Antidiabetic Efficacy of Aldimine Derivatives Synthesised from Vanillin and Primary Amines using *Malpighia emarginata* Fruit Extract

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ABSTRACT

The aim of the present study is to analyse the antidiabetic potentials of three aldimines synthesised from vanillin- aniline, vanillin - p-nitroaniline and vanillin - p-toluidine using Malpighia emarginata fruit extract. The three aldimines are synthesised by solvent-free method. The synthesised aldimines are characterized by UV-Visible and FT-IR spectral techniques. The in vitro antidiabetic activities of the synthesised aldimines are determined by alpha glucosidase inhibition assay method and the IC_{50} values are found to be 205.36, 166.26and 235.96µg/mL respectively. The obtained results revealed that the synthesised aldimine shows slight antidiabetic activities. Thus, the biological activities of the synthesised aldimines will trigger more interest in the synthesis of these types of compounds from the easily available starting materials.

***Keywords**: Green synthesis, Malpighia emarginata extract, Aldimines, Antidiabetic activity*

1. Introduction

Green chemistry, also known as sustainable chemistry, focuses on designing and optimizing processes and products to reduce or eliminate the production and use of toxic substances. This approach includes using alternative reaction media to replace the hazardous and expensive solvents typically used in organic synthesis [1]. Recently, solvent-free organic reactions have gained popularity, because most solvents are toxic, flammable, and significantly increase the overall synthesis cost. Solvent-free conditions, such as those for condensation, cyclization, oxidation, and reduction reactions, demonstrate versatility across different chemical transformations. These reactions usually require shorter reaction times, simpler and more efficient work-up procedures, improved selectivities, and easier separations and purifications compared to conventional solvents [2].

Aldimines are key intermediates for synthesizing various bioactive products and are fundamental materials for creatin chiff base ligands, which are used as chiral auxiliaries in asymmetric synthesis [3]. Aldimines exhibit various biological activities due to the azomethine linkage, responsible for antibacterial, antifungal, herbicidal, and clinical properties [4,5]. Traditional methods of aldimine synthesis generate hazardous waste, longer reaction time, and produce low yield [6]. Thus, modifying the synthetic method using green techniques, such as

solvent-free approaches and grindstone methods, is employed to maximize yield [7]. Recently, fruit juice has been recognized as a potential organic solvent for synthesizing compounds of pharmaceutical interest [8]. Fruit juice is increasingly used in various organic transformation reactions due to its non-toxic, safe, inexpensive, and environmentally friendly nature [9]. Bioactive compounds such as enzymes, polyphenols, vitamins, and carotenoids can be extracted from fruit and vegetable waste and used as catalyst for organic compound synthesis [10].

Based on a literature review, this study focuses on the solvent-free synthesis of three aldimines synthesised from vanillin - aniline, vanillin - *p*-nitroaniline and vanillin - *p*-toluidine using *Malpighia emarginata* fruit extract. *Malpighia emarginata* fruits are rich in vitamin C, carotenes, thiamine, riboflavin, niacin, proteins, and mineral salts, including iron, calcium, and phosphorus [11]. These fruits are one of the best natural sources of vitamin C, surpassing guava, cashew, orange, and lemon [12]. They are also beneficial for liver problems, diarrhoea, dysentery, cough, and cold. The aldimines synthesized using *Malpighia emarginata* fruit extract are characterized by UV-Visible and FT-IR spectral techniques. The synthesised aldimines exhibit slight antidiabetic activities.

2. Materials and Methods

Fresh and ripened *Malpighia emarginata* fruit were collected from the college campus. Vanillin, aniline, *p*-nitroaniline and *p*-toluidine used for the synthesis of aldimines were procured from Merck. Double-distilled deionized water was used for the preparation of the *Malpighia emarginata* fruit extract. Analytically graded ethanol was used for the recrystallisation of the synthesized aldimines.

2.1. Preparation of *Malpighia emarginata* Extract

Fresh and ripened *Malpighia emarginata* fruit was used for the preparation of the extract. 25 g of fresh ripened fruit was taken and washed thoroughly with double-distilled deionized water. The fruit was grinded using pestle and mortar. The resulting extract was filtered using Whatman filter paper and the filtrate was collected and then centrifuged for about 8000 rpm for about 10 minutes. The supernatant extract was collected and used for the synthesis of aldimines.

2.2 Synthesis of Aldimines from *Malpighia emarginata* Extract

Equimolar amount of vanillin (0.1 mol) and aniline (0.1 mol) were taken in a clean beaker. About 2 mL of *Malpighia emarginata* extract was added to the mixture and stirred well at room temperature. The pale-yellow precipitate was formed immediately after the addition of the fruit extract. The aldimine obtained was washed with distilled water and recrystallized from

ethanol. Similar procedure was adopted for the synthesis of aldimines from vanillin - *p*-nitroaniline and vanillin - *p*-toluidine.

2.3 Characterization Techniques

The absorption spectral measurement of the synthesised aldimine was carried out using Shimadzu UV-1800 spectrophotometer. FT-IR analysis of the synthesised compound was carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratio and the spectrum were recorded using Shimadzu IR Affinity.

2.4 Antidiabetic Activity

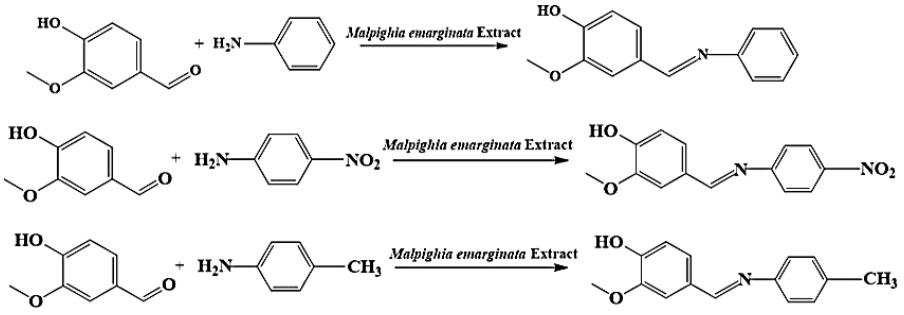
The antidiabetic activities of the synthesised aldimines were determined using alpha glucosidase inhibition assay method. About 400 µL of α-glucosidase (0.067 U/mL) was preincubated with different concentrations of the aldimine for 30 min. Then 200 µL of 3.0 mM *p*-nitrophenyl glucopyrano side (pNPG) used as substrate dissolved in 0.1 M sodium phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 30 min and stopped by adding 2 mL of 0.1 M Na_2CO_3. The alpha glucosidase activity was determined by measuring the yellow-colour ed*p*-nitro phenol released from pNPG at 400 nm. The results were expressed as percentage of inhibition. Same procedure was done with acarbose (1 mg/mL stock) which was used as standard.

$$\textbf{Inhibitory activity (\%)} = (B\text{-}T/B\text{-}C) \times 100$$

Where, B is the absorbance of blank. T is the absorbance in the presence of test substance. C is the absorbance of control.

3. Results and Discussion

The role of *Malpighia emarginata* fruit extract in the synthesis of biologically active aldimines from vanillin, aniline, *p*-nitroaniline and *p*-toluidine is reported in this section. The synthesised aldimines are characterized by UV-Visible and FT-IR spectral analysis. This solvent-free approach is non-polluting and does not employ any toxic materials, quantifying it as a green approach for the synthesis of aldimines (**Scheme 1**).



Scheme 1. Synthesis of aldimines from vanillin, aniline, *p*-nitroaniline and *p*-toluidine

3.1 Absorption Spectral Analysis

The formation of aldimines using *Malpighia emarginata* extract is preliminary confirmed by UV-Visible spectrophotometric analysis. The aldimine synthesised from vanillin and an iline shows absorption bands at 220, 232, 278 and 325 nm due to π-π* and n-π* transitions (Fig. 1). The aldimine synthesised from vanillin and *p*-nitroaniline shows absorption band at 232, 281 and 391 nm due to π-π* and n-π* transitions (Fig. 2). The aldimine synthesised from vanillin and *p*-toluidine sows absorption band at 235, 286 and 319 nm due to π-π* and n-π* transitions (Fig. 3). The higher energy bands in the region at 278-286 nm in the three aldimines is attributed to π-π* transition of the azomethine group [13]. Thus, the absorption spectral data confirms the formation of the aldimines.

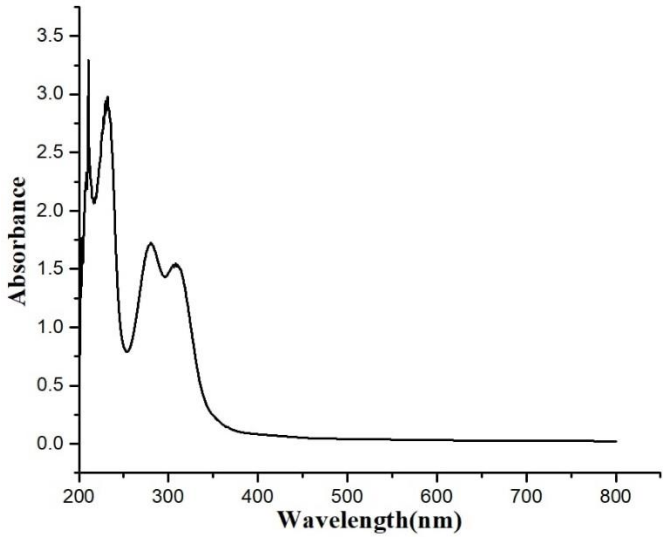


Fig. 1 UV-Visible spectrum of aldimine from vanillin and aniline

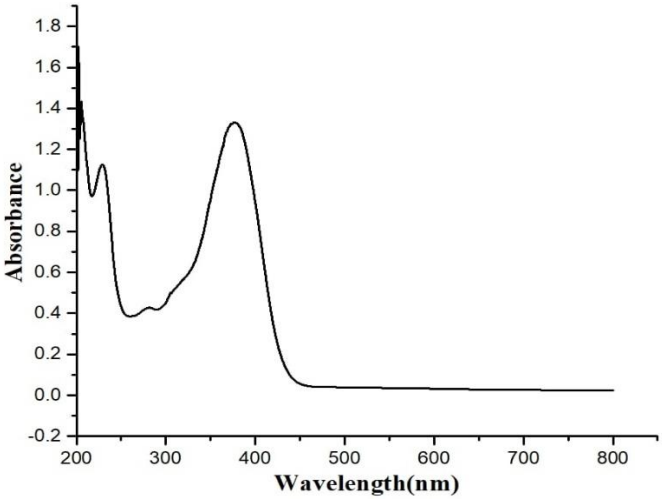


Fig. 2 UV-Visible spectrum of aldimine from vanillin and *p*-nitroaniline

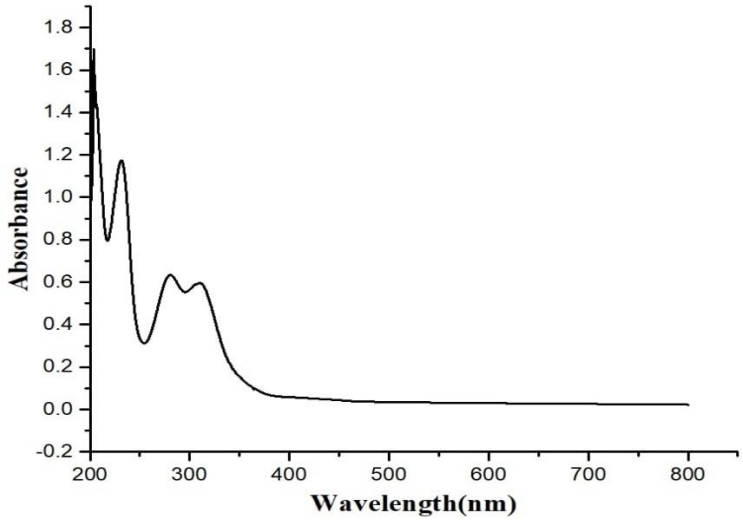


Fig. 3 UV-Visible spectrum of aldimine from vanillin and *p*-toluidine

3.2 FT-IR Spectral Analysis

The FT-IR spectrum of aldimine synthesised from vanillin and aniline using *Malpighia emarginata* extract shows absorption bands 3526, 2924, 2853, 2742, 1674, 1587, 1288, 970, 923, 872, 816, 769, 714, 614 and 520 cm^{-1} respectively (Fig. 4). The band at 3526 cm^{-1} is due to O-H stretching of the hydroxyl group. The weak bands at 2924, 2853 and 2742 cm^{-1} are due to the C-H stretching of alkenes and aromatic system. The band at 1674 cm^{-1} is due to the presence of azomethine group, this indicates the formation of aldimine. The weak band at 1587 cm^{-1} is due to the stretching vibration of aromatic C=C bond. The band at 1288 is due to C-O cm^{-1}stretching vibration of methoxy group. Aromatic C=C and aliphatic C-H bending vibrations occur at 970, 923, 872, 816, 769, 714, 614 and 520 cm^{-1} respectively. The FT-IR spectral data confirms the formation of aldimine from vanillin and aniline.

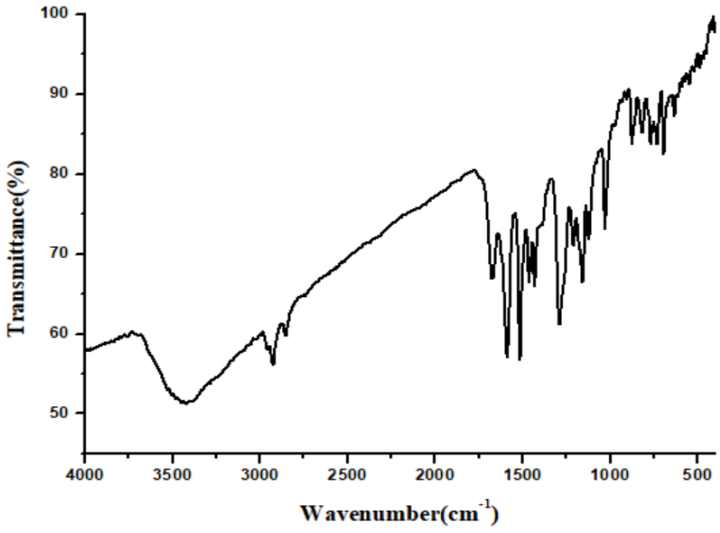


Fig. 4 FT-IR spectrum of aldimine synthesised from vanillin and aniline

The FT-IR spectrum of aldimine synthesised from vanillin and *p*-nitroaniline shows the following absorption data 3479, 3082, 2926, 2853, 2706, 1925, 1672, 1593, 1512, 1292, 1113 961, 869, 759, 696 and 548 cm^{-1} (Fig. 5). The band at 3479 cm^{-1} is due to the O-H stretching vibration of the hydroxyl group. The band at 3082 cm^{-1} is due to the stretching vibration of the aromatic C-H group. The band at 2926, 2853 and 2706 cm^{-1} are due to C-H stretching of the alkene and the aromatic system. The band at 1925 cm^{-1} is due to the C-H bending vibration of the aromatic system. The IR band at 1672 cm^{-1} due to the presence of the azomethine group. Bands at 1593 and 1512 cm^{-1} are due to the stretching vibration of aromatic C=C bond. The band at 1292 cm^{-1} is due to C-O stretching of the methoxy group. The band at 1113 cm^{-1} is due to the ring stretching. Aromatic C=C and aliphatic C-H bending vibrations occur at 961, 869, 759, 696 and 548 cm^{-1}.

The FT-IR spectrum of aldimine synthesised from vanillin and *p*-toluidine shows the following absorption data 3470, 2924, 2853, 2736, 2527, 2477, 1996, 1904, 1674, 1589, 1514, 1429, 1283, 1123, 837, 744, 686, 621 and 531 cm^{-1} (Fig. 6). The band at 3470 cm^{-1} is due to the O-H stretching of hydroxyl group. Weak bands at 2924, 2853, 2736, 2527 and 2477cm^{-1} is due to the C-H stretching of alkene and aromatic system. The band at 1674 cm^{-1} is due to the presence of azomethine group. Absorption bands at 1589, 1514 and 1429 cm^{-1} are due to the stretching vibration of aromatic C=C bond. The band at 1283 and 1123 cm^{-1} due to C-O stretching of methoxy group. Aromatic C=C and aliphatic C-H bending vibrations occur at 837, 744, 686, 621 and 531 cm^{-1}. Thus, all the UV-Visible absorption and FT-IR spectral data of the three synthesised aldimines from *Malpighia emarginata* fruit extract confirms the formation of aldimines.

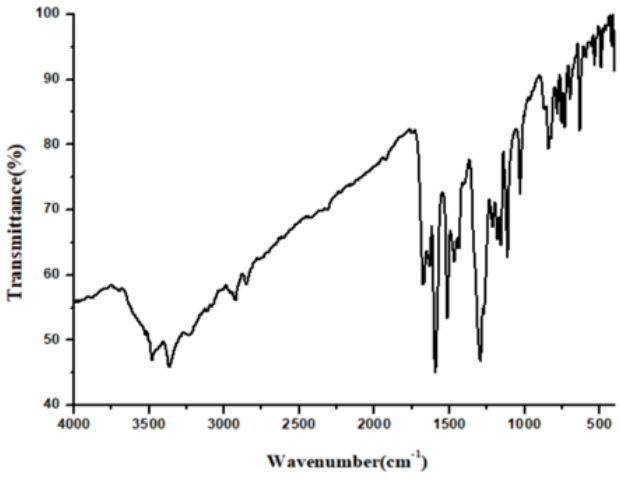


Fig. 5 FT-IR spectrum of aldimine synthesised from vanillin and *p*-nitroaniline

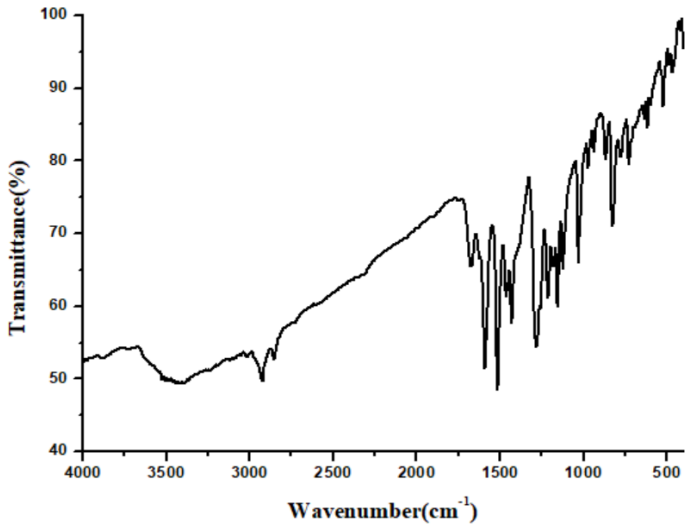


Fig. 6 FT-IR spectrum of aldimine synthesised from vanillin and *p*-toluidine

3.3 Antidiabetic Activities of Aldimines

The antidiabetic activities of the aldimines synthesised from vanillin - aniline, vanillin - *p*-nitroaniline and vanillin - *p*-toluidine at different concentrations are determined and tabulated in Tables 1 - 3. The IC_{50} values for the aldimines synthesised from vanillin and aniline is 205.36 µg/mL, vanillin and *p*-nitroaniline is 166.26 µg/mL and vanillin and *p*-toluidine is 235.96 µg/mL. The IC_{50} value of acarbose is 16.72 µg/mL. The synthesised aldimines show higher IC_{50} values compared to that of the standard acarbose. Compared to the IC_{50} values of the three aldimines, the aldimine synthesised from vanillin and *p*-nitroaniline shows lower IC_{50} value, this is due to the presence of electron withdrawing nitro group. The presence of electron donating methyl group in *p*-toluidine increases the IC_{50} value. Lower IC_{50} value reflects better antidiabetic activity. Thus, the obtained results reveal that the synthesized aldimines produce slight antidiabetic activity as compared to that of acarbose.

Table 1. Antidiabetic activity of aldimine synthesised from vanillin and aniline

Concentration (µg/mL)	Absorbance at 400 nm	% of Inhibition
1.5	1.751	2.33
3.125	1.652	7.96
6.25	1.597	11.09
12.5	1.518	15.59
25	1.362	24.46
50	1.302	27.87
100	1.202	33.56
IC_{50}	**205.36 µg/mL**	

Table 2. Antidiabetic activity of aldimine from vanillin and *p*-nitroaniline

Concentration (µg/mL)	Absorbance at 400 nm	% of Inhibition
1.5	1.735	3.24
3.125	1.642	8.53
6.25	1.574	12.40
12.5	1.474	18.09
25	1.321	26.79
50	1.257	30.43
100	1.151	36.46
IC_{50}	**235.96 µg/mL**	

Table 3. Antidiabetic activity of aldimine from vanillin and *p*-toluidine

Concentration (µg/mL)	Absorbance at 400 nm	% of Inhibition
1.5	1.719	4.15
3.125	1.624	9.56
6.25	1.502	16.50
12.5	1.426	20.82
25	1.292	28.44
50	1.211	33.05
100	1.091	39.87
IC_{50}	**166.26 µg/mL**	

4. Conclusion

An eco-friendly route for the synthesis of aldimines using *Malpighia emarginata* extract has been investigated. The synthesised aldimines are characterized by UV-Visible and FT-IR spectral analysis techniques. The higher energy bands in the region at 278-286 nm in the three aldimines is attributed to π-π* transition of the azomethine group. The FT-IR bands in the range of 1672-1674$cm^{-1}$are due to the presence of azomethine group, the spectral data confirms the formation of aldimines. The antidiabetic activities of the synthesised aldimine show slight activity than that of the control. This solvent-free approach is non-polluting and does not employ any toxic materials, quantifying it as a green approach for the synthesis of aldimines.

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