Antihyperglycu-lipidemic and Antioxidant activities in Aqueous methanol Extract of Some Vegetables Peel: An in vitro Analysis

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ABSTRACT

Objective: To examine phytochemical constituents, antihyperglycemic, antihyperlipidemic and free radical scavenging activities in 50% methanol extract of peels of Cucumis melo var. chito (CM), Cucumis pubescens (CP), Lagenaria siceraria (LS), Luffa acutangula (LA), and Momordica charantia (MC) applying in vitro methods. Material and Methods: Total polyphenol (gallic acid equivalent; GAE), flavonoid (rutin equivalent; RE) and anthocyanin (cyanidin-3-glucoside equivalent; CGE) content were estimated. The 2,2-diphenyl-1-picrylhydrazyl (DPPH); 2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS\textsuperscript{+}); nitric oxide (NO) scavenging; nitro blue tetrazolium (NBT) reduction; advanced glycation end-products (AGEs), intestinal \(\alpha\)-glucosidase, pancreatic lipase and liver Protein-tyrosine phosphatase \(1\beta\) (PTP \(1\beta\)) inhibitory activities were examined in vitro. Results: Peel of CM (1190 \(\mu\)GAE/g) and MC (923 \(\mu\)GAE/g) was rich in polyphenol and flavonoids (68.8, 145.1 \(\mu\)GAE/g respectively). Anthocyanin content was more in MC (167 mg CE/100 g) than others. LS potently scavenged ABTS\textsuperscript{+} (SC\textsubscript{50}, 99 \(\mu\)g) and DPPH (SC\textsubscript{50}, 0.6 mg) radicals. Inhibition of vesperlysine-like AGEs (71.5%) was significant (p<0.006) than pentosidine-like AGEs (71.5%) by LA peel. Inhibition of pentosidine-like AGEs was significant (p<0.009) by CM than CP peel. MC reduced (84.5 %) only vesperlysine-like AGEs. Intestinal \(\alpha\)-glucosidase was inhibited (23.02 %) by MC peel only. Pancreatic lipase was inhibited by CM (74.5%) and CP (58.6 %) peels. The LS reduced PTP \(1\beta\) (32.1 %) more potently than others. Conclusion: Vegetable peels present variable degree of antioxidant, antihyperglycemic and antihyperlipidemic activities.

Key words: Advanced glycation end products, Antioxidant, Intestinal \(\alpha\)-glucosidase, Porcine pancreatic lipase, Protein-tyrosine phosphatase \(1\beta\).

INTRODUCTION

Recent clinical and experimental observations disclose that consumption of vegetables and their juice is helpful in control and management of epidemic of glycemic and lipidemic disorders in a simple and cost effective manner.\textsuperscript{1-3} However, most of the times, vegetables are peeled before cooking and consumption. Interestingly, some research results disclose that vegetable peels display potent antioxidant\textsuperscript{4}, antidiabetic\textsuperscript{5} activities and cardioprotective properties. However, the possible mechanism by which vegetable peels display antioxidant, antidiabetic and cardioprotective properties is not investigated. This research presents analysis of phytochemical constituents, various free radical scavenging activities as a measure of antioxidant capacity; intestinal \(\alpha\)-glucosidase, Protein-tyrosine phosphatase \(1\beta\) and advanced glycation end-products inhibitory properties relating to the antidiabetic activities, and porcine pancreatic lipase inhibitory activity as cardioprotective property (antihyperlipidemic) in some common vegetable peels.

MATERIALS AND METHODS

Chemicals

Reagents of high quality were obtained from Sigma-Aldrich Chemicals (St Louis, MO). Other chemicals of analytical grade were purchased from Merck Limited (Mumbai, India) and S.D. Fine Chemicals Ltd (Mumbai, India).

Plant material and extraction procedure

Fruits of Cucurbitaceae family vegetables namely, bitter gourd [Momordica charantia (Molina) Standl.), LS]; ridge gourd [Luffa acutangula (L.), LA]; kachri melon [Cucumis pubescens Wild., CP] and mother Mary’s pie melon [Cucumis melo var. chito C. Morren, (CM)] (Figure 1) were purchased from vegetable shops in Hyderabad city. The vegetable fruits were taxonomically indentified and authenticated by Prof. Ajmeera Ragan [Botany Department in Kakatiya University, Warangal (TS)]. Voucher specimens (MC-KUW 1321, LS-KUW 1322, LA-KUW 1323 and CP-CM-KUW 1324) were deposited in the departmental herbarium. Fruits were washed thoroughly with water and peels were removed with the help of stainless steel peeler. Peels of respective vegetables were dried for 24 h in incubator (Innova 4230 refrigerated incubator shaker, New Brunswick Scientific Edison, NJ, USA) at 37°C and ground to powder. Peel powder (1 g) was soaked in 8 mL of HPLC grade methanol and Milli Q purified water (1:1) as described by Slanc et al.\textsuperscript{6} Extracts were stored at 4°C for analysis.

PHYTOCHEMICAL ANALYSIS

Total phenol content

Phenol content was measured according to Tiwari et al (2013). With suitable modifications.\textsuperscript{7} Total polyphenol content was measured spectrophotometrically (BioTek synergy4 multi-mode microplate reader, BioTek Instruments, Inc Winooski, VT, USA) at 765 nm and results were expressed in terms of \(\mu\)g/mL of gallic acid equivalent.

Total flavonoid content

Flavonoid content was determined by the procedure previously described by Tiwari et al.\textsuperscript{7} with suitable modifications. Total flavonoid con-
tent was recorded spectrophotometrically at 430 nm and results were expressed as of µg/mL rutin equivalent.

**Total anthocyanin content**

Anthocyanin content was determined as described by Tiwari et al. (2011) with suitable modifications. Absorbance at 510 nm and 700 nm were recorded at different pH separately. Results were expressed as milligrams of anthocyanins per 100 g of extract. 8

**DPPH free-radical scavenging activity**

DPPH free-radical scavenging activity was determined according to previously reported technique. 8,9 Absorbance was recorded at 517 nm. Several serial dilutions of respective extract were prepared and percentage scavenging concentration 50% (SC 50) was calculated.

**ABTS** free-radical scavenging activity

ABTS' free-radical scavenging activity was performed according to Tiwari et al (2001). 10 Decolorized ABTS' absorbance was measured spectrophotometrically at 734 nm. The percentage of ABTS' scavenging activity was expressed in terms of trolox equivalent. Suitable regression analysis was applied for calculation of SC 50.

**Nitric oxide scavenging assay**

Nitric oxide scavenging assay was carried according to Tiwari et al (2013). 11 The absorbance of chromophore formed was measured at 546 nm spectrophotometrically and percentage of nitric acid scavenging activity was reported.

**Nitro blue tetrazolium (NBT) reducing assay**

Nitro blue tetrazolium reducing assay was determined according to Tiwari et al (2013). 11 The reduction of NBT was measured at 560 nm using a BioTek synergy 4 multimode microplate reader (BioTek Instruments Inc, Winooski, VT, USA). The NBT reducing activity was obtained in terms of ascorbic acid equivalent μg/mL.

**Rat intestinal α-glucosidase inhibition**

Rat intestinal α-glucosidase enzyme inhibitory activity was performed as described by Tiwari et al (2013). 11 After incubation for 10 min, released p-nitrophenol was measured spectrophotometrically at 405 nm and results were expressed as percentage rat intestinal α-glucosidase inhibition.

**Pancreatic lipase inhibition**

Pancreatic lipase inhibition was determined according to procedure described by Mc Dougall et al (2009). 11 Absorbance was read at 405 nm spectrophotometrically and percentage inhibition of pancreatic lipase was reported.

**Advanced glycation end products (AGEs) inhibitory activity**

AGEs screening was performed according to Poormina et al (2015). 12 Aminoguanidine (5 mg/mL) was used as standard. Both vespertine-like (λ ex 370 nm; λ em 440 nm) and pentosidine-like (λ ex 335 nm; λ em 385 nm) AGEs inhibition was determined according to Sero et al., (2013) 25 by using BioTec synergy 4 multimode microplate reader (BioTec Instruments Inc, Winooski, VT, USA. Results were expressed as percentage inhibition of AGEs.

**Protein-tyrosine phosphatase 1β (PTP 1β) inhibition**

PTP 1β activity was determined according to the method described by Tiwari et al (2013). 11 Absorbance was read spectrophotometrically at 405 nm and results were expressed as percentage inhibition.

**Statistical analysis**

All the data were expressed as mean ±SD. One way ANOVA followed by Tukey’s multiple comparison tests was applied to compare differences within gourd vegetable’s peel and t-test with Welch’s correction was applied to compare differences between melon vegetable’s peel. The criterion for statistical significance was p<0.05. Statistical analyses were performed by using GraphPad PRISM Version 5.01(GraphPad Software, Inc. California, USA).

**RESULTS AND DISCUSSION**

**Polyphenol and antioxidant activities**

Polyphenol, flavonoids and anthocyanins are primarily responsible for free radicals scavenging antioxidant activities, and play important role in prevention and treatment of metabolic disorders such as cardiovascular and diabetes. 13 Fruits and vegetables are the major sources of phenolic compounds in human diet.

Data presented in Table 1 shows that vegetable’s peel are rich source of polyphenol. The total polyphenolic content in MC peel was significantly

**Figure 1:** Representative photographs of vegetables
(p<0.001) high than that present in LS and LA peels. Although peels of LA were thinner than peels of LS, the total polyphenol content in LA peels was recorded four times (p<0.001) higher than in LS peels. Contrarily however, the thick peels of CM displayed two times more (p<0.0006) polyphenol content than CP. The total flavonoid content in MC was sixteen times higher (p<0.001) than that present in peels of LS and LA. In contrast to the total polyphenol content, the flavonoid content in LS and LA peels did not differ significantly. Similarly to the total polyphenol content, the flavonoid content in CM was recorded twice more (p<0.0002) than that present in CP. The anthocyanin content in MC was significantly (p<0.001) high when compared with LS and LA. The anthocyanin content was recorded significantly (p<0.001) high in LS than in LA. Similarly, the anthocyanin concentrations in CP were detected more (p<0.001) than that present in the CM peel. Our analysis shows that among gourd vegetables viz. MC, LS and LA, the peel of MC was rich source of polyphenols, flavonoids and anthocyanins. Between the melons CP and CM, the peel of CM was rich in polyphenols and flavonoids.

Free radicals scavenging antioxidant activities

Mixture of variety of polyphenolic compounds present in plant materials provide protection against postprandial oxidative stress by virtue of their free radicals scavenging antioxidant activities. The structural diversity of polyphenolic compounds displays varying degree of free radicals scavenging and antioxidant activities on different targets. It is advised therefore to analyze antioxidant activity of a compound or the mixture of compounds applying different test models in order to gauge their optimum antioxidant potentials. The mono cation of 2, 2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+) is generated by oxidation of ABTS with potassium persul fate and is reduced in the presence of hydrogen-donating antioxidants. The decolorization assay of ABTS+ is applicable in identification of the both lipophilic and hydrophilic antioxidant compounds. Aqueous methanol extract of all the vegetable peels scavenged ABTS+ radical potently (Table 2). However, when compared with their SC50 values, the extract of LS was found twice more potent (p<0.01) than MC peel extract and four times more potent (p<0.001) than LA peel extract. The extract of CM peel displayed significantly (p<0.0006) high ABTS+ radical scavenging capacity than CP peel extract (Table 2).

The 2, 2-diphenyl-1-picrylhydrayzyl (DPPH-) radical is one of the few stable organic nitrogen centered radicals. Sanchez-Moreno (2002) suggests that DPPH- radical scavenging assay is an easy and accurate method with regard to measuring antioxidant capacity of fruit and vegetable juices. Applying this method, we found that extract of LS peel potently (p<0.001) scavenged DPPH- radical than MC extract. The LA and CP peel extracts could not scavenge DPPH- radical more than 32% at 3.1mg RME concentration (Table 2). Peel extract of CM was stronger (p<0.0005) than CP in scavenging DPPH- radical (Table 2).

Nitric oxide (NO) reacts with superoxide radical (O2) to form peroxynitrite anion. Decomposition of this molecule generates hydroxyl radical and nitrogen dioxide which play a major role in oxidative cell damage. Therefore, consumption of foods that possess NO scavenging activity appears beneficial to human health. Data presented in table 2 shows that there were significant differences in NO scavenging potential among different vegetable peels. LS peel extract displayed higher (p<0.001) NO scavenging capacity than MC and LA peel extracts. The differences between NO scavenging activity of MC and LA extract were also significant, where the MC peel extract was more potent (p<0.01) than the LA. The NO scavenging potential in CM peel extract was 28% higher (p<0.0002) than that present in CP peel extract (Table 2).

Nitro blue tetrazolium (NBT) has been applied for detection of ascorbic acid which is a potent antioxidant and cellular reductant present in plant leaves. Our analysis finds that peels of MC and CM possess potent NBT reducing power followed by LS (Table 2). The NBT reducing power in MC peel was significantly higher than that present in LS (p<0.05) and LA (p<0.01). The NBT reducing capacity in CM peel was recorded four times higher (p<0.004) than that present in CP peels.

The advanced glycation end products (AGEs) are considered highly damaging compounds generated during hyperglycemic and hyperlipidemic conditions. Although, AGEs are formed primarily under oxidative stress conditions, they have also been implicated in increasing generation of free radicals and disturb antioxidant systems. AGEs are held responsible for development of diabetic complications, neurological disorders and accelerating ageing process. Therefore, prevention of AGEs formation presents exciting opportunities in extenuating development of diabetic complications and neurological disorders along with slowing the ageing process. AGEs present vast structural differences and
### Table 2: Different biological activities (in vitro) in vegetables peel

| Ext. | % ABTS<sup>a</sup>
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<tr>
<td></td>
<td>scavenging&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>(SC&lt;sub&gt;v&lt;/sub&gt;)</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>MC</td>
<td>98.4±0.5 (190±2)&lt;sup&gt;1,1&lt;/sup&gt;</td>
</tr>
<tr>
<td>LS</td>
<td>98.0±0.6 (99±5)&lt;sup&gt;1,2&lt;/sup&gt;</td>
</tr>
<tr>
<td>LA</td>
<td>98.7±0.3 (390±90)&lt;sup&gt;1,2&lt;/sup&gt;</td>
</tr>
<tr>
<td>CP</td>
<td>97.3±0.2 (350±16)&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>CM</td>
<td>96.2±0.7 (130±18)&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Note:** MC, Momordica charantia L., bitter gourd; LS, Lagenaria siceraria (Molina) Standl., bottle gourd; LA, Luffa acutangula L., ridge gourd; CP, Cucumis pubescens Wild, kachri melon; CM, Cucumis melo var. chito (C. Morren), Mother Mary's pie melon. Values represent means ± SD, n=3.

One way ANOVA followed by Tukey's multiple comparison tests was applied to compare differences within gourd vegetable's MC, LS and LA peels and t-test with Welch's correction was applied to compare differences between melon vegetable's CP and CM peels and differences between v-AGEs vs p-AGEs in LR. Degree of significance for similar symbols, *p<0.05, **p<0.01, ***p<0.005, ****p<0.0005.

1Percentage inhibition at 1.25 mg raw material equivalent (RME) extract concentration for ABTS<sup>+</sup> and 3.125 mg for DPPH scavenging.

2Percentage scavenging of NO (nitric oxide radical) at 2.5 mg RME extract concentration.

3Percentage scavenging of NO (nitric oxide radical) at 6.25 mg RME extract concentration.

4Percentage inhibition at 1.25 mg raw material equivalent (RME) extract concentration for ABTS<sup>+</sup> and 3.125 mg for DPPH scavenging. Values in parentheses represent radical scavenging concentration 50% (SC<sub>50</sub>). SC<sub>50</sub> for ABTS<sup>+</sup> scavenging are presented in μg RME and for DPPH scavenging, values are presented in mg RME. Trolox was taken as standard radical scavenger and it's SC<sub>50</sub> for ABTS<sup>+</sup> and DPPH was 0.35 μg and 32.7 μg respectively.

5Percentage scavenging of NO (nitric oxide radical) at 6.25 mg RME extract concentration.

6Activities represent at 2.5 mg RME extract concentration.

7Activities represent at 6.25 mg RME extract concentration.

8Values in parentheses represent radical scavenging concentration 50% (SC<sub>50</sub>). SC<sub>50</sub> for ABTS<sup>+</sup> scavenging are presented in μg RME and for DPPH scavenging, values are presented in mg RME. Trolox was taken as standard radical scavenger and it's SC<sub>50</sub> for ABTS<sup>+</sup> and DPPH was 0.35 μg and 32.7 μg respectively.

9Activities represent at 6.25 mg RME extract concentration.

10AGEs represent advanced glycation end-products, v-AGEs represent vesperlysine type-AGEs and p-AGEs represent pentosidine type-AGEs.

11PTP<sub>1β</sub> represents protein tyrosine phosphatase 1β.

12Na, not active; ns, not significant.

Table:<br>have recently been grouped in two major types (the vesperlysine-like AGEs and pentosidineline-like AGEs by Sero et al (2013).<sup>23</sup> The pentosidine-like AGEs are found primarily in plasma and erythrocytes whereas as the vesperlysine-like AGEs are mainly shown in the lens of diabetic subjects (Grillo and Colombatto, 2008).<sup>24</sup> Therefore, identification of specific type of AGE inhibitors may help directing their therapeutic importance in specific type of diabetic complications. Our analysis finds that MC peel extracts could inhibit formation of only vesperlysine-like AGEs (Table 2). LS peel extracts did not display AGEs inhibitory activity. Extracts of LA peels inhibited both, vesperlysine-like AGEs and pentosidine-like AGEs. However, inhibitory activity towards vesperlysine-like AGEs was significantly high (p<0.006) than for pentosidine-like AGEs (Table 2). The AGEs inhibitory activity of CP and CM was not significantly different for vesperlysine-like AGEs and pentosidine-like AGEs. However, CM peel extract inhibited pentosidine-like AGEs more potentially (p<0.009) than CP peel extracts.

**Enzyme inhibitory activities in peel extracts**

The intestinal α-glucosidase inhibitors and pancreatic lipase inhibitors have shown remarkable effect in reducing development of postprandial hyperglycemia and hyperlipidemia respectively, in clinical settings. However, there are several disadvantages associated with synthetic drugs.<sup>26</sup> Therefore, identification and development of inhibitors for these enzymes from dietary sources have become an area of recent interest.<sup>27,28</sup> Only MC peel could display intestinal α-glucosidase inhibitory activity in our analysis (Table 2). Peel extracts of CP and CM displayed pancreatic lipase inhibitory potentials. The pancreatic lipase inhibitory activity in CM peel extract was significantly high (p<0.01) than that present in CP peels extract (Table 2).

Popov (2011), observed that the hallmark phenomenon of insulin resistance in type 2 diabetes and leptin resistance in obesity, are associated with increased activity and expression of Protein-tyrosine phosphatase 1β (PTP<sub>1β</sub>).<sup>29</sup> Furthermore, inhibition of PTP<sub>1β</sub> activity has been found to reduce incidences of hyperglycemia and hyperlipidemia.<sup>30</sup> Recently, vegetables were identified as rich natural resources of PTP<sub>1β</sub> inhibitors.<sup>31</sup> Data presented in table 2 shows that vegetable peels displayed varying degree of PTP<sub>1β</sub> inhibitory potentials. The extract of LS and CM peels displayed better PTP<sub>1β</sub> inhibitory activity than other vegetable's peels (Table 2). The PTP<sub>1β</sub> inhibitory activity was significantly high (p<0.003) in CM peel extract than present in CP peel. Interestingly, the PTP<sub>1β</sub> inhibitory activity was found significantly associated with ABTS<sup>+</sup> (Pearson r = -0.8337, p<0.0397) and DPPH (Pearson r = 0.9162, p< 0.0144) radicals scavenging activities in peel extracts.

**CONCLUSION**

Peels of vegetables were found to be rich source of polyphenols, flavonoids and anthocyanins. The aqueous methanol extract of vegetable peels reported in this study, displayed multifaceted free radicals scavenging and antioxidant potentials. Peels of MC, LA, CP and CM displayed varying but potent AGEs inhibitory activity. This property may help attenuate disorders related with metabolism, oxidative stress and ageing. Furthermore, presence of intestinal α-glucosidase inhibitory ac-
tivity in MC, and pancreatic lipase inhibitory activity in CP and CM may become helpful in reducing postprandial hyperglycemic and hyperlipidemic burden induced by carbohydrate and lipid rich diet respectively. Interestingly, the presence of PTP1β inhibitory activity in vegetable peels and its association with free radical scavenging properties, presents exciting opportunity for their incorporation in diet, for they can attenuate development of oxidative stress together with insulin resistance and leptin resistance.

ACKNOWLEDGEMENT
This research was financially supported by grants SMiLE-CSC-0111 and NAPAHA-CSC-130 from CSIR, New Delhi. DAK thanks D’CSIR-IICT for award of SPF under SMiLE project.

CONFLICT OF INTEREST
Authors declare that they have no conflict of interest financial or otherwise.

ABBREVIATIONS USED

| CM                        | Cucumis melo var. chito,     |
| CP                        | Cucumis pubescens           |
| LS                        | Lagenaria siceraria          |
| LA                        | Luffa acutangula             |
| MC                        | Momordica charantia          |
| CGE                       | Cyanidine-3-glucose equivalent |
| RE                        | Rutin equivalent             |
| GAE                       | Gallic acid equivalent       |
| DPPH                      | 2,2-diphenyl-1-picylhydrazyl |
| ABTS*                     | 2,2-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) |
| NO                        | Nitric oxide                 |
| NBT                       | Nitro blue tetrazolium       |
| AGEs                      | Advance glycation end-products |
| PTP 1β                    | Protein-tyrosine phosphatase 1β |
| SD                        | Standard deviation           |

ANOVA: Analysis of variance

REFERENCES
PICTORIAL ABSTRACT

• Vegetable peels are rich source of antioxidant activities and phytochemicals.
• Peel of LA, CP, CM inhibited formation of various AGEs.
• MC peel inhibited 23% intestinal α-glucosidase enzyme.
• Peel of CP and CM could inhibit 58% and 74% pancreatic lipase respectively.
• All the peels displayed varying degree of PTP1β inhibition.

SUMMARY

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