated aqueous solution of picric acid) (Annapoorani *et al.*, 2013).

### 3. Results and Discussion

#### Sensory characters of *Momordica charantica* products

The sensory properties of new or improved foods are usually tested by human beings to ensure that they have acceptable and desirable properties before they are launched onto the market.

**Table 1:** Mean scores of acceptability of standard and momordica charantica incorporated biscuits

Sample	appearance	colour	taste	donenes	flavour	crispines	brittleness	Over all acceptability	F-Value
S1	8.2 ±0.78	8.1 ±0.8	7.7±0.8	8.3± .67	8.2±0.7	7.7± 0.67	8± 0.81	8± 0.94	28.33 *
PIA	7.1± 0.99	6.8±0.7	6.8±1.0	7.5±0.52	7.9±0.7	6.9± 0.73	6.9 ±0.87	7.1 ±0.73	
PIB	6.3 ±0.94	6± 0.81	5.4± .69	7.1±0.73	7.6±0.7	7.6±0.84	6±0.8	6±0.8	
P1C	6.3±0.94	5.6±1.0	4±0.81	7±0.66	7.6±0.9	5.6±0.96	5.4±1.17	4.7±0.94	
P2A	7.1±0.87	6.4±0.8	6.2±0.6	7.4±0.51	8.2±0.7	6.9±0.78	7±0.66	6.5±0.70	
P2B	6.5±0.84	6.5±0.8	6±0.66	7.3±0.67	8.2±0.7	6.5±0.84	6±0.81	5.4±0.51	
P2C	6.3±0.67	6.3±0.7	5.4±0.8	7.2±0.63	7.8±0.7	6.3±0.67	5.9±0.87	5.2±0.63	
<b>F -Value</b>	<b>20.24*</b>								

Incorporated biscuits P1A, PIB and PIC secured less scores compared with standard(S1) biscuit. However high acceptability found in P1A biscuit among the P1 sample incorporated biscuits with the scores 7.1 ±0.99, 6.8 ±0.78, 6.8± 1.03, 7.5 ±0.52, 7.9 ±0.73, 6.9± 0.87 and 7.1± 0.73 for the attributes appearance, colour, flavour, taste, doneness, crispiness, brittleness and over all acceptability respectively. Comparing the P2 sample incorporation, P2A was highly acceptable than the other P2 sample incorporated biscuits with the scores of 7.1± 0.87, 6.4± 0.69, 6.2 ±0.63, 7.4± 0.51, 8.2 ±0.78, 6.9 ±0.78, 7 ±0.66 and 6.5 ±0.70 for the attributes appearance, colour, flavour, taste, doneness, crispiness, brittleness and over all acceptability respectively. Perhaps the comparative results of the P1A and P2A samples indicates that P1 sample was highly acceptable than the P2 sample. However the statistical analysis revealed a significant difference between the sample and among the attributes at P < 0.05 percent level. The highly acceptable samples of two different processed products were used for further analysis.

#### Comparison of physiochemical properties of *Momordica charantica* and its products

The physiochemical properties such as moisture, pH, ash, acid number, saponification number, peroxide value, total sugar of P1 and P2

**Table 2:** Physiochemical Properties of *Momordica Charantica* Biscuits

Properties	S1	P1A	P2A
Moisture (%)	7 ±0.05	6.8 ±0.12	7± 0.22
pH	6.58± 0.05	6.54±0.01	6.57± 0.01
Ash (%)	2.7 ±0.28	3.0 ±0.05	3.0± 0.15
Acid number	0.37 ±1.62	0.65 ±1.61	0.74 ±1.61
Saponification number	128.0 ±1.60	130.8 ±1.6	125.2 ±1.61
Peroxide value	13.3± 5.7	16.6 ±5.7	23.3 ±5.7
Total sugar (mg/g)	19.1 ±0.11	18.4 ±0.2	16.8± 0.2

The moisture content of Standard, P1A and P2A biscuits found to be 7 ±0.05, 6.8±0.12 and 7±0.02 respectively. The lower the moisture contents of a product, the better the self stability of a product. Hence, low moisture percentage ensures higher life stability in dried products. The pH of standard biscuit was 6.58 ±0.05, whereas P1A and P2A samples contain 6.54±0.01, 6.57±0.01 respectively. The ash content of P1A and P2A samples found to be 3.0± 0.05 and 3.0± 0.15. The ash content of food material could be used as an index of mineral constituent of the food ([www.sciencedomain.org](http://www.sciencedomain.org)). The P1A and P2A have the acid number of 0.65± 1.61 and 0.74 ±1.61 respectively. When compared to the standard biscuits (0.37 ±1.62) the acid number is slightly higher. Mean saponification number of the developed biscuits namely standard, P1A and P2A were found to be 128.0 ±1.60, 130. 8±1.61

respectively. Higher the saponification value the lesser the quality of the product. The saponification value of *Momordica charantia* seed oil is 185.08 indicated that it contained mainly high molecular mass fatty acids. The lower amount of Unsaponifiable matter (1.19%) shows the lower amount of hydrocarbon. The predominant fatty acid was -eleosteroid acid with percentage of 59%. They also had substantial amount of stearic acid (23%) and other fatty acids like linoleic acid, palmitic acid, oleic acid were less than 10% ( Arora *et al.*,2012). Mean peroxide value of the developed samples indicates that standard, P1A and P2A have  $13.3 \pm 5.7$ ,  $16.6 \pm 5.7$  and  $23.3 \pm 5.7$  values respectively. The mean total sugar content of P1 and P2 was  $10.1 \pm 0.11$  and  $14.4 \pm 0.11$  respectively. In P1A and P2A the mean total sugar was  $18.4 \pm 0.2$  and  $16.8 \pm 0.2/100g$ . When comparing the total sugar content of standard biscuit ( $19.1 \pm 0.11$ )g per 100g of incorporated samples contains less total sugars that may be attributed to the addition of *momordica charantica* powders.

### Phytochemicals in *Momordica Charantica* Incorporated Products Compared With Standard Biscuits

Phytochemicals are non- nutritive plant chemicals that have protective or disease preventive properties. Table-III gives the results of phytochemical screening done in processed *momordica charantica* powders ( P1 and P2) incorporated and unincorporated biscuits.

**Table 3:** Phytochemical in *momordica charantica* powder products from different extraction

Sample	Flavonoid	Terpenoid	Cardiac glycosides	Steroid	Tannin	Saponin	Alkaloid
Aqueous extraction							
S1	-	-	-	-	-	-	-
P1A	+	-	+	-	+	+	-
P2A	+	-	+	-	+	+	-
Diethyl ether							
S1	-	-	-	-	-	-	-
P1A	-	-	+	-	-	+	+
P2A	-	-	+	-	-	+	+
Ethyl acetate							
S1	-	-	-	-	-	-	-
P1A	-	+	-	-	-	+	+
P2A	-	+	-	-	-	+	+
Ethanol							
S1	-	-	-	-	-	-	-
P1A	+	-	+	+	+	-	-
P2A	+	-	+	+	+	-	-
Chloroform							
S1	-	-	-	-	-	-	-
P1A	+	+	-	+	-	-	+
P2A	+	+	-	+	-	-	+

In aqueous extract, Terpenoid, steroids and alkaloids were absent in P1 and P2 biscuits which may be due to the heat interaction with those compounds. Diethyl ether extract of both the P1 and P2 samples showed the presence of cardiac glucosides, saponin, and alkaloids whereas they were absent in flavonoid. Cardiac glucosides, saponin, and alkaloids were present in P1A and P2A samples and rest of the phytochemicals where found absent in both the products of P1 and P2. Ethyl acetate extract of P1A and P2A showed the presence of terpenoids, saponin and alkaloids. Ethanol extract of the P1A and P2A contains flavonoid, cardiac glucosides, tannin and steroids.

### Antimicrobial Property

**Table 4:** Inhibitory effects of aqueous extraction of *Momordica Charantica* powder and products

Samples	Zone of Inhibition in cm				
	Fungus		Bacteria		
	Alternaria	Aspergillus	H1kkb	E.coli	Staphylococcus
<b>Drug</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>2</b>
<b>S1</b>	-	-	-	-	-
<b>P1A</b>	<b>0.5</b>	<b>0.3</b>	<b>0.2</b>	<b>0.3</b>	<b>0.4</b>
<b>P2A</b>	<b>0.5</b>	<b>0.5</b>	<b>0.2</b>	<b>0.5</b>	<b>0.4</b>

The zone of inhibition of standard drug tetracycline and trioderm was found to be 2cm in both the type of organisms (bacteria and fungus). The zone of inhibition of P1A against fungus is 0.3- 0.5cm whereas P2A inhibited 0.5 cm against fungus. The aqueous extract of P1A and P2A inhibited 0.2 to 0.5 cm against bacterias such as Klebsilla Pneumonia, E. coli and staphylococcus. Though the zone of inhibition level of the biscuits were low when compared to the standard drug, the ability of the *momordica charantica* still persists which may be attributed to the phytochemicals of *momordica charantica* exist in the biscuits too.

**Table 5:** Inhibitory effect of ethanol extraction of *Momordica charantica* biscuits

Samples	Zone of Inhibition in cm				
	Fungus		Bacteria		
	Alternaria	Aspergillus	H1kbb*	E.coli	Staphylococcus
Drug	2	2	2	2	2
Standard	-	-	-	-	-
PIA	0.1	0.2	0.1	0.1	0.3
P2A	0.1	0.2	0.5	0.1	0.2

**\*H1kbb-Klebsilla Pneumonia**

The zone of inhibition of standard drug tetracycline and trioderm was found to be 2cm in both the type of organisms. The ethanol extract of P1A and P2A biscuits inhibited the growth of cultures of bacteria and fungus that ranged from 0.1 to 0.5cm. Among the P1 and P2 samples, P2 products are more potent than P1 samples.

#### 4. Conclusion

The phytochemical screening and qualitative estimation of the *momordica charantica* biscuits showed that they are rich in steroid, flavonoid, alkaloid, saponin, tannin and cardiac glucosides. The antimicrobial studies, justifies the potentiality of *momordica charantica* incorporation in biscuits could be a suitable vehicle of supplementing various bioactive compounds.

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