Phytochemical Constituents of Pectin Extracted from Peels of *Cola milleni*, *Theobroma cacao* and *Irvingia gabonensis*


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ABSTRACT

The Phytochemical constituents of the pectin extracted from the peels/husk of *Cola milleni*, *Theobroma Cocoa* and *Irvingia gabonensis* were evaluated using the standard methods. The results obtained showed the presence of saponin, tannin, phenol, flavonoid, glycoside, steroid, cardenolide and terpenoid. The quantitative phytochemical contents of the extracted pectins from *Cola milleni*, *Theobroma Cocoa* and *Irvingia gabonensis* were as follows: Saponin and glycoside (36.00, 52.00 and 55.10), tannin (05.23, 07.40 and 08.20), steroid (00.47, 01.50 and 02.53), alkaloid (10.90, 01.72 and 08.53), terpenoid (11.73, 14.60 and 13.10). Total flavonoid gives (10.17, 14.07 and 05.94). In comparison, Irvingia mango pectin has the highest phytoconstituents followed by Cocoa pectin and finally Cola milleni pectin.

The results obtained indicates that the three pectin samples contain appreciable quantities of bioactive phytochemical compounds which may combat various ailments like Cancer, diabetics or cardiovascular diseases which have developed resistant to already known antibiotics.

Keywords: Phytochemicals screening, *Cola milleni*, *Irvingia gabonensis*, *Theobroma cacao*, pectin, extracts.

1. INTRODUCTION

Phytochemicals are compounds that act as free radical scavengers to help eliminate the highly charged oxygen molecules that are byproducts of metabolized Oxygen (Khalid, 2007), and are believed to offer various health benefits (Van Duyn and Pivonka, 2000; Min et al., 2013).

There is only limited scientific evidence that phytochemicals have biological activity or nutritional value (Sneader, 2000). Non digestible dietary fibers from plant foods, often considered as phytochemicals are now generally regarded as a nutrient group having approved health claims for reducing the risk of some types of cancer and coronary heart disease (Onawumi, 1997). According to the American Cancer Society, "no evidence has shown that phytochemicals taken as supplements are as good for long-term health as the vegetables, fruits, beans, and grains from which they are extracted having the same health benefits as dietary phytochemicals having biological properties like stimulation of the body immune system, antimicrobial, anticancer effect, hormone metabolism and detoxification properties (Patthamakanokporn et al., 2008). Cardiac glycosides support strength and the rate of heart contraction when it is failing but when it is too high, it is toxic to the heart (wang and wang 2008). Trease and Evans (1996) reported that tannins had been widely used as an application to sprains, bruises and superficial wounds, while steroids are used as main treatment of certain inflammatory conditions like inflammation of blood vessels and inflammation of muscles. (Pourmorad, et al 2006 and Ugwu, et al, 2013) have shown that flavonoid and total phenols are free radical scavengers that prevent oxidative cell damage and possess strong anticancer
activities, they induce mechanism that affect cells and inhibit tumor invasion. In this research work, the phytochemical constituents of the extracted pectin from three fruit peels were determined to establish evidence on bioactive components of the extracts.

Monkey Cola, (*Cola milleni*) is a deciduous shrub or tree that is native to the West Africa. It is called monkey kola but locally known as Obi-Edun in Yoruba and Achi okokoro in Igbo. The pods are bright red in colour and have seeds which are covered by a pulp which has a sweet taste and mucilaginous. The pulp is the part which is commonly licked. The fruit, bark and leaves of monkey cola have antimicrobial and antioxidant properties. (Borokini, *et al*, 2014). The plant contains phytochemicals such as saponins, alkaloids and peptides. The health benefits includes: Young leaves eaten as vegetables provides nutrients to the body system, bark and leaves are used to treat skin diseases like ringworm, scabies, the leaves also is used to treat gonorrhea and dysentery. Orisakeye and Olugbade (2012).

African mango, (*Irvingia gabonensis*) is a tree found in the rain forests of West Africa. Fruits of this tree are protein rich and resemble mangoes. It is called wild mango, bush mango, dika, or ogbono. All parts of the tree are used for a variety of purposes. The fleshy part and the pulp of the fruit are consumed as food or used to make jams, juice and wine. The seeds are eaten raw or used to prepare different foods and supplements. The roots, bark, leaves and seeds are used to make traditional medicine. The health benefits are: it promotes weight loss and burning of fat, lowering bad cholesterol and raising the good cholesterol; lowers blood sugar levels, reduces blood pressure. The bark of the tree helps in relieving pain (Randa, 2017).

Cocoa, *Theobroma cacao* tree is a native to the amazon basin. Cocoa was first domesticated in Equitorial South America before being domesticated in Central America. It grows in a limited geographical zone of about 20°C to the North and South Equator. Nearly 70% of the world crop today is grown in West Africa. Health wise, *Theobroma cacao* has been known for treating fever, improving heart strength, treatment of kidney disorder and to increase appetite. It was thought to be nourishing, improve digestion, life lengthening and health preserving. Leaves from the flower were even used to treat skin problems such as burns and stomach complaints. The plant is rich in antioxidant thereby making the blood healthy and act as mood boosting. (Kiran, 2018).

The benefit of cocoa plant includes relief from high blood pressure, cholesterol, obesity, constipation, diabetes, bronchial, asthma, cancer, chronic fatigue syndrome and various neurodegenerative diseases. It's beneficial for quick wound healing, Skin care and helps to improve cardiovascular and brain health. It also helps in treating Copper deficiency. It possesses mood enhancing properties and exerts protective effects against neurotoxicity. (Kiran, 2018).

2.0 MATERIALS AND CHEMICALS

*Cola milleni, Irvingia gabonensis, Theobroma cacao* fruit samples were collected from Idasen farm in Owo Local Government area, Ondo State, Nigeria. All the chemicals and reagents used were of analytical grade obtained from Sigma Aldrich.

2.1 METHODS

2.1.1 Sample Preparation
The peels/husk from the samples (Cola milleni, Irvingia gabonensis and Theobroma cacao) were sun dried for one week and ground to powder using blender. The powdered samples were sieved with a fine mesh of size 14mm. The sieved samples were kept in an air tight container prior to extraction process.

### 2.1.2 Extraction of Pectin

Extraction of pectin from the samples was performed under acid condition using the methods of Koubala et al, 2008, with little modification. The dried powder was subjected to extraction by mixing with acidified distilled water inside a water bath set at different temperatures ranges from 50°C - 100°C and different time from 30 – 150 mins. After contact time reflux, the samples were filtered through cheesecloth and cooled; it was then centrifuged for 20 mins at 3,500 rpm. Ethanol 96% was added to the supernatant and allowed to stand for one hour for pectin precipitation. The precipitated pectin was separated by filtration, washed thrice its volumes with absolute ethanol and washed twice with water to remove impurity. The extracts were separately dried in an oven at 50°C and the pectin yield was determined. The dried pectin samples were stored in aluminium foils at 4°C until used.

### 2.3 Qualitative Phytochemical Analysis

The extracted Pectins were analyzed for the following Phytochemicals:

#### 2.3.1 Test for Tannin

Tannin analysis was carried out using the method of Sofowora, (1999). About 0.5g of the extract was stirred with 100ml distilled water, filtered and ferric chloride reagent was added to the filtrate, a blue black green or blue green precipitate was taken as evidence for presence of tannin.

#### 2.3.2 Test for Saponin

The ability of Saponin to produce frothing in aqueous solution was used as screening test for Saponin. About 0.5g of extract was shaken with distilled water in a test tube frothing which persist on warming was taken as preliminary evidence for the presence of Saponin.

#### 2.3.3 Test for Alkaloid

About 0.5g of the extract was stirred in 5ml 1% aqueous HCl on a steam water bath, 1ml of the filtrate was treated with a few drops of Dragendorf reagent, blue black turbidity was taken as preliminary evidence for the presence of alkaloid.

#### 2.3.4 Test for Steriod

About 20ml of acetic anhydride was added to 0.5g of the extract and filter, 2ml of Conc. H₂SO₄ was added to the filtrate. There was a colour change from violet to blue or green which indicate the presence of steroid.
2.3.5 Test for Terpenoid

About 0.5g of the extract was mixed with 20ml of chloroform and filtered 3ml of conc. \( \text{H}_2\text{SO}_4 \) was added to the filtrate to form a layer. A reddish brown colour at the interface was observed which indicate the presence of terpenoid.

2.3.6 Test for Cardiac Glycosides

The followings were carried out to test for cardiac glycosides

2.3.6.1 Legal’s test- The extract was dissolve in pyridine and a few drops of 2% sodium nitroprusside with few drops of 20% NaOH were added. A deep red colouration which faded to a brownish yellow indicates the presence of cardenolides.

2.3.6.2 Lieberman’s test - 20ml of acetic anhydride was added to 0.5 g of the extract and filter, 2ml of conc. \( \text{H}_2\text{SO}_4 \) was added to the filtrate. There was a colour change from violet to blue or green which indicate the presence of steroids nucleous. (i.e. a glycone portion of the cardiac glycosides.)

2.3.6.3 Salkowski’s test- 0.5 g of the extract was mixed with 20 ml of chloroform and filtered 3ml of conc. \( \text{H}_2\text{SO}_4 \) was added to the filtrate to form a layer. A reddish brown colour at the interface was observed which indicate the presence of steroidal ring.

2.3.6.4 Keller- Killiani’s test- 0.5g of the extract was dissolve in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layed with 1ml of conc. \( \text{H}_2\text{SO}_4 \). A brown ring was obtained at the interface indicating the presence of a deoxy sugar characteristic of cardenolides. A violet-green ring may appear below the brown ring while in the acetic acid layer; a green ring may form just above the brown ring and gradually spread throughout this layer, confirming the presence of glycosides. (Olusupo et al, 2017)

2.4. Quantitative Determination of Total Phenol

The total phenol content of the extract was determined by the method of Singleton et. al., (1999). 0.2ml of the extract filterate was mixed with 2.5ml of 10% Folin ciocalteau’s reagent and 2ml of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45°C for 40mins, and the absorbance was measured at 700nm in the spectrophotometer, Trihydroxylbenzoic acid was used as standard phenol (galic acid).

2.3.3 Determination of Total Flavonoid

The total flavonoid content of the extract was determined using a colorimeter assay developed by (Bao, 2005). 0.2ml of the extract was added to 0.3ml of 5% NaNO\(_3\). After 5min, 0.6ml of 10% AlCl\(_3\) was added and after 6min, 2ml of 1M NaOH was added to the mixture followed by the addition of 2.1ml of distilled water. Absorbance was read at 510nm against the reagent blank.

2.4 Statistical Analysis

The experimental results were expressed as mean ± standard Deviation (SD) of three replicates. Data obtained were statistically analyzed using one way Analysis of Variance (ANOVA), a tool in statistical packages for Social sciences (SPSS 14.0). The level of significance was set at \( P < 0.05 \). Means were separated with Duncan Multiple Range Test (DMRT).

3.0 RESULTS AND DISCUSSION
3.1 RESULTS

Table 1: Phytochemical screening of the extracted pectin in the three samples

<table>
<thead>
<tr>
<th>Test Parameters</th>
<th>Cola</th>
<th>Cocoa</th>
<th>Irvingia mango</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponin</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: Highly present +++
      Moderately present ++
      Slightly present +

Table 2: Quantitative Phytochemical Contents of the Pectin samples

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Cola</th>
<th>Cocoa</th>
<th>Irvingia mango</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponin &amp; Glycoside</td>
<td>36.00 ± 0.20</td>
<td>52.00 ± 0.20</td>
<td>55.10 ± 0.40</td>
</tr>
<tr>
<td>Tannin</td>
<td>05.23 ± 0.06</td>
<td>07.40 ± 0.00</td>
<td>08.20 ± 0.00</td>
</tr>
<tr>
<td>Steroid</td>
<td>00.47 ± 0.06</td>
<td>01.50 ± 0.00</td>
<td>02.53 ± 0.06</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>10.90 ± 0.80</td>
<td>01.72 ± 0.34</td>
<td>08.53 ± 0.06</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>11.73 ± 0.58</td>
<td>14.60 ± 0.10</td>
<td>13.10 ± 0.00</td>
</tr>
<tr>
<td>Total Flavonoid</td>
<td>10.17 ± 0.58</td>
<td>14.07 ± 0.15</td>
<td>05.94 ± 0.25</td>
</tr>
</tbody>
</table>

3.2 DISCUSSION

The preliminary phytochemical screening of the extracted pectins showed the presence of phytochemical constituents like tannin, saponin, flavonoid, terpenoid, steroid, glycoside, and alkaloid. The presence of these secondary metabolites in the samples provides information about their potentials for novel drug delivery. The quantitative results of phytochemical analysis of pectin extract in table 2 shows that the sum of the concentrations of phytonutrients are more abundant in *Irvingia mango* and Cocoa (93.40 and 91.29) pectins than in *Cola milleni* (74.50) pectin extract.

The Saponin and glycoside contents are present at appreciable level in Irvingia mango and Cocoa pectin (55.10 and 52.00) while it is moderately present in cola milleni (36.00). Reports by Osagie, (1998), shows that high presence of saponin in sample have a beneficial effect of cholesterol lowering, deleterious properties, and exhibit structure dependent of medicinal activities.

Steroid and alkaloids were very small in cola milleni and cocoa pectin but moderately present in Irvingia sample. Presence of steroid in large amount is very toxic (Malcolm, 1991) but, these pectin samples have very minute amount which makes it safe for human consumption. Flavonoid is present in appreciable quantity in the three samples in the range of (10.17 ± 0.58,
14.07 ± 0.15, and 05.94 ± 0.25) for cola milleni, cocoa and Irvingia samples. Tannin is present in the range of (05.23 ± 0.06, 07.44 ± 0.00 and 05.94 ± 0.00) for cola, cocoa and Irvingia. High percentage of glycosides in sample is toxic to the heart, (wang and wang 2008). Glycosides, whose presence in samples helps in supporting strength and in controlling the rate of heart contraction, are moderately present in the three pectins which is an indication that the samples are a good material for drug formulation.

This research work shows that the three pectin samples were rich in phytochemicals which may contribute to the health benefits of pectins.

4.0 CONCLUSION

This research shows that the pectin extracted from the three samples contain phytochemical compounds which can serve as medicinal therapeutic purposes and as food without plausible toxicity to body tissues.

ACKNOLEDGEMENTS

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