Enzymatic Synthesis and Characterization of Quercetin Occurring in Onion (Allium cepa L.) Outer Skin

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A B S T R A C T

The rapidly increasing trend of onion outer skin waste (OOSW) generation from the food industry compelling the scientists to search for possible ways of their utilization as it is not suitable for fodder in high concentrations. The best possible way could be to use these waste as a new and natural source of high value added products related to health benefits owing to the presence of flavonoids especially the powerful antioxidants, anti-inflammatory, anti-cancerous and colouring compounds quercetin. For this, it is very essential to synthesize and characterize the quercetin occurring in OOSW. In this study, the quercetin was synthesized successfully from OOSW by Vacuum Assisted Soshet Extraction (VASE) method followed by enzymatic hydrolysis for purification. The purity study and structural characterization of synthesized quercetin were carried out by analyzing HPLC, UV-Visible, Fourier Transform Infrared (FTIR), Nuclear Magnetic Resonance (NMR) (H and C) spectra. Differential scanning Calorimetry (DSC), Thermo Gavimetric Analyzer (TGA) and Scanning Electron Microscope (SEM) were used for thermal and surface morphology characterization of quercetin. The results not only confirmed the structure of quercetin but also its thermal stability and smooth surface morphology with needle like regular shape profile. The antibacterial activity of quercetin in terms of zone of inhibition (ZOI) and bacterial reduction percentage was investigated against Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria. The investigated quercetin showed good antibacterial activity and are found to be a promising non-toxic material for use in food industry as packaging and in medical industry as medical material.

Introduction

Quercetin (3, 3’, 4’, 5, 7- pentahydroxy flavone) is a natural bioactive flavonoid found abundantly in vegetables and fruits has proven beneficial impact on human health. The growing evidence suggesting that quercetin has therapeutic potential for the prevention and treatment of different diseases, including cardiovascular disease, cancer and neurodegenerative disease. Mechanistically, quercetin has been shown to exert antioxidant, anti-inflammatory and anticancer activities in a number of cellular and animal models as well as in humans through modulating the signaling pathways and gene expression involved in these processes. Hertog et al. (1992) reported that outer skin of onion ranked the highest in quercetin content amongst 28 vegetables and 9 fruits. This information compels us to think for the extraction of quercetin present in onion outer skin which is generally thrown out as waste.

Onions (Allium cepa L.) are the second most important horticultural crop worldwide after tomatoes and its production is increasing every year due to increasing consumer demand (Benitez et al., 2011). In recent years, the consumption of onion has increased by more than 25% due to its flavour and health benefits. In addition, there is also an increase in demand for processed onions which ultimately has led to an increase in the generation of large quantities of waste (Han et al., 2007). Since, OOSW has strong aroma and provides the media for growth of phytopathogens, it cannot be used for fodder and landfill disposal (Sala et al., 2013). Moreover, due to large moisture content its disposal by combustion is expensive (Roldain et al., 2008).

Therefore, proper utilization of OOSW is a major issue. Several studies (Slimestad et al., 2007; Kim and Kim, 2006; Yao et al., 2004; Sellappan and Akoh, 2002; Griffiths et al., 2002; Soltof et al., 2009; Pérez-Gregorio et al., 2010; Kiasmous et al., 2009; Jin et al., 2011) have shown that OOSW contains high level of dietary flavonoids especially quercetin in much higher concentrations which may exhibit health promoting effects as it has many pharmaceutical properties like anti-microbial, antioxidant, anti-carcinogenic, anti-mutagenic, anti-diabetic etc. In addition, it has also the potentiality to behave like a dyestuff for textile material (Deo and Paul, 2000). Literature review over the past decade reveals the progress regarding extraction of quercetin and its related glycosides from total onions (Verma et al., 2018; Kwak et al., 2019).
The isolation of essential bioactive substances from OSSW also has been studied as the way to utilize or evaluate the huge parts of the resources (Suh et al., 1999).

So, the synthesis of valuable component quercetin from OSSW should be worth enough not only from economic and environmental benefits point of view but also for experiencing the beneficial impact on human health. The present paper therefore aims at synthesis and characterization of major bioactive substance quercetin present in OSSW in order to enhance the understanding of the same for its biological activities in the broader spectrum of research.

Materials and Methods

Materials

Onion outer skin waste (OOSW) (supplied by Sheoraphuly local market, Hooghly, West Bengal, India), reagent grade ethanol (50% aqueous), citric acid, cellulase, pectinase, xylanase, deionized water, standards of rutin, quercetin, quercetin-3-glucoside and kaempferol of purity 98.5% (supplied by Unichem agency, Kolkata, West Bengal, India) were used. Two bacterial cultures S. aureus (accession no. ATCC 29213) and E. coli (accession no. ATCC 25922) supplied by University of Calcutta, Kolkata, West Bengal, India were used for study.

Methods

Preparation of OSSW powder

Firstly, OSSW was washed thoroughly with plain water to remove the dirt, dust and foreign particles adhered onto it. Then they were dried under direct sunlight and ground into small unit and then into powder form with the help of mini grinding machine. The wastages were removed by using fine mesh of cloth made up with nylon 66 and finally the weight was taken. The yield was found to be 82.5 g per 100 g of OSSW.

Preparation of crude extract of OSSW

82.5 g of OSSW powder was extracted with 600 ml of ethanol (50% aqueous) at 80°C for 16 cycles using Vacuum Assisted Soxhlet Extraction Method (VASEM). The extract was then treated with 12 ml of citric acid for 20 min at 80°C followed by desolventization. The amount of crude extract obtained was 30 ml. After drying, the yield was found to be 82.5 g per 100 g of OSSW.

Synthesis of major bioactive component quercetin from crude extract of OSSW

The crude extract of OSSW contains main colouring component quercetin (3, 5, 7, 3’, 4-pentahydroxy flavone), quercetin-3-glucoside, kaempferol and tannin/rutin (Lokhande et al., 1998). The isolation of main functional component (quercetin) from extracted solid mass of OSSW was carried out as per the method described elsewhere (Kittur et al., 2003). Cellulase, pectinase and xylanase of activities 1.951 IU/µg of protein, 235 IU/µg of protein and 2.57 IU/µg of protein were added to 1% solution of crude extract of OSSW at concentrations of 0.48, 0.11 and 0.75 mg of protein/g of OSSW respectively.

Enzymatic hydrolysis was performed with a citrate phosphate buffer (pH 4.5) by stirring (200 rpm) at 45°C for 48h. After enzymatic hydrolysis, the component parts i.e. quercetin-3-glucoside, rutin and kaempferol were hydrolyzed and got dissolved. Then the solution was centrifuged at 10,000 x g. The precipitate was then dissolved in deionized water and passed through MF-Millipore Membrane (Merck 0.22 µm pore size) filter using high pressure vacuum pump filtration, lyophilized (Lyophilizer, e-Gen Biotech, Pune, India) and stored at 20°C for further use. The yield of quercetin was found to be 2.42 g.

Chromatographic conditions employed and related sample preparation

Commercially, it is very difficult to get quercetin in pure form. For this reason, High Performance Liquid Chromatography (HPLC) of the material was carried out at each step during synthesis to receive the single peak which is the signal of purity. HPLC analysis was performed on HPLC-UV system (Karlsruhe, Germany) equipped with an auto-sampler quaternary pump and VWD UV detector. Stationary phase used was reverse phase C18 column (4.6 x 250 mm I.D., particle size 5 µm) with mobile phase consisting of methanol and 0.4% phosphoric acid (49:51 v/v) at 25°C. The flow rate was 1.0 ml/min with 20 µl injections. In HPLC –UV system, if we click on the peak in the chromatogram we can obtain the UV spectra of the corresponding sample through the special task mode of inbuilt LC Chem station software.

For sample preparation, 2.0 g of sample after each treatment of synthesis was taken and then 20 ml of methanol was added and refluxed twice keeping 30 min time as constant for each reflux. The mixture was then filtered and the residue was washed thoroughly with 10 ml of methanol. Then the solution was mixed with 5 ml of 25% hydrochloric acid (HCl) and refluxed for 30 min. The solution was then cooled and filtered through 0.45 µm Millipore filter prior to HPLC analysis.

UV-Visible spectra

The UV-Visible spectra were recorded over 200-600 nm range using double beam UV-Vis spectrophotometer (Double R optics DR-324C).

Characterization

Structural characterization

The structural characterization of quercetin obtained from OSSW was carried out by analyzing Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) (1H and 13C) spectra.

The FTIR spectrum of quercetin was recorded on Perkin Elmer RX 1 FTIR Spectrometer, keeping air as reference. Prior to analysis, the sample was mixed with potassium bromide (KBr) at a ratio of 1:100 (by weight) followed by uniaxially pressing into pallets with a Specac compressor (Specac Inc., Snyama, USA). In the analysis, a resolution of 4 cm⁻¹ was chosen and data collection was made in the wave number range of 4000-400 cm⁻².

NMR spectra of quercetin were recorded on a Bruker AVANCE 600 spectrometer (Bruker Biospin GmbH, Karlsruhe, Germany) with a 5 mm TCI cryo probe equipped with z-gradients upto 53 G/cm and operating at 600.13 MHz respectively at 25°C. Samples were dissolved in 0.7 ml dimethyl sulfoxide and transferred in Schott Economic 5 mm NMR tubes. Results were analysed in ACD labs software for spectra calibration and peak assignment.

Thermal characterization

The thermal characterization of quercetin was carried out by Differential Scanning Calorimetry (DSC) (Perkin Elmer, Jade DSC, USA) and Thermogravimetric Analyzer (TGA) (Perkin Elmer, Pyris 6TG, USA). The temperature range was set up from 25-450°C for DSC and 25-700°C for TGA under nitrogen atmosphere at a flow rate of 40 ml/min at a heating rate of 10°C/min.

Surface morphology characterization

The surface morphology of quercetin was studied on Scanning Electron Microscope (SEM) (Zeiss EVO 50, with...
Figure – 1: HPLC chromatogram of a: crude extract of OOSW and b: quercetin

Figure – 2: UV-Visible spectrum of a: crude extract of OOSW and b: quercetin
Figure – 3: FTIR spectrum of quercetin

Figure – 4: $^1$H NMR spectrum of quercetin

Figure – 5: $^{13}$C NMR spectrum of quercetin
Figure – 6: TGA thermogram of quercetin

Figure – 7: DSC thermogram of quercetin
Figure – 8: Scanning electron micrograph of quercetin a: under normal condition b: after heating at 229°C, c: after heating at 321°C and d: after heating at 321°C

Figure – 9: AFM image of quercetin synthesized from OOSW

Figure – 10: Effect of concentration of quercetin on zone of inhibition

Figure – 11: Effect of concentration of quercetin on bacterial reduction percentage
Figure 12: Effect of quercetin on skin irritation a: without quercetin, b: non abraded skin with quercetin at 24 h, c: non abraded skin with quercetin at 48 h, d: non abraded skin with quercetin at 72 h, e: on abraded skin without quercetin, f: abraded skin with quercetin at 24 h, g: abraded skin with quercetin at 48 h and h: abraded skin with quercetin at 72 h.

<table>
<thead>
<tr>
<th>Primary Irritation Index (PII)</th>
<th>Evaluation</th>
</tr>
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<tbody>
<tr>
<td>0.00</td>
<td>No irritation</td>
</tr>
<tr>
<td>0.04 – 0.99</td>
<td>Irritation barely perceptible</td>
</tr>
<tr>
<td>1.00 – 1.99</td>
<td>Slight irritation</td>
</tr>
<tr>
<td>2.00 – 2.99</td>
<td>Mild irritation</td>
</tr>
<tr>
<td>3.00 – 5.99</td>
<td>Moderate irritation</td>
</tr>
<tr>
<td>6.00 – 8.00</td>
<td>Severe irritation</td>
</tr>
</tbody>
</table>

Table 1: Evaluation of Primary Irritation Index (PII) (Gotmare et al., 2018)

<table>
<thead>
<tr>
<th>Description of sample</th>
<th>Rat skin score</th>
<th>PII</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td></td>
<td>Erithema</td>
<td>Oedema</td>
</tr>
<tr>
<td>5% sodium dodecyl sulphate (Positive control)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Phosphate buffered saline</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sample under test</td>
<td>0</td>
<td>0</td>
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</tbody>
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Table 2: Skin irritation test of quercetin on rat skin
Antibacterial activity

Antibacterial activity was carried out using the well diffusion assay as per the method prescribed by National Committee for Clinical Laboratory Standards (NCCLS). The qualitative evaluation of antibacterial activity of quercetin against Gram-positive bacteria *Staphylococcus aureus* (S. aureus) and Gram-negative bacteria *Escherichia coli* (E. coli) was assessed by determining the Zone of Inhibition (ZOI) of bacterial growth as per AATCC 147 test method. The quantitative evaluation of antibacterial activity of quercetin against the said two bacterial strains was performed as per AATCC 100-2004 test method. Bacterial colonies recovered on the agar plate were counted and the bacterial reduction percentage was calculated by using the following formula:

\[
\text{Percentage reduction of bacteria (RE)} = \frac{B - A}{B} \times 100
\]

where, A is the number of colonies formed in presence of quercetin after inoculation over 24 h of contact period and B is the number of colonies formed in absence of quercetin after inoculation over 24 h.

Toxicity test

In order to confirm the non-toxicity of samples, skin irritation study was carried out using Draize patch test method. The patch test as described by Draize et al., 1944 involved single applications of the test substance to the shaved skin on the back of white rat, Wister race. Accurately measured quantities (0.5 mg) of substance were applied to the skin under 25.4 mm square gauge patches which were secured with adhesive tape. To prevent any volatile constituents from evaporating, the trunks of the rats were wrapped in rubberized cloth. The animals were placed in restrainers and the patches left intact for 24 h. This procedure ensured that the skin was in contact with the test substance for the entire period of treatment and that the patches were not dislodged by the movement of the animals. In addition to applying the patches to areas of intact skin, patches were applied to areas of skin which have been abraded by scoring through the stratum corneum but not sufficiently to cause bleeding. Test areas were examined at 24, 48 and 72 h after treatment and scores allocated according to the degree of erythema and oedema formation. The average of combined scores given for the areas of intact and abraded skin is referred to as the Primary Initiation Index (PII). The use of positive and negative control is essential in animal tests. 5% sodium dodecyl sulphate was employed as positive control and phosphate buffered saline as negative one. The degree of irritancy was categorized as per PII shown in Table I.

Results and Discussion

Analysis of chromatogram generated after HPLC

The HPLC Chromatogram of crude extract of OOSW and quercetin are shown in Figures 1a and b. During synthesis and purification of quercetin, the material obtained at each step i.e. after treatment of the OOSW powder with 50% aqueous ethanol followed by treatment with citric acid and after enzymatic hydrolysis using cellulase, pectinase and xylanase was identified by analyzing the HPLC chromatogram recorded. The crude extract obtained by treating OOSW powder with ethanol and citric acid was analyzed with HPLC and the chromatogram so obtained showed five numbers of peaks against different retention time like 7.25, 17.08, 18.52, 19.87 and 26.13 min. The identification of constituents was done by comparing the acquired peaks in the spectrum against retention time with the peaks in the reported spectrum (Careri et al., 2003). After comparing, it was observed that the peaks shown in Figure 1a are assigned for rutin, quercetin-3-glucoside, kaempferol, quercetin and accumulated phenolics. When the material was hydrolyzed with cellulase, pectinase and xylanase and analysed with HPLC, it gave out only a single peak against retention time 10.07 min (Figure 1b) which clearly indicates not only the presence of quercetin but also its purity. By clicking on the chromatogram peak of quercetin, the UV-Visible spectrum of quercetin having a single peak at 268 nm was observed which was also supported by the UV-Visible spectrum of quercetin.

UV-Visible spectra analysis

The UV-Visible spectra of crude extract of OOSW and quercetin are shown in Figure 2a and b. In Figure 2a, the absorption peaks were noticed at 206, 264, 360 and 432 nm for the crude extract of OOSW. Out of them, the highest absorption peak was seen against 264 nm. It was found that these peaks are assigned for rutin, quercetin, quercetin-3-glucoside and kaempferol when compared with the UV spectra of the corresponding standard samples. The enzymatic hydrolysis of crude extract of OOSW showed a single absorption peak at 268 nm (Figure 2b) which was for quercetin as the absorption peak was nearer to 264 nm. This clearly indicates the absence of other chemicals and impurities within quercetin. Therefore, it can be said that the synthesized quercetin is pure in nature.

Structural characterization

Analysis of Fourier Transform Infrared (FTIR) spectrum

The FTIR spectral features of quercetin are shown in Figure 3. FTIR analysis revealed peaks at 3318 and 1250-1050 cm\(^{-1}\) which were consistent with the presence of hydroxyl groups. The peak at 1663 cm\(^{-1}\) was attributed to the stretching vibration of carbonyl group (Singh and Mendhulkar, 2015). Several other peaks were observed at 1611, 1561, 1522 and 1450 cm\(^{-1}\) which were consistent with the presence of phenyl ring skeleton. The peaks observed at 1611, 1522 and 1450 cm\(^{-1}\) also suggested the presence of an oxygen containing heterocycle (Bakkialakshmi and Barani, 2013).

Analysis of Nuclear Magnetic Resonance (NMR) spectra

NMR Spectroscopy is a comprehensive study involving the renovation of chemical structure of a compound architecture by generating detailed information about carbon and hydrogen atoms in the structure. The synthesized strain fractions were further analyzed for structural characterization by \(^1\)H and \(^13\)C NMR.

\(^1\)H NMR spectrum of quercetin revealed three types of protein signals (Figure 4): aromatic proton, hydroxy protons of the analyte and a solvent proton signal. The UV spectra of the corresponding standard samples. The enzymatic hydrolysis of crude extract of OOSW showed a single absorption peak at 268 nm (Figure 2b) which was for quercetin as the absorption peak was nearer to 264 nm. This clearly indicates the absence of other chemicals and impurities within quercetin. Therefore, it can be said that the synthesized quercetin is pure in nature.
Thermal characterization
Analysis of TGA thermogram

The TGA thermogram as shown in Figure 6 had undergone four decompositions between 28.81 and 667.6°C. The first stage of weight loss (39.702%) was observed over the temperature ranging from 28.81-116°C which is due to the elimination of absorbed/bound water/moisture owing to vaporization of water molecule from the structural network (Martinez-Comacho et al., 2010). The above decomposition can also be attributed to the strong water absorbent nature of quercetin. The second stage of degradation initiates at 116°C, picks up its acceleration at 167°C and continues up to 238°C. The weight loss occurred in the second stage of degradation was 9.989% which is due to loss of hydroxyl groups. The third stage of degradation occurred around 238-338°C with 9.832% weight loss which is mostly associated with the pyrolysis of oxygen containing groups. (Islam et al., 2018). The fourth stage weight loss (9.026%) observed over the temperature ranging from 338-667.8°C was mostly associated with the degradation of aromatic rings of the ligand. At the end of the experiment, the total weight loss was found to be 68.549% indicating that 31.451% of quercetin remained as residue which also confirmed the thermal stability of quercetin.

Analysis of DSC thermogram

The DSC thermogram of quercetin (Figure 7) exhibited a sharp endothermic peak centered at 129.4°C. The peak was attributed to the loss of water associated with the hydrophilic group (-OH) of quercetin. This suggests that the sample was not completely anhydrous and some bound water was still not removed when dried in the desiccator which was confirmed by the results obtained from TGA. Another broad endothermic peak at 321°C was associated with the decomposition of aromatic rings present in quercetin. The glass transition temperature (Tg) was observed at 48°C. The higher delta H (13.34.0286 J/g) of quercetin indicated large portion of ordered structure of quercetin resulting in lower mobility of chain molecules of quercetin due to its comparatively more crystalline behaviour with the high ordered structure of quercetin, quercetin cannot move with more ease resulting in higher Tg (Shanthi and Kothai, 2015). The higher onset temperatures are associated with higher thermal stability. From the obtained onset temperature and higher value of Tg it is concluded that quercetin was found to be highly thermally stable.

SEM and AFM analysis of synthesized quercetin

The scanning electron micrograph of quercetin (Figure 8) was characterized as needle like crystals of smaller size and regular shape with an apparently smooth surface (Figure 8a). There is an effective reduction of crystal particle size with the increase in temperature to 129°C. It was also observed that with the increase in temperature, the crystals of quercetin seem to aggregate with another into a closely packed arrangement. This effect is highly pronounced especially at 229°C (Figure 8b). The aggregation of crystals can be attributed to the dehydration of quercetin at temperatures greater than 100°C. When the temperature reached at 321°C, the aggregation starts melting and forming a shrunk mass of reduced volume due to hydrolysis of oxygen containing groups and elimination of gases from the substrate (Figure 8c). At higher temperature, it forms a cracked lump (Figure 8d) owing to the decomposition of aromatic rings which are clearly evident from DSC and TGA profile.

The result of AFM is shown in Figure 9. The thin smooth layer of quercetin consists of large globules approximately 42 nm large and each containing 5 to 6 smaller units. The size of the smaller unit was found to be 3-5 nm in height and 10-15 nm in width.

Antibacterial activities of quercetin synthesized from OOSW

Quercetin is a pentahydroxy flavone having the five hydroxy groups placed at the 3-, 3′-, 4′-, 5′- and 7-positions. The mechanism of antibacterial activity of quercetin has not been fully understood. However, the existence of hydroxyl group at position 3 in the C-ring is believed to be responsible for the antibacterial activity of quercetin (Wu et al., 2013).

The qualitative evaluation of antibacterial activity of quercetin was carried out by estimating the zone of inhibition against Gram positive (S. aureus, accession number ATCC 29213) and Gram-negative (E. coli, accession number ATCC 25922) bacteria under different concentrations of quercetin (0.5-3%). From Figure 10 it is observed that the zone of inhibition (ZOI) increases significantly with the increase in concentration of quercetin upto 2.0% for both types of bacterial strain, after that the increase is found to be insignificant in nature. Initially at 0.5% concentration of quercetin the ZOI was found to be 14.4 mm for S. aureus and 12.7 mm for E. coli which starts increasing significantly with the increase in concentration upto 2.0% (ZOI = 21.2 mm for S. aureus and 17.8 mm for E. coli) thereafter, the increase was found to be insignificant till 3.0% concentration of quercetin. The ZOI was seen to be always higher in case of S. aureus than E. coli which means that the inhibitory effect of quercetin is very much prominent on Gram-positive bacteria S. aureus than Gram-negative bacteria E. coli. The quantitative analysis of the percentage reduction in Gram positive (S. aureus) and Gram-negative (E. coli) bacteria with the treatment of quercetin synthesized from OOSW is shown in Figure 11. Quercetin showed maximum reduction in bacteria (91.74% for S. aureus) at 2.5% concentration which is 88.73% for E. coli and 2.0% concentration. Quercetin was seen to be more effective on S. aureus than E. coli.

In bacteria, the gram stain provides an important classification system as several properties can be correlated with the cell envelope. Cell walls and membranes are important defensive barriers for bacterial resistance to the external environment. In particular, the bacterial cell wall plays an important role in maintaining the bacterium’s natural shape. The components of the cell membrane produce different adsorption pathways for quercetin against Gram-positive and Gram-negative bacteria (Lesniak et al., 2013). Gram-positive bacteria have a relatively thick (about 20 to 80 nm) continuous cell wall (often called the sacculus), which is composed largely of teichoic acids, polysaccharides and peptidoglycolipids) are covalently attached to the peptidoglycans. The peptidoglycans layer is composed of network with plenty of pores which allow foreign molecules to penetrate into the cell without difficulty. In contrast, the peptidoglycans layer in Gram-negative bacteria is thin (about 7.5 to 10 nm thick). In most Gram-negative bacteria, the membrane structure is anchored non-covalently to lipoprotein.
molecules (Braun’s lipoprotein), which in turn are covalently linked to the peptidoglycans. The lipopolysaccharides of the Gram-negative cell envelope form part of the outer leaflet of the outer membrane structure. Because of the layered structure, the outer membrane is a potential barrier against foreign molecules (Abou-Zeid et al., 2011). This makes E. coli comparatively more resistive against quercetin than S. aureus.

Toxicity test

The toxicity test was conducted (ethical clearance certificate memo no. 238/MBHMCH/CH/ADM/19 dated 22.08.19 issued by Chairman, Institutional Ethical Committee, M. B. Homeopathic Medical College & Hospital, Howrah, West Bengal, India) on eight number of rats in order to evaluate the non irritancy of quercetin. Table 2 and Figure 12 a to h clearly reveals the Primary Irritation Index (PII) of quercetin and it was found to be zero. This also confirms that the synthesized quercetin is non-irritant in nature when compared with material of positive control.

Conclusion

A novel bioactive material quercetin was successfully synthesized from OOSW by vacuum assisted Soxhlet extraction method (VASEM). The structural characteristic peaks as shown in FTIR and NMR (¹H and ¹3C) not only confirmed the formation of quercetin but also its structure. HPLC and UV-Visible studies showed a single peak against 10.08 min retention times and at 268 nm thereby verifying its purity. The DSC and TGA studies clearly indicated high thermal stability of quercetin. The quercetin was found to be regularly shaped with an apparent smooth surface and needle like crystals of smaller size in AFM and SEM. The antibacterial activity of quercetin in terms of zone of inhibition and bacterial reduction percentage was investigated against Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria. Quercetin showed good antibacterial activity on both types of bacterial strains but the effect was stronger on S. aureus than E. coli. Therefore, from the results it can be concluded that quercetin is strongly supported as an antibacterial material having potential application in medical fields to prevent infection, particularly during healing of wounds, in food industry as food preservatives and also in textile industry for the development of protective textile materials.

References


