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Phenolic compounds in drumstick peel for the evaluation of antibacterial, hemolytic and photocatalytic activities

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ABSTRACT

Most of the wastes emitted from the food processing industries are not utilized for any further purpose. The economic value of the food waste is very less when compared to the collection or reuse or discard. To increase the economic value we have to design the food waste as useful product or applicable in most of the current field. Nothing is waste in this world with this concept we have investigated the phytochemical analysis of drumstick peel (*Moringa oleifera*). The result supports the presence of phenols, alkaloids, flavanoids, glycosides and tannins. Since various functional groups containing molecules are present in the extract; it has been further subjected to antibacterial and hemolytic activities. To analysis the antibacterial studies we have employed human pathogenic *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) bacterium. The result of antibacterial activity clearly shows that it possesses significant activity on both bacterial cultures. The hemolytic activity was performed on red blood cells (RBCs). From this result we observed that drumstick peel extract has been considered as non-toxic on RBCs. Malachite green was selected to perform photocatalytic activity. The results stated that the drumstick peel extract possessed good behaviour towards photocatalytic investigation. The malachite green was degraded upto 99.7% using drumstick peel extract.

1. Introduction

The whole plants and plant extracts are having many favourable and advantages in many fields such as biological, medicinal and nanotechnology [1]. The plant extracts were act as a good source for extracting many bioactive compounds which can be useful to treat many diseases [2]. The *Moringa oleifera* is belongs to Moringaceae family and it can be consider as one of the important medicinal plant in many countries. Every portion of this tree for example pods, roots, leaves and stem were behaving in positive aspects towards biological field [3]. The *M. oleifera* has been deliberated as one of the abundant sources for the minerals, nutrition, vitamins and amino acids [4]. The qualitative phytochemical literature resulted that the *M. oleifera* plant extract is rich in phenols, β-sitosterol, flavanoids, tannins and alkaloids [5]. Also it can be act as an immune modulator [6], helps in controlling the blood sugar [7] and cholesterol level [8]. Especially, *M. oleifera* helps to fair antiproliferative activity on cancerous human alveolar cells [9] and pancreatic cancer cells [10]. The *M. oleifera* extract and bioactive compounds which are isolated from the different parts were shown strong antimicrobial [11], anti-inflammatory [12], hepatoprotective [13] and other therapeutic effects [14]. Earlier studies reported that green synthesis of metal and metal oxide nanoparticles can be achieved using the extract of *M. oleifera* leaves [15].

Malachite green (MG) has been utilized as one of the dyestuff and aquatic microbial agent. It also represented as basic green and the chemical formula is $C_{23}H_{25}N_2Cl$. The suitable solvent for MG is water, ethanol and blue-green solutions. MG is one of the useful ingredients in aquaculture industry due to its low cost and better efficiency against protozoa and fungal organisms [16]. Also, it is commonly used in the cotton, silk, paper, leather, paint industries [17]. But MG is the one of the most dangerous dye which affects the human cells and causes tumor formation in liver due to its toxicity [18] and genotoxic effects [19]. Because of this reason, the toxic MG dye should eliminate from the contaminated water and its effluents. Different purification methods were reported to degrade the dye from waste water such as biological purification [20], adsorption method [21], photocatalytical degradation [22], sunlight irradiation [23] and sonochemical degradation [24]. Currently, degradation of the toxic dyes using photocatalyst gaining more importance due to the less toxic to nature and low cost. Among the verities of photocatalyst, the naturally available and cost effective plant

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extracts have been consider as good source to degrade the MG dye. The plant extracts are the abundant source for the presence of different biological and catalytic amount of bioorganic compounds. The *M. oleifera* is also one of the important sources for different chemical constituents. In this present work, we have decided to find a novel and easily available photocatalyst to degrade MG dye. As a result we found drumstick peel extract as a good photocatalyst to degrade the MG dye. Further we explored the drumstick peel extract for antibacterial activity against human pathogenic bacterium. In addition to that we herein report the non-toxicity nature of drumstick peel using haemolytic assay. Also we have done qualitative phytochemical analysis and GC–MS analysis of drumstick peel extract to find the active ingredient which in present in it.

2. Materials and Methods

2.1. Drumstick Collection

Drumsticks were purchased from the local market, Vellore (12.9202° N, 79.1333° E), Tamil Nadu, India. The peel was authenticated as BSI/SRC/S/23/2013-14/tech. 1116 from Botanical Survey of India, Coimbatore.

2.2. Preparation of Drumstick Peel Extract

The peel was separated from the drumsticks and cleaned several times with distilled water and powdered using electrical grinder. The extract was prepared by methanol by maceration method, solvent was distilled off and the extract was concentrated to syrupy consistency under controlled temperature using distillation apparatus.

2.3. Phytochemical Screening of Drumstick Peel Extract

The different chemical groups which are present in *drumstick* peel were identified using preliminary phytochemical analysis [25].

2.3.1. Phenol Test

2.3.1.1. Ferric Chloride Test. About 1 mL of *drumstick* peel extract was added to the few drops of ferric chloride solution, intense green color formation indicates the presence of phenol.

Table 1
Phytochemical test of petroleum ether extract of drumstick peel extract.

Sl. no	Test	Extract
1	Phenol	
	❖Ferric chloride test	+
2	Alkaloids	
	❖Dragendorff's test	+
	❖Wagner's test	+
	❖Hager's test	−
	❖Mayer's test	+
3	Carbohydrates	
	❖Molisch's test	+
4	Flavanoids	
	❖Shinoda test	−
	❖Alkaline reagent test	+
	❖Zinc hydrochloride test	+
5	Glycosides	
	❖Liebermanns Butchard test	+
	❖Legal test	−
	❖Keller Killani test	+
6	Tannins	
	❖Ferric chloride test	+

2.3.2. Alkaloid Test

2.3.2.1. Mayer's Test. Mayer's reagent prepared using 1.358 g of $HgCl_2$ mixture in 60 mL H_2O and 5 g of KI in 10 mL of H_2O. The Mayer's reagent (2 mL) was added to 1 mL of drumstick peel extract and heated few min in the presence of HCl. The yellow formation as precipitate concludes alkaloids presence in drumstick peel.

2.3.2.2. Wagner's Test. Wagner's reagent prepared using the mixture of Iodine and KI. The drumstick peel extract was added to the Wagner's reagent and 2 mL of HCl followed by heat the mixture for few min. The formation of brown or reddish color precipitate is the evidence for the presence of alkaloids.

2.3.2.3. Dragendroff's Test. The potassium Iodide and bismuth nitrate were used for the preparation of Dragendroff's reagent. The drumstick peel extract was mixed with 1 mL of Dragendroff's reagent and heated gently. A positive will show turbid orange color.

2.3.2.4. Hager's Test. The drumstick peel extract (2 mL) was added to the 2 mL of the Hager's reagent. The formation of precipitate indicates the presence of alkaloids.

2.3.3. Test for Carbohydrates

2.3.3.1. Molisch's Test. About 2 mL of Molisch's reagent was added to the methanolic extract of drumstick peel and heated gently for 2 min. The formation of violet ring in the test tube indicates the presence of carbohydrates.

2.3.4. Test for Flavonoids

2.3.4.1. Alkaline Reagent Test. The sodium hydroxide solution (1 mL drop by drop) was added to 1 mL of drumstick peel extract and it forms the intense yellow color. The yellow color should turn into colourless on addition of dil. HCl.

2.3.4.2. Shinoda Test. The few fragments of magnesium ribbon were added to the drumstick peel extract and then around 1 mL of Con. HCl was added drop wise. The crimson red color appearance indicates the presence of flavanoids.

2.3.5. Test for Glycosides

2.3.5.1. Borntrager's Test. About 1 mL of drumstick peel extract was hydrolyzed with dil. HCl and heated for 30 s. Then ferric chloride solution was added in it and boiled for 5 min followed by allow the mixture to cool it. About 1 mL benzene was added after cooling, benzene layer was appeared. Then ammonia solution was added to the benzene layer and the development of pink color shows glycosides presence in drumstick extract.

2.3.5.2. Keller Killani Test. The drumstick peel extract (1 mL) was auxiliary to glacial acetic acid (1 mL) containing trace elements of ferric chloride. Then the mixture was transferred to the small test tube and about 5 mL Con. H_2SO_4 was added slowly. Blue color appears in the acetic acid layer indicated the presence of glycosides.

2.3.5.3. Legal Test. This test has done to identify the presence of cardiac glycosides. Few drops of sodium nitropruside and sodium hydroxide were added to the drumstick peel extract. The pink color turns to blood red color indicates the presence of glycosides.