beads. These results highlight the need to develop dissolution media that are more representative of the gastrointestinal tract, especially where physiologically sensitive biopharmaceuticals are concerned.

Conclusions BCG entrapped in alginate enhances viability in SGF for up to 2 hours compared with naked BCG. However, incubation in SIF resulted in large losses in viable BCG, possibly due to the cocktail of enzymes in the SIF. Ongoing in vivo studies will investigate the suitability of this approach to oral tuberculosis vaccination of wildlife and the efficacy of the vaccine.

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Short Papers in Pharmacognosy and Pharmaceutical Chemistry

9

Analysis of linear polyamine natural product conjugates

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Objectives The applications of linear polyamines, for example spermidine and spermine and their conjugates, in drug delivery are continuing to be investigated. Our studies of organic nano-bioparticles as vehicles for non-viral gene delivery have afforded a library of semi-synthetic polyamine conjugates, which are efficient at DNA condensation and delivery. However, their quantitative analysis is challenging as they lack any chromophore. In order to analyse these polyamine conjugates, fluorescence derivatization has been applied.

Methods We have developed methods for poly-derivatization with a panel of extrinsic fluorophores (e.g. dansyl chloride (Minocha and Long 2004), o-phthalaldehyde (OPA), 9-fluorenyl-methoxycarbonyl chloride (FMOC; Koros et al 2007) and fluorescamine) followed by high-performance liquid chromatography (HPLC) with both fluorescence and UV absorption detection. These methods enable the analytical optimization of synthetic routes. Reaction and chromatographic conditions were optimized for each fluorophore using a series of model mono- and diamines and finally applied to natural and semi-synthetic polyamines. The structures of the resulting derivatives were confirmed by off-line high-resolution electrospray ionization mass spectrometry (H ESI-MS). Linear responses were obtained over the concentration range 0.01-1.00 mm. The relative quantum yields of the polyamine-fluorophore derivatives were examined to measure any intramolecular fluorescence quenching. We have also developed an alternative methodology for distinguishing the similarly functionalized polyamines spermidine and spermine (tri- and tetra-amines) by preparing hexahydropyrimidines (Chantrapromma et al 1980) before derivatizing with a chromophoric reagent and HPLC separation.

Results Our results show that synthesis of polyamine derivatives in quantitative yield depends on the reaction conditions: time, temperature and the molar ratio of derivatization reagent to substrate amine. Off-line mass spectrometry analysis of the products demonstrated complete derivatization of both primary and secondary amino groups with dansyl and FMOC fluorescent derivatives and of primary amine groups for OPA and fluorescamine derivatives. Under the H ESI-MS ionization conditions used, the dansyl derivatives often showed double protonation as the cations $\left[\frac{M+2H}{2}\right]^{2+}$ in addition to the expected monovalent ions [M+H]⁺. Presumably this is because this chromophore contains basic amino groups that can be protonated easily, whereas FMOC derivatives gave predominantly [M+Na]⁺ ions. Dansyl derivatization of polyamines showed no apparent steric hindrance. The OPA reaction with polyamines is rapid, but the products have poor stability. Derivatization with fluorescamine gave multiple products (HPLC analysis). The chromatographic separation of poly-dansyl derivatives of spermidine (three) and spermine (four) as mono- and dihexahydropyrimidines (as di-dansyl derivatives) showed retention times for spermidine, spermine and their hexahydropyrimidines of 11.60, 17.80, 7.50 and 10.00 minutes respectively (HPLC, Luna C8, 5 μ m, 150 × 4 mm, isocratic 70:30 acetonitrile/water, $\lambda_{ex} = 310$ nm, $\lambda_{em} = 550$ nm).

Conclusions This methodology provides a useful way to analyse these important natural products and their semi-synthetic analogues which lack any chromophores. These compounds are being developed as efficient vehicles for gene delivery and as anti-cancer lead compounds where quantitative analysis is important.

Acknowledgement We thank the CRN for financial support (studentship to SB).

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10

Investigation of scopoletin biosynthesis during post-harvest physiological deterioration in cassava roots using stable isotopic labelling

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Objectives Coumarins are pharmacologically active and have roles in plant defence. Despite their importance, key aspects of the biosynthesis of these secondary metabolites remain unresolved. Here we exploit the observation that the accumulation of scopoletin and its glucoside scopolin increases in cassava roots (Manihot esculenta Crantz) during post-harvest physiological deterioration to test alternative pathways for the biosynthesis of these hydroxycoumarins.

Methods Cassava roots (cv. Mcol22 and NGA 19), within 2 hours of harvesting, were fed with different labelled intermediates on the postulated biosynthetic pathway of scopoletin (e.g. trans-cinnamic-d₇, trans-cinnamic-3,2',3',4',5',6'-d₆, trans-cinnamic-2',3',4',5',6'-d₅, trans-cinnamic-2-d, transcinnamic-18O2, p-coumaric-2-13C, caffeic-2-13C and ferulic-2-13C acids). Also, competition feeding experiments were carried out with a mixture of transcinnamic-2',3',4',5',6'-d5 acid with each of 2',4'-dihydroxycinnamic, caffeic and ferulic acids. Post-harvest physiological deterioration was allowed to occur. Ethanolic extracts of the deteriorated roots were separated (high-performance liquid chromatography) and analysed (high-resolution electrospray ionization mass spectrometry).

Results Deuteriated cinnamic acids were incorporated, and typically 29% of the scopoletin was deuteriated. Incorporation (in both scopoletin and scopolin) of only three deuterons when fed with trans-cinnamic-d7 and trans-cinnamic-d6, and non-deuteriated scopoletin when cinnamic-2-d acid was fed, indicates that the pathway involves exchange of the 2-hydrogen atom in cinnamic acid, and strongly supports our thesis that the *trans-cis* isomerization step is enzymic, and not photochemical, as found in vitro and in other plants where four deuterons would have been found in the labelled product. Incorporation of p-coumaric-2-13C, caffeic-2-¹³C and ferulic-2-¹³C acids in the biosynthesis of scopoletin gave an average increase of 14% ¹³C-labelled scopoletin and ¹³C-labelled scopolin. There was no reduction in label incorporation when either caffeic or ferulic acids were fed in competition to deuterium-labelled cinnamic acid whereas competition with unlabelled trans-2',4'-dihydroxycinnamate caused a decrease from 4.6 to 3.4% (compared with the labelled scopoletin isolated when the roots were fed with labelled cinnamic acid only). Trans-cinnamic- $^{18}O_2$ was incorporated (5%) in the biosynthesis of scopoletin with only one ¹⁸O-labelled oxygen atom in the product.

Conclusions The ready accumulation of scopoletin and scopolin in cassava roots during post-harvest physiological deterioration makes it a good model to investigate their biosynthesis. We have shown that the E-Z isomerization step (of the cinnamic acid derivative) during the biosynthesis is enzymic. Furthermore, all three proposed pathways (as found in different plants) are operating in cassava, but the pathway via 2',4'-dihydroxycinnamate is likely to be the predominant one. A pathway via a spirolactone-dienone (quinol) intermediate has been previously established in Streptomyces niveus for novobiocin biosynthesis in elegant work by Kenner and co-workers (Bunton et al 1963), and also proposed from UV studies in cultures of the plant Ammi majus L. (Apiaceae, Bishop's flower, large bullwort) (Matern 1991) following work by Grisebach and Ollis (1961). The absence of doubly enriched ¹⁸O-scopoletin means that the lactonization step is through ortho-hydroxylation not via a spirolactone-dienone intermediate, where both ⁸O-atoms would be incorporated in the final product.

Acknowledgement We acknowledge the financial support of the Egyptian Government (studentship to SALB).

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An evaluation of the chemotaxonomy of Lignosus rhinocerus

11

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Objectives To identify a chemotaxonomic marker compound that may be suitable for evaluating the quality and safety of traditional herbal medicinal products (THMPs) containing Lignosus rhinocerus. The use of THMPs has increased over the last 10 years in Malaysia. However, the quality and safety of these products are still questionable since they usually contain a range of herbal constituents which are complex and may also contain adulterants. Hence, it is essential to identify a chemotaxonomic marker compound for a herbal plant that could give an indication and confirmation (qualitative determination) about the presence or absence of a claimed species in THMPs. For this study, *L. rhinocerus* has been chosen for chemotaxonomic evaluation. This fungus, in the Malay language known as *kulat susu rimau*, has been used as a traditional herbal medicine for the treatment of cough, cold and asthma.

Methods Six sample solutions, namely *L* rhinocerus, Reishi (Ganodema lucidum), Maitake (Grifola frondosa), Coriolus (Coriolus versicolor) and Long Heh (Cordyceps sinensis), were prepared by refluxing 2.0 g of sample with a mixture of solution containing 0.5 M methanolic sodium hydroxide (2 mL), methanol (18 mL) and diethyl ether (20 mL) for 1 hour. The mixture was filtered into a separating funnel and distilled water (20 mL) was added. The filtrate was extracted with diethyl ether (30 mL). The combined ether layer was then evaporated to dryness and the residue was reconstituted with 10% v/v of dichloromethane in methanol (2 mL). The resulting solution was then analysed using high-pressure liquid chromatography with a photodiode array detector. A C₈ column (150 mm × 4.6 mm) and solvent mixture of methanol and water (95:1% v/v) as the mobile phase were used. The detector wavelengths were set at 220, 280 and 350 nm and the run time of the analysis was 40 minutes. A standard solution of ergosterol (100 μ g/mL) was used in this analysis.

Results A biochemical compound ergosterol has been detected in all sample solutions while ergosta-4,6,8(14),22-tetraen-3-one (ergone) might be present in this fungus. These compounds are secondary metabolites that derive from the metabolism of glucose to acetate (a precursor of steroids). Ergosterol interacts with form all membranes (Frisvad et al 1998). Ergosterol has been reported as a major fungus-derived steroid (Zhang et al 2007) while ergone has been reported as a marker compound for THMPs containing *Polyporus sclerotium* (Yuan et al 2003). Further isolation and identification of the other two compounds will be carried out. These two compounds have the potential to be chemotaxonomic marker compounds since they were only detected in *L. rhinocerus* sample solution.

Conclusions Ergosterol and ergone are two biochemical compounds that have been found in *L. rhinocerus*. However, ergosterol is present as a cell-wall component in most, if not all, fungi and is therefore not much use except as an indicator of fungal contamination in higher plants. As for ergone, it is inappropriate as a chemotaxonomic marker compound but it could be used as a general marker compound for a screening test of THMPs containing *L. rhinocerus*.

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12

Eco-friendly method for assay of ibuprofen tablets using sodium salicylate as a hydrotropic solubilizing agent

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Objectives Concentrated aqueous solutions of a large number of hydrotropic agents, such as niacinamide, urea, sodium benzoate, sodium salicylate, sodium glycinate and sodium gentisate, have been employed to enhance the aqueous solubilities of poorly water-soluble drugs. The Indian Pharmacopoeia (2007) method of assaying ibuprofen tablets involves the use of chloroform, which is toxic, a pollutant and costly. The main objective of the present investigation was to replace this organic solvent with a hydrotropic solution of sodium salicylate (2.5 m), which solubilizes the ibuprofen (a poorly water-soluble drug) from the tablet powder in order to carry out titrimetric analysis.

Methods There was a more than 90 times enhancement in the solubility of ibuprofen in 2.5 M sodium salicylate solution compared with its aqueous solubility. Therefore, it was thought worthwhile to employ 2.5 M sodium salicylate solution to solubilize ibuprofen for titrimetric analysis. Twenty tablets of ibuprofen (formulations 1 and 2) were weighed and finely powdered. The tablet powder equivalent of about 500 mg of ibuprofen was taken in a conical flask. One hundred millilitres of 2.5 M sodium salicylate solution were added and the flask was shaken for about 5 min to solubilize the ibuprofen from tablet powder and the solution was titrated with 0.1 M sodium hydroxide using 0.2 mL of phenolphthalein solution as an indicator. Necessary correction was made by conducting a blank determination (for 100 mL of 2.5 M sodium salicylate solution) and the amount of ibuprofen was calculated. For recovery studies, the same procedure was repeated using 60 and 100 mg of ibuprofen bulk drug as the spiked drug together with the pre-analysed tablet powder equivalent to 500 mg of ibuprofen. Tablet powders were also assayed by the Indian Pharmacopoeial method employing chloroform and ethanol. Each type of analysis was performed three times.

Results The mean percentages of drug estimated by the Indian Pharmacopoeial method were $102.31 \pm 0.771\%$ (formulation 1) and $100.72 \pm 2.013\%$

(formulation 2) whereas the mean values estimated by the proposed method were 100.88 \pm 0.401% (formulation 1) and 99.33 \pm 1.320% (formulation 2). These values are very comparable and close to 100%, indicating the accuracy of the proposed method. The results of the analysis using the standard Indian Pharmacopoeia method. The mean percentage recoveries by the proposed method were 98.47 \pm 1.700 and 100.91 \pm 1.008% in the case of formulation 1 and 99.55 \pm 0.888 and 101.62 \pm 0.720% in the case of formulation 2, which are very close to 100%. This further confirms the accuracy of the proposed method. The satisfactorily low standard deviation, percentage coefficient of variation and standard error validated the method.

Conclusions Thus, it may be concluded that the proposed method of analysis is new, rapid, simple, cost-effective, eco-friendly, safe, accurate and reproducible. By proper choice of hydrotropic agents the use of organic solvents in analysis may be discouraged to a large extent. Hydrotropic agent did not interfere in the proposed method. The tablets of ibuprofen can be analysed with the proposed method in routine practice. An advantage is that the organic solvent is precluded but not at the expense of accuracy. The proposed method is worth adopting in the pharmacopoeias.

13

Schiff-base conjugates of 5-aminosalicylic acid with enhanced free radical-scavenging activity

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Objectives 5-Aminosalicylic acid (5-ASA) used in the treatment of inflammatory bowel disease is known to be an effective scavenger of reactive oxygen and nitrogen species and is thought to be able to decrease nuclear factor κ B (NF- κ B) activation. It is known to accumulate in the bowel during treatment, suggesting that its ability to scavenge reactive species may be responsible for its activity in the treatment of inflammatory bowel diseases. 5-ASA is normally delivered in a conjugated form: previously Schiff-base dextran derivatives of 5-ASA have been synthesized that may bypass the stomach and small intestine intact but could release 5-ASA via secondary amine deaminase present in colonic microflora (Ahmad et al 2006). The aim of this project is to develop low-molecular-mass 5-ASA Schiff-base conjugates to (1) deliver 5-ASA to be released via enzymes in the colonic microflora and (2) synthesize Schiff-base 5-ASA conjugates that contain hydroxyl-rich moieties and have enhanced free radical-scavenging activity.

Methods The Schiff-base conjugates of 5-ASA were synthesized by adapting the method previously described for *p*-aminosalicylic acid (Patole et al 2006). The corresponding aldehydes used were 2,4-dihydroxybenzaldehyde, 2,3-dihydroxybenzaldehyde and 4-hydroxy-3-methoxybenzaldehyde as hydroxyl-containing conjugates. In addition, a conjugate of 4-dimethylaminobenzaldehyde was synthesized as a derivative deficient of hydroxyl functionality. The free radical-scavenging capacity of the synthesized compounds, 5-ASA and Trolox (a vitamin E analogue with previously established anti-oxidant activity) was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) discoloration method completed in triplicate (Silva et al 2006). The mean percentage inhibition of DPPH discoloration relative to the control at approximately steady state was calculated for each compound and plotted against concentration. From these graphs, the IC50 value (the concentration required to reduce DPPH discoloration by 50%) was determined for each compound. The lower the IC50 value, the greater the free radical-scavenging activity of the compound.

Results All target compounds were synthesized in yields of greater than 95% and characterized using appropriate analytical techniques. Reduction of DPPH by Schiff-base 5-ASA derivatives was observed as a reduction of absorption and indicative of the anti-radical capacity of the compounds. All compounds tested showed anti-oxidant activity in the DPPH assay. The 5-(((2,3-dihydroxylphenyl) methylidene)amino) salicylic acid Schiff-base conjugate showed highest free radical-scavenging activity; other synthesized Schiff-base conjugates were comparable to 5-ASA.

Conclusions The 5-(((2,3-dihydroxylphenyl)methylidene)amino) salicylic acid Schiff-base conjugate showed enhanced free radical-scavenging activity compared with 5-ASA. The catechol ring present in this structure may be responsible for its enhanced scavenging activity compared with 5-ASA and the other compounds synthesized. Further work is ongoing to assess the release of 5-ASA in colonic microflora.

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Exploration of the anti-ageing effect of Ocimum sanctum on a D-galactose-induced ageing mouse model

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Objectives In the Ayurvedic system of medicine, one of the important *Rasayana* drugs is *Ocimum sanctum*, which is commonly known as Holy Basil and widely used as an anti-oxidant and to delay ageing. To corroborate the traditional claims, we studied the effect of *Ocimum sanctum* on lipid peroxidation and anti-oxidant enzyme status in a mouse model of D-galactose-induced ageing.

Methods Female albino mice (*Mus musculus*) were placed in five groups, each group consisting of eight animals. Ageing stress was induced by subcutaneous injections of 5% D-galactose 0.1 mL per day for 15 days. Three experimental groups received extract of *O. sanctum* orally in doses of 100, 200 and 400 mg/kg of body weight, in addition to 5% D-galactose. The experimental procedures and research protocol used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC). To assess oxidative stress in the tissues all animals were killed by cervical dislocation. Different organs like the heart, liver and brain were dissected out and weighed and used for the ensuing investigations. Tissue levels of lipid peroxidation, superoxide dismutase, reduced glutathione and protein content were estimated. Protein content was determined by the Lowry method.

Results Lipid peroxidation in the form of malonaldehyde increased significantly (P < 0.01) in all organs in the ageing group. Maximum percentage inhibition was observed with the 400 mg/kg dose. The percentages of inhibition of lipid peroxidation at the 100, 200 and 400 mg/kg doses in liver were 52.8 ± 0.57 , 60.9 ± 0.59 and $70.1\pm0.62\%$ respectively, and the control and the mice treated with D-galactose alone had 49.4 ± 0.5 and $25.98 \pm 0.4\%$. Similar results were seen in brain and heart. D-Galactose challenge in these animals caused a significant depletion in superoxide dismutase (SOD) and glutathione levels. Brain, heart and liver SOD and glutathione levels were significantly elevated in the experimental groups. SOD contents in liver were 3.93 ± 0.72 , 4.38 ± 0.46 and 4.34 ± 0.06 units of SOD/mg of protein respectively for the 100, 200 and 400 mg/kg doses, whereas the control and D-galactose-treated mice showed 4.07 ± 0.27 and 1.24 ± 0.26 units of SOD/mg of protein in liver respectively. Similar values of $3.8 \pm 0.1, 4.1 \pm 0.3$ and 4.2 ± 0.2 , and $3.9 \pm 0.2, 4.6 \pm 0.4$ and 4.29 ± 0.3 units of SOD/mg of protein were obtained for the experimental groups in brain and heart respectively. Glutathione reductase activities in liver at the doses of 100, 200 and 400 mg/kg of body weight were 70.1 \pm 1.4, 77.0 \pm 2.4 and 82.4 \pm 3.2 units/mg of protein, in brain they were 71.0 \pm 2.1, 76.3 \pm 2.5 and 81.2 \pm 2.6 units/mg of protein and in heart they were 68.7 \pm 1.2, 73.3 \pm 2.9 and 78.9 \pm 2.4 units/mg of protein respectively. In control and D-galactose-treated mice levels in the liver were 60.9 ± 1.7 and 47.3 ± 1.07 units/mg of protein respectively. These findings show that O. sanctum is a potential therapeutic agent for ageing.

Conclusions In conclusion, *O. sanctum* is highly protective against oxidative damage. It has a multidimensional role, as it scavenges free radicals, balances the anti-oxidant enzyme system and stimulates metabolism of oxidative waste products such as lipid peroxides.

15

Synthesis and evaluation of a series of methanesulphonate derivatives of 4-hydroxyphenyl ketone-based compounds as potential inhibitors of types 1 and 3 17β -hydroxysteroid dehydrogenase

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Objectives In the fight against hormone-dependent prostate cancer, the type 3 isozyme of 17β -hydroxysteroid dehydrogenase (17β -HSD3) is an important biochemical target as it catalyses the conversion of androstenedione to testosterone. However, there is currently no crystal structure for 17β -HSD3. To aid the drug-discovery process, we previously undertook the derivation of the transition state of the reaction catalysed by 17β-HSD3 (Olusanjo and Ahmed 2007). We have also previously designed, synthesized and evaluated a series of inhibitors based on the 4-hydroxyphenyl ketone backbone that were found to be potent (Lota et al 2006). Consideration of the structure-activity relationship suggested that these compounds utilized a H-bonding group at the active site close to the C16 and C15 area of the steroid backbone. We concluded that the use of a bulky group in place of the hydrogen atom in the hydroxy functionality of the 4-hydroxyphenyl ketone-based compounds would result in a decrease in H-bonding and therefore a decrease in inhibitory activity. Here we report the synthesis, biochemical evaluation and rationalization of the inhibitory activity of a range of methanesulphonate derivatives of 4-hydroxyphenyl ketones. We also evaluated the compounds against type 1 17 β -hydroxysteroid dehydrogenase (17 β -HSD1) and 3 β -hydroxysteroid dehydrogenase (3 β -HSD).

Methods In the synthesis of the inhibitors, Friedel–Crafts acylation of phenol using a range of acid chlorides was undertaken, following which the compounds were reacted with methanesulphonyl chloride to give the target compounds. Biochemical evaluation of the synthesized compounds (final concentration 100 μ M) was taken from a procedure in the literature using rat testes homogenate and radiolabelled oestrone for 17 β -HSD1, androstenedione for 17 β -HSD3 and dehydroepiandrosterone for 3 β -HSD (Lota et al 2006). To rationalize the inhibitory activity, compounds were constructed using the CAChe computer program and superimposed on to the previously reported transition state.

Results The reactions proceeded in good yield (typically 60%) and without any major problems. Consideration of the inhibitory activity shows that the compounds are weak inhibitors of 17 β -HSD3. For example, the methanesulphonate derivative of 4-hydroxynonanophenone was found to possess approximately 20%, approximately 25% and less than 10% inhibition against 17 β -HSD3, 17 β -HSD1 and 3 β -HSD respectively: the non-methanesulphonate derivative was previously reported to possess approximately 81% inhibitory activity under similar conditions. Superimposition of the methanesulphonate derivatives on to the transition state for 17 β -HSD3 shows that the compounds undergo steric interaction and that the mode of binding does not allow H-bonding to take place, resulting in weaker binding to the active site and therefore weak inhibitory activity.

Conclusions The results of our study have added further support to our approximate model of the 17β -HSD3 active site; in particular, the existence of an additional H-bonding moiety that is able to undergo H-bonding interaction with the 4-hydroxy moiety in compounds such as 4-hydroxynonanophenone but is unable to undergo similar interaction in the methanesulphonate derivative, thereby resulting in an overall decrease in inhibitory activity.

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16

The use of herbs and spices in the Gujarati Hindu community in the London borough of Redbridge

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Objective Herbal medicinal products or medicinal plants are a common source of self-treatment in many cultures (Williamson 2002). The present study identified the herbs and spices utilized in the Gujarati Hindu community in the London borough of Redbridge.

Methods A questionnaire posing both open and closed questions was employed in three favourable sites, encountering a high number of individuals, allowing quantitative and qualitative data to be obtained. The time period chosen was ideal as it coincided with the holy festivals of Diwali and Navaratri. Medicinal plants and their use were particularly targeted, as were the participants' knowledge about them and their employment in Western countries. The research conducted was as accurate and impartial as possible, with efforts to eliminate any possible bias.

Results In total, 150 interviews were conducted and the results were compiled in a table. The study indicated that medicinal plants are still employed on a frequent basis, equally by all ages, citing Curcuma longa (also known as haldi) as the most commonly used in this European city. The seeds of the plants are frequently utilized, principally using the oral route, to alleviate digestive complaints. The study confirms that the Gujarati Hindu community is very much in favour of the utilization of medicinal plants, regarding them as excellent and safe healthcare products, and seeing them as important in healing diseases. It has been established that medicinal plant use is linked to the traditions of the community, and that extensive use is encouraged. Medicinal plants are mainly purchased, considered good value for money and easily obtainable. Self-treatment with herbal medicines is initially employed to alleviate minor illnesses. Predictably, varied answers were obtained from both genders, giving an insight into their distinct opinions. Additionally, with regards to the influence of Western life on adolescence and growing generation, the study shows equal argument in favour of and against youths abandoning traditions and ancestral beliefs for Western culture and ethics, with a remarkable diversity of attitudes corresponding to the different age groups.

Conclusions The research clearly highlights the opulence of information that exists within this immigrant community; however, as the youth of today are losing cultural customs, what knowledge will be evident in years to come is unknown. In terms of conducting further research, time would undoubtedly offer better findings and the use of additional locations could also be employed, allowing new observations to be made.

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