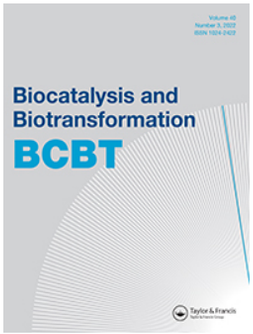

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REVIEW ARTICLE

Biotransformation of curcumin and structure–activity relationship (SAR) of its analogues: A systematic review

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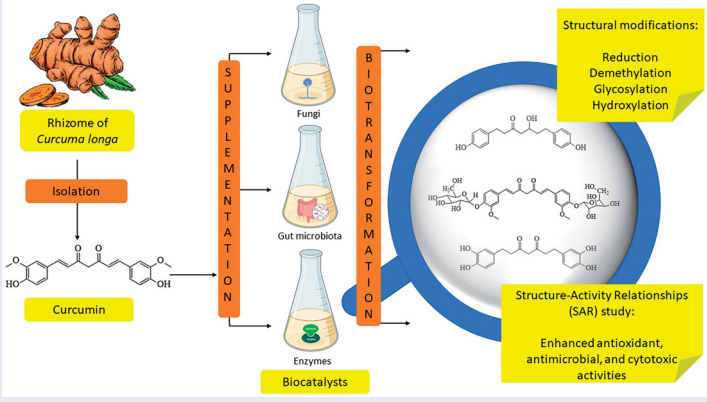
ABSTRACT

Curcumin has been widely acclaimed for several pharmacological properties, such as antioxidant, antimicrobial, anticancer, and anti-inflammation. Curcumin's poor aqueous solubility, bioavailability, and cellular uptake hamper its ability to display maximum pharmacological effect in the human body. Synthesis of curcumin analogues to enhance its properties can be achieved through biotransformation. Greener, simpler, and higher selectivity and specificity make biotransformation an alternative approach when preparing curcumin analogues for the structure–activity relationship (SAR) study intended for drug design. This work systematically reviews the biotransformation of curcumin by utilizing fungi, gut microbiota, and enzymes. The SAR study of curcumin and its analogues for several bioactivities is also highlighted.

GRAPHICAL ABSTRACT



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KEYWORDS
Biotransformation; curcumin; biocatalysts; structure–activity relationship; systematic review; gut microbiota

1. Introduction

Curcumin, or systematically known as (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6- heptadiene-3,5-dione, is the major constituent found in the rhizome of *Curcuma longa*. It falls under the diarylheptanoid class due to its 1,7-diphenylheptane carbon skeleton. It exists as a keto-enol tautomer with a major keto form in neutral or acidic solutions and a stable enol form in alkaline solution. Curcumin is widely used as a spice, food preservative, and food colouring agent in golden powder form (Omosa et al. 2017). Curcumin possesses various bioactivities, such as antioxidant (Bustanussalam et al. 2015), anticancer (Gurung et al. 2017), anti-inflammatory (Burapan et al. 2017), and antimicrobial (Akram et al. 2010) activities. Despite all those advantages, curcumin has poor water solubility, structural instability, and low bioavailability, which hamper its ability in pharmacological studies (An et al. 2017). The low bioavailability of curcumin seems to be caused by its poor pharmacokinetic properties, which result in a very low and sometimes undetectable presence in the blood serum after administration (Rodrigues et al. 2015). It has been reported that curcumin derivatives showed better potency than curcumin; thus, there is great interest in developing methods to obtain its derivatives.

Research interest in producing curcumin derivatives to increase its bioavailability and reduce its toxicity

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Table 1. Summary of reaction conditions for biotransformation of curcumin.

Biocatalysts	Metabolites	Incubation conditions			Refs.
		Time	Temp. (°C)	Rotation per minute (rpm)	
Fungi					
A. alternata	**2–4**	14 d	27	100	Younis et al. 2016
B. bassiana	**2–6**	5 d	27	80	Martin et al. 2017
	5	7 d	28	250	Zeng et al. 2010
C. blackesleeana	**7, 8**	14 d	27	100	Younis et al. 2016
C. elegans	**9, 10**	14 d	27	100	Younis et al. 2016
Diaporthe sp.	**4, 14, 15**	2 d	28	90	Maehara et al. 2011
O. brasiliensis	**12**	5 d	37	140	Majeed et al. 2019
P. brevicompactum	**11**	14 d	27	100	Younis et al. 2016
P. kudriavzevii	**2, 3**	1 d	30	200	Zhang et al. 2013
R. chinensis	**3, 13**	1 d	25	120	Zhang et al. 2010
R. oryzae	**2–4**	5 d	27	80	Martin et al. 2017
Gut microbiota					
Human faecal microbiota	**2, 18, 19**	1	37	200	Tan et al. 2015
P. anomala	**3, 4, 20–23**	14 d	25	100	Herath et al. 2007
Mice faeces	**2, 24–30**	2 d	37	200	An et al. 2017
Colonic microbiota	**2, 31–33**	1 d	37	200	Bresciani et al. 2020
Mouse gut microbiota	**3, 34–40**	1 d	37	NM	Sun et al. 2020
Human intestinal *bacterium Blautia* sp.	**34, 41**	6 & 24 hr	37	NM	Burapan et al. 2017
Human colonic microbiota	**3, 42–58**	1 d	NM	13,000	Lou et al. 2015
Enzymes					
Amyloglucosidase from *Rhizopus* sp.	**59**	3 d	68	NM	Vijayakumar et al. 2005
β-glucuronidase	**59**	35 hr	40	150	Prasad et al. 2015
One-pot multienzyme comprised of UMP kinase, acetate kinase, UDP-*α*-*D*-glucose synthase, phosphomannomutase, *N*-acetyl-*D*-glucosamine kinase and glycosyltransferase	**13, 59–61**	5 min	37	12,000	Gurung et al. 2017

NM: not mentioned.

has increased lately, including curcumin conversion by microbial biotransformation. The use of microorganisms to produce synthetic curcumin analogues has been a breakthrough in scientific studies. This is because synthetic reactions often require extreme conditions, such as temperature and pressure, where these chemical reactions are usually non-environmentally friendly and industrially undesirable in comparison with microbial transformation, which can be conducted at near-neutral pH, ambient temperatures, and atmospheric pressures (Jayeshkumar and Sakthivel 2015).

Biotransformation refers to the organic reactions involving biocatalysts that can structurally modify a chemical compound (Lin and Tao 2017). Biotransformation approaches that use biocatalysts have gained interest in industries. Apart from being an environmentally friendly process, it has a promising future for further studies due to the large and diverse microbes available and its extensive scope in chemical reactions. In addition, the products obtained through these processes can also be considered natural if the substrate is of natural origin (Majeed et al. 2019). Substrate, enzymes, and stability in operation are among several constraints associated with biotransformation, but it may be overcome through exploration of microbial biodiversity (Bustanussalam et al. 2015).

This review article presents an insight into the biotransformation of curcumin catalysed by microorganisms, including fungi, gut microbiota, and enzymes. This article also highlights the structure of metabolites, structural modification, and the bioactivities and structure–activity relationship (SAR) of these metabolites. The number of articles found related to this topic was quite scarce prior to 2001. Thus, most of the articles obtained were systematically searched between 2001 and 2020 to ensure that this review included all the newest updates on the biotransformation of curcumin. This review also summarises the reaction conditions of each biocatalyst (Table 1), which helps to narrow down the biocatalyst options needed for the biotransformation of curcumin in future studies.

2. Systematic review search strategy

Two search strategies were employed in this systematic review: (i) systematic search strategy and (ii) manual search strategy. The systematic search was conducted by searching articles using leading

databases, such as Scopus, ScienceDirect, PubMed, Scielo, Lilacs, and Medline. The keywords used were "biotransformation," "curcumin," "bioactivities," "metabolites," and "biocatalyst." The synonyms of these keywords were also generated to ensure a rigorous search was conducted. The synonyms are (a) biotransformation: microbial biotransformation, enzymatic biotransformation, biological conversion, biological transformation, bioconversion, biomimetic transformation, and microbial transformation; (b) curcumin: curcuminoids, *Curcuma longa*, and diarylheptanoids; (c) biocatalyst: accelerator and catalyst; (d) bioactivity: active biologically, pharmacological activity, and biological activity; and (e) metabolites: biotransformed products, analogues, derivatives, and biotransformation products. Meanwhile, two sources were used as supporting databases for the manual search strategy. The selected sources were Google Scholar and ResearchGate. These databases are needed to obtain additional sources, such as non-indexed journals and articles that cannot be retrieved from the leading databases. The manual search techniques used were handpicking and snowballing techniques.

The protocols for performing the systematic review were developed following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement. The first step was to exclude duplicate articles, read the titles and abstracts, and apply the inclusion and exclusion criteria. All articles from this stage were read in full and the inclusion and exclusion criteria were applied again. Following this step, the remaining articles were finally chosen for review. The inclusion of articles considered the following criteria: (i) the timeline of publication must be from 2001 to 2020, (ii) type of publication – original journal and review articles, (iii) only English articles, (iv) articles must present biotransformation of curcumin, and (v) articles must present the bioactivity of curcumin and the biotransformation metabolites. For the exclusion criteria, the following criteria were used: (i) book chapters, book reviews, and conference proceedings; (ii) full-text articles not found; (iii) articles without one of the keywords in the title and abstract; and (iv) articles without the presence of biotransformation of curcumin. Figure 1 summarises the flow diagram of the systematic search strategies.

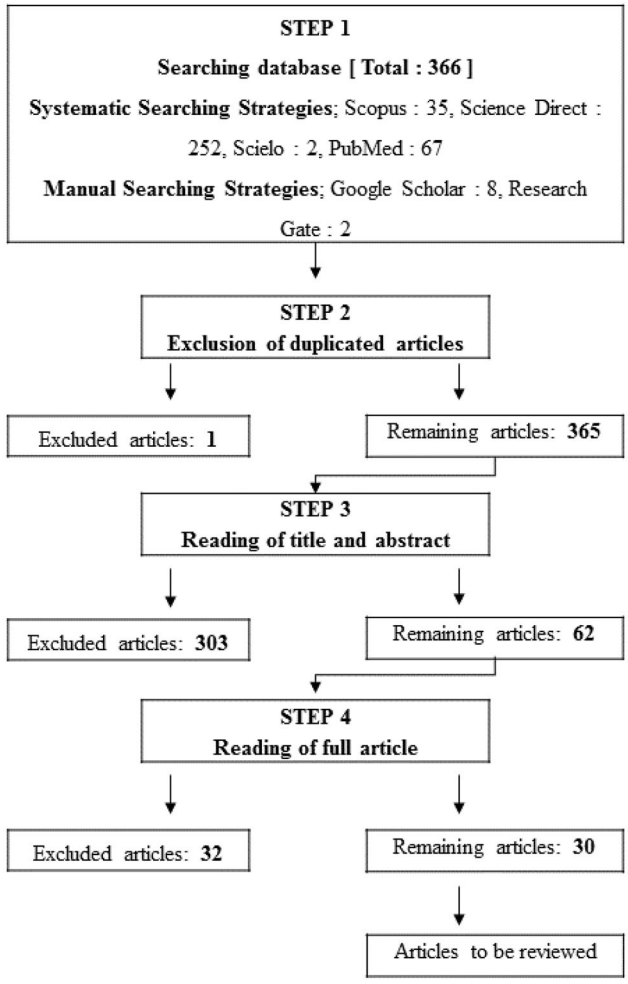


Figure 1. Flow diagram of the systematic search strategies.

3. Biocatalysts for biotransformation of curcumin

A biocatalyst is defined as a catalyst of natural origin that can be used in the transformation of organic compounds, such as enzymes (Wilding et al. 2012), and biocatalysts are currently utilized for the production of products in different fields, such as pharmaceuticals, fine chemicals, and food ingredients (Molinari et al. 2011). An ideal biocatalyst should have high selectivity, wide availability, and low cost, and it should also be easily reused as these parameters directly affect the overall cost of the process (Aguieiras et al. 2018). Through systematic search, the types of biocatalysts that have been utilized for biotransformation of curcumin consist of fungi, gut microbiota, and enzymes (Figure 2). It can be observed that fungi dominated the chart as the most frequently used biocatalyst for the biotransformation of curcumin. Fungi offer numerous biocatalyst selections for biotransformation reactions, and they are more convenient to handle than gut microbiota and isolated enzymes.

3.1. Biotransformation by fungi

The incubation of curcumin **(1)** with *Rhizopus oryzae* yielded three metabolites, namely 1,7-bis(4-hydroxy-3-

methoxyphenyl)heptane-3,5-dione [tetrahydrocurcumin] (**2**), 5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptan-3-one [hexahydrocurcumin] (**3**), and 1,7-bis(4-hydroxy-3-methoxyphenyl)heptane-3,5-diol (**4**) [octahydrocurcumin] (Martin et al. 2017). The metabolites **2 − 4** are formed due to the hydrogenation of diene moieties and the reduction of either one or both ketone moieties to hydroxyl. Meanwhile, the incubation of **(1)** with *Beauveria bassiana* also yielded metabolites **2 − 4** with additional glucopyranoside metabolites identified as curcumin-4′-*O*-4‴-*O*-methyl-*β*-*D*-glucopyranoside (**5**) and octahydrocurcumin-4′-*O*-4‴-*O*-methyl-*β*-*D*-glucopyranoside (**6**) (Martin et al. 2017). Zeng et al. (2010) also reported the isolation of metabolite **5** from the incubation of **1** by *B. bassiana*. A soil yeast strain, *Pichia kudriavzevii*, was also able to transform **1** to metabolites **2** and **3** (Zhang et al. 2013). All biotransformation reactions are depicted in Scheme 1.

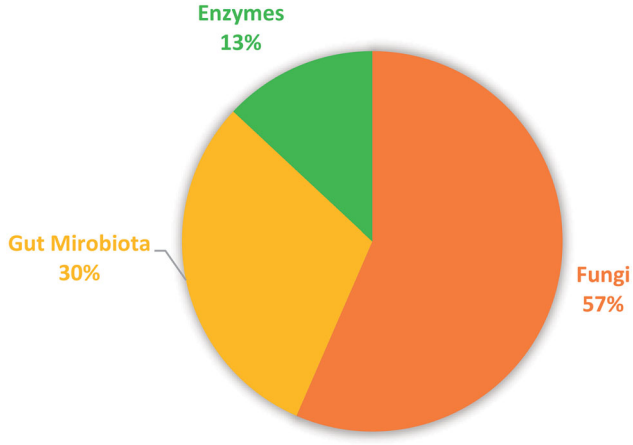
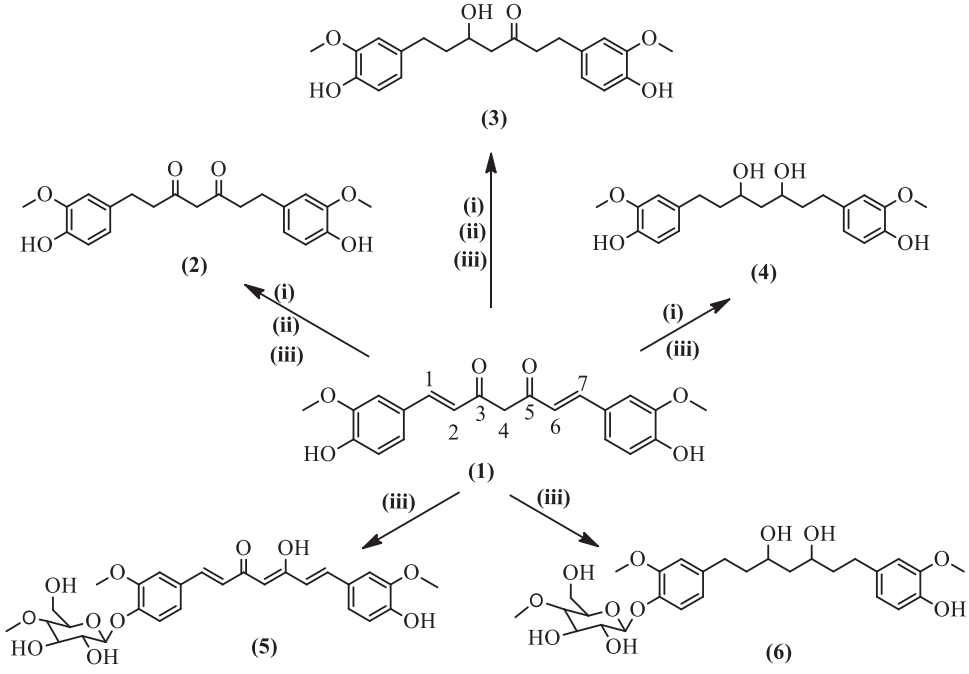


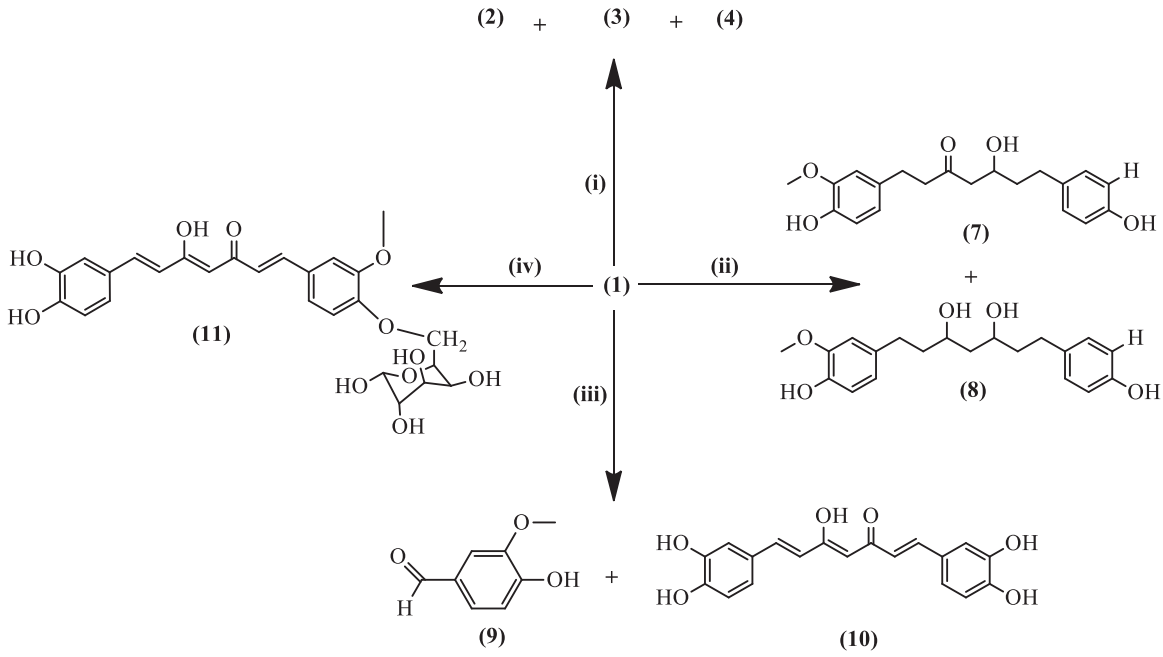
Figure 2. Types of biocatalysts for biotransformation of curcumin.

The ability of four endophytic fungi species (*Alternaria alternata, Penicillium brevicompactum, Cunninghamella blackesleeana*, and *Cunninghamella elegans*) as the biocatalysts for biotransformation of **1** was also investigated (Younis et al. 2016). *A. alternate* successfully reduced **1** to yield several products (i.e. compounds **2 − 4**). Meanwhile, *P. brevicompactum* afforded the demethoxylation of methoxyl group to phenyl hydrogen and the reduction of oxo moieties to alcohol to produce major metabolites, demethoxyhexahydrocurcumin **(7)** and demethoxyoctahydrocurcumin **(8)**, respectively. *C. blackesleeana* catalysed the hydrolysis of **1** to vanillin **(9)** and the demethoxylation of dimethoxyl group to diol, producing didesmethylcurcumin **(10)**. Meanwhile, only one glucosylated metabolite identified as curcumin-4′-*O*-*β*-*D*-glucoside **(11)** was produced from the biotransformation of **1** by *C. elegans* (Scheme 2) (Younis et al. 2016).

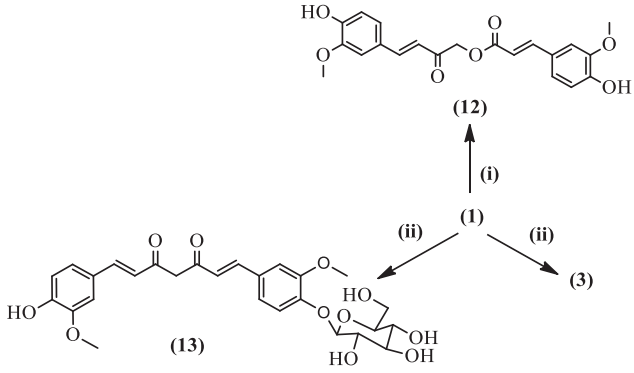
The utilization of an endophytic fungus identified as *Ovatospora brasiliensis* isolated from *Curcuma caesia* successfully transformed curcumin **(1)** to calebin-A **(12)** (Scheme 3). The structure of calebin-A **(12)** is



Scheme 1. Biotransformation of curcumin by (i) *R. oryzae*, (ii) *P. kudriavzevii*, and (iii) *B. bassiana*.



Scheme 2. Biotransformation of curcumin by (i) *A. alternata,* (ii) *P. brevicompactum*, (iii) *C. blackesleeana*, and (iv) *C. elegans.*



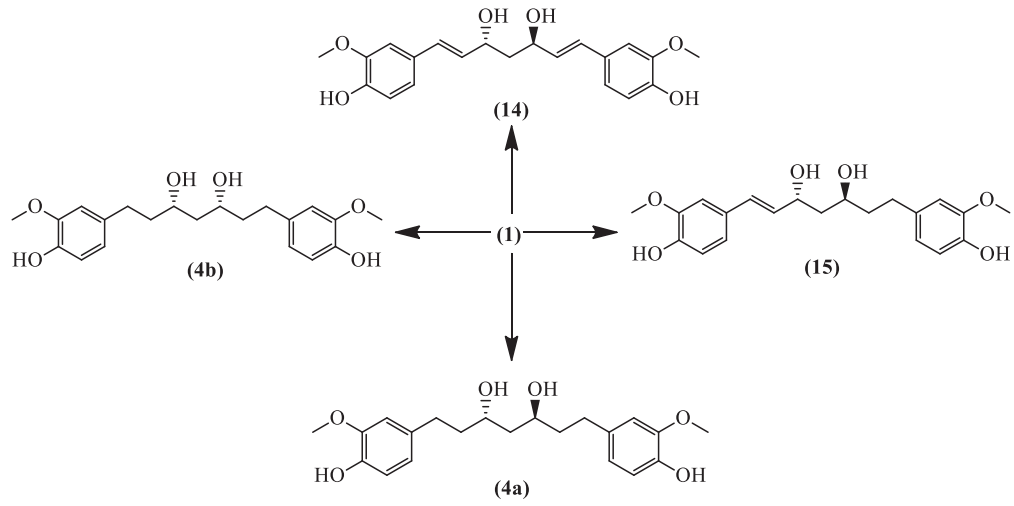
Scheme 3. Biotransformation of curcumin by (i) *O. brasiliensis* and (ii) *R. chinensis*

unique due to the oxidation of one keto group to an ester and is postulated to be due to the enzymatic activity of Baeyer-Villiger monooxygenase (Majeed et al. 2019). In another study, it was found that curcumin could be efficiently converted into glucosides by filamentous fungi, including *Gibberella fujikuroi var. fujikuroi*, *Absidia coerulea*, *Aspergillus niger*, *Rhizopus chinensis*, *C. elegans*, and *Saccharomyces cerevisiae* (Zhang et al. 2010). *R. chinensis*, *C. elegans*, and *A. coerulea* afforded curcumin glucosides as the biotransformation products. Among them, *R. chinensis* was the most potent strain and was selected for preparative-scale biotransformation to isolate the products. A glucosylated metabolite, namely curcumin 4′-*O*-*β*-*D*–glucoside **(13)**, together with metabolite **3**, were successfully isolated from the preparative-scale biotransformation (Scheme 3) (Zhang et al. 2010).

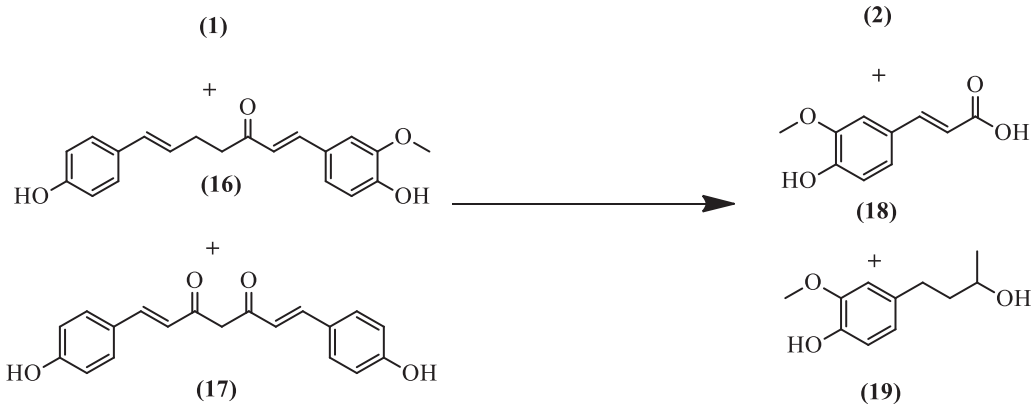
The biotransformation of curcumin **(1)** utilizing *Diaporthe* sp. as the biocatalyst afforded four reduced metabolites. The structure of all metabolites was characterized as (3*R*,5*R*)-tetrahydrocurcumin **(14)**, neohexahydrocurcumin **(15)**, (3*S*,5*S*)-octahydrocurcumin **(4a)**, and meso-octahydrocurcumin **(4b)** (Scheme 4) (Maehara et al. 2011). (3*S*,5*S*)-octahydrocurcumin **(4a)** and meso-octahydrocurcumin **(4b)** are the stereoisomers of octahydrocurcumin **(4)**. The transformation of **1** involves the reduction of dioxo functionalities to diol afforded compound **(14)**. Further reduction of **14** involves the hydrogenation of double bond between C-6 and C-7 in curcumin **(1)** to afford compound **15**.

3.2. Biotransformation by gut microbiota

The fermentation of curcumin (**1**), demethoxycurcumin **(16)**, and bisdemethoxycurcumin **(17)**, or commonly



Scheme 4. Biotransformation of curcumin by *Diaporthe* sp.



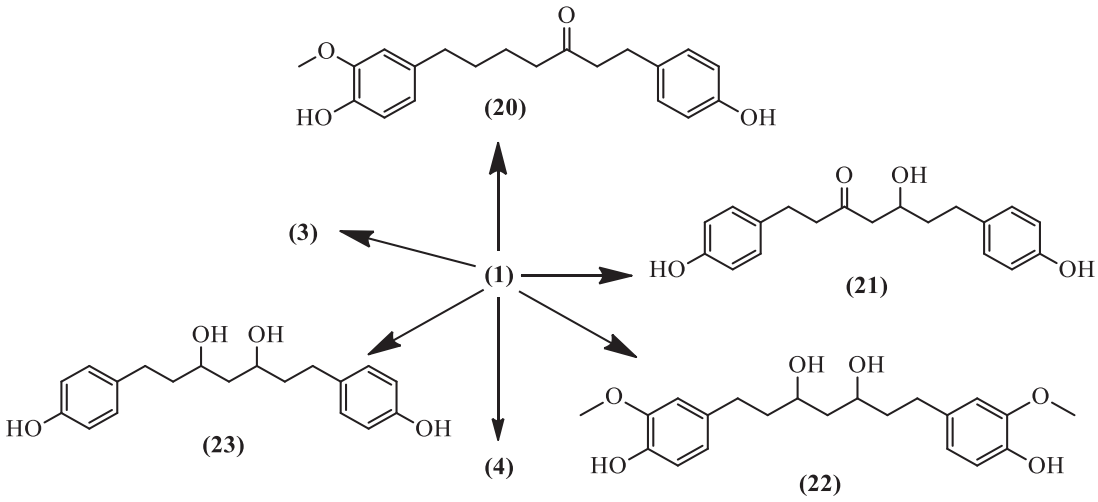
Scheme 5. Biotransformation of curcuminoids by human faecal microbiota.

called curcuminoids in human faeces, yielded three metabolites comprising of dihydroferulic acid **(18)**, 1-(4-hydroxy-3-methoxyphenyl)-2-propanol **(19)**, and metabolite **2** (Scheme 5) (Tan et al. 2015). The analyses of microorganisms isolated from human faeces revealed that *Escherichia coli* exhibited the highest curcumin-metabolizing activities via NADPH-dependent curcumin reductase (Hassaninasab et al. 2011). Metabolites **18** and **19** lost halves of the structure compared to the original substrates. The ketone and double bond functionalities were also reduced for metabolite **19**.

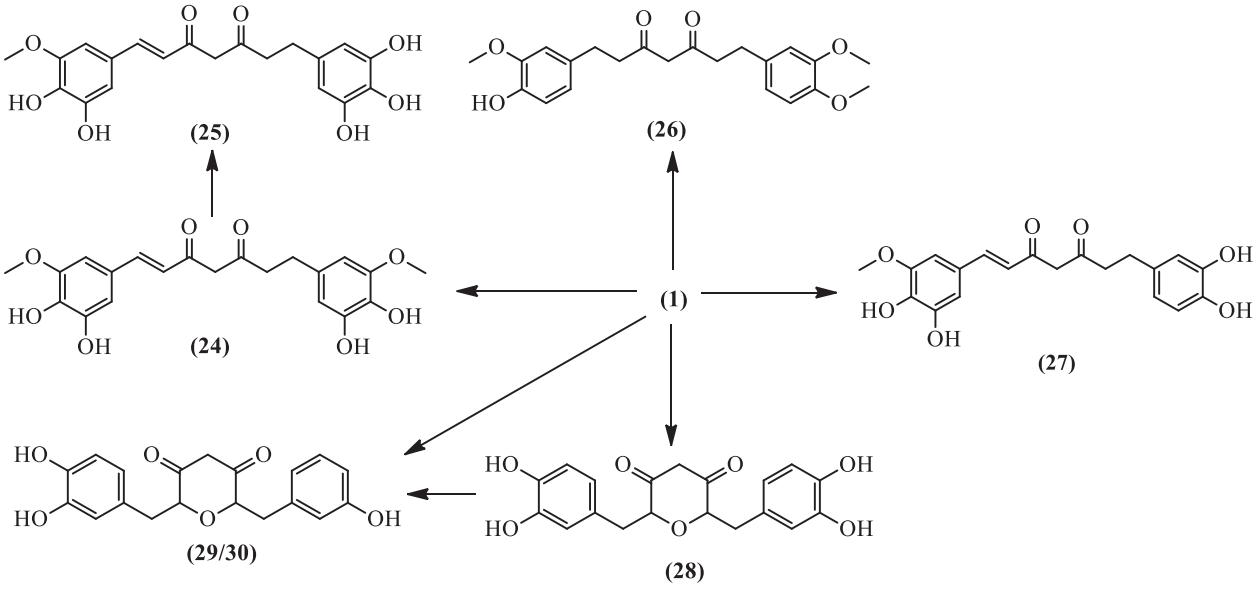
The microbial biotransformation of curcumin **(1)** with *Pichia anomala* found in the intestinal tract successfully yielded metabolites **3** and **4** together with other four metabolites, namely 5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)heptan-3-one **(20)**, 5-hydroxy-1,7-bis(4-hydroxyphenyl)heptane-3-one **(21)**, 1,7-bis(4-hydroxy-3-methoxyphenyl)heptan-3,5-diol **(22)**, and 1,7-bis(4-hydroxyphenyl)heptane-3,5-diol **(23)** (Scheme 6) (Herath et al. 2007). The transformation of curcumin **(1)** involves either the mono- or dihydroxylation of ketone groups and the change of unsaturated diene functionalities to become fully saturated with hydrogens.

Microbiota from mice faeces showed the capability of transforming curcumin to seven metabolites (compounds **2** and **24 − 30**), as shown in Scheme 7 (An et al. 2017). It afforded the addition of one hydroxyl group to the diphenyl rings as in metabolites **24** and **25**. No polar metabolites from the reduction of the ketone moiety to hydroxyl were isolated from this biotransformation. Instead, this biotransformation afforded unique metabolites **28 − 30**. It is postulated that the addition of one oxygen atom to one of the double bonds in the heptane linker leads to the cyclization to form pyrandione ring.

The fermentation of curcumin in an *in vitro* human faecal model afforded four metabolites identified as tetrahydrocurcumin **(2)**, demethyltetrahydrocurcumin **(31),** bis(demethyl)tetrahydrocurcumin **(32)**, and bis-



Scheme 6. Biotransformation of curcumin by *P. anomala*.

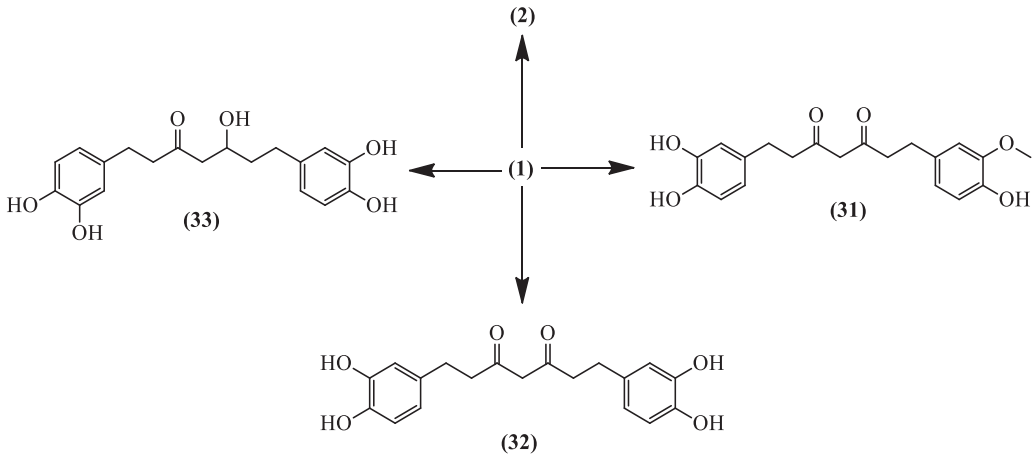


Scheme 7. Biotransformation of curcumin by microbiota from mice faeces.

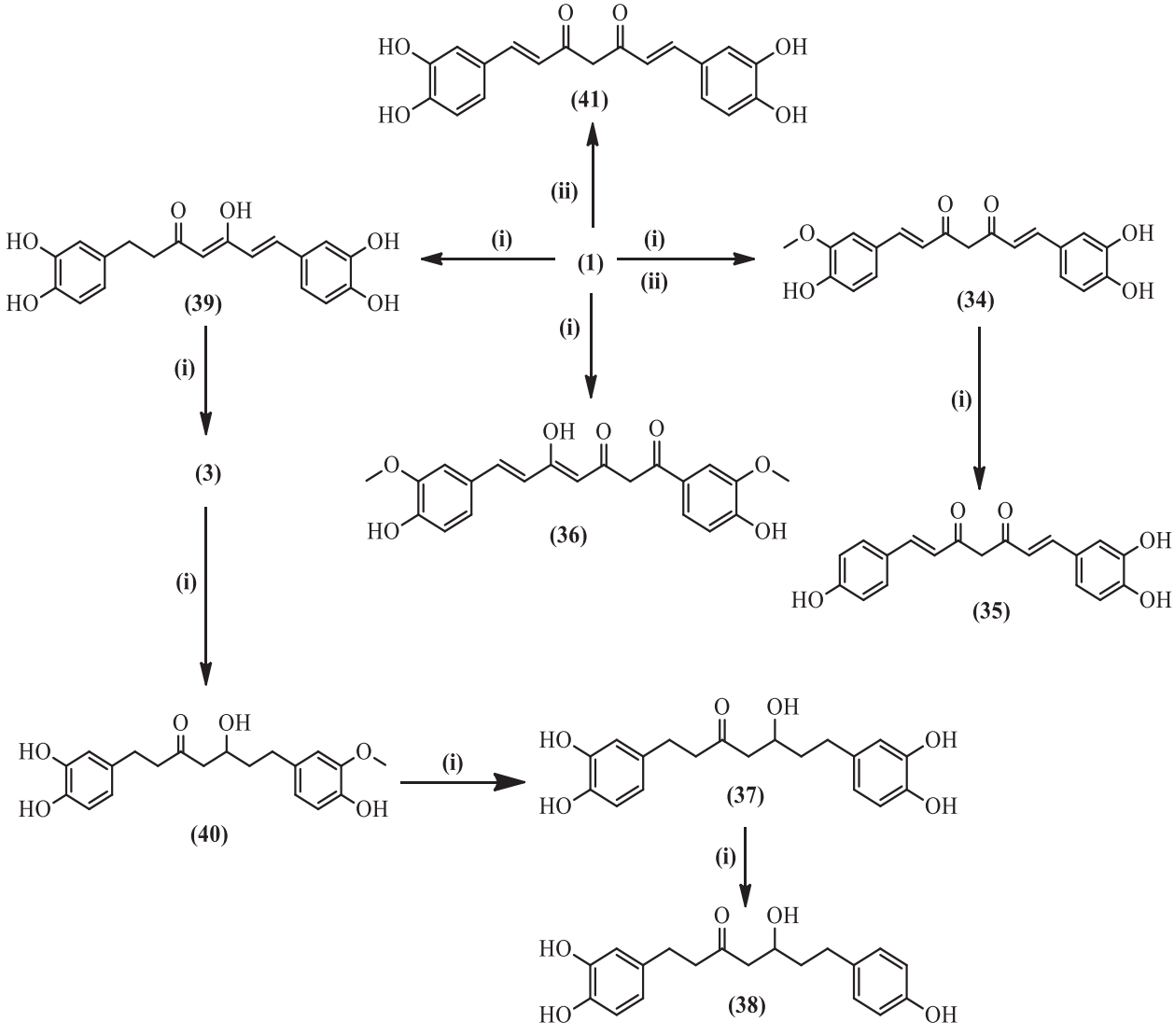
(demethyl)hexahydrocurcumin **(33)** (Scheme 8) (Bresciani et al. 2020). All metabolites detected were principally derived from the reduction of the double bonds, as well as the demethylation of the methoxy groups of curcumin **(1)**. The extra hydroxyl groups in the phenyl ring for metabolite **33** indicated that hydroxylation reaction occurred.

Sun et al. (2020) investigated the interactions of curcumin with the gut microbiota of mice where metabolites **(3)** and **(34 − 40)** were found (Scheme 9). The monohydroxylation of the methoxyl group of curcumin **(1)** afforded metabolite **34** and further removal of the methoxyl group resulted in the formation of metabolite **35**. Meanwhile, the keto-enol tautomerism from of one the curcumin **(1)** keto functionalities was also formed in metabolite **(36)**, and one alkene functionality oxidized to ketone. The keto-enol tautomerism was formed for metabolite **(39)**, but the alkene functionality was reduced to alkane. Further reduction of **39** afforded saturated metabolite **3**. Metabolites **40** and **37** were the products of the hydroxylation of methoxyl groups in metabolite **3**, while the removal of one hydroxyl group at the phenyl ring afforded metabolite **38**, a para-disubstituted curcumin derivative. A study on the conversion of curcumin **(1)** by the human intestinal *Bacterium blautia* sp. identified bisdemethylcurcumin **(41)** and metabolite **34** as the biotransformation products (Scheme 9) (Burapan et al. 2017).

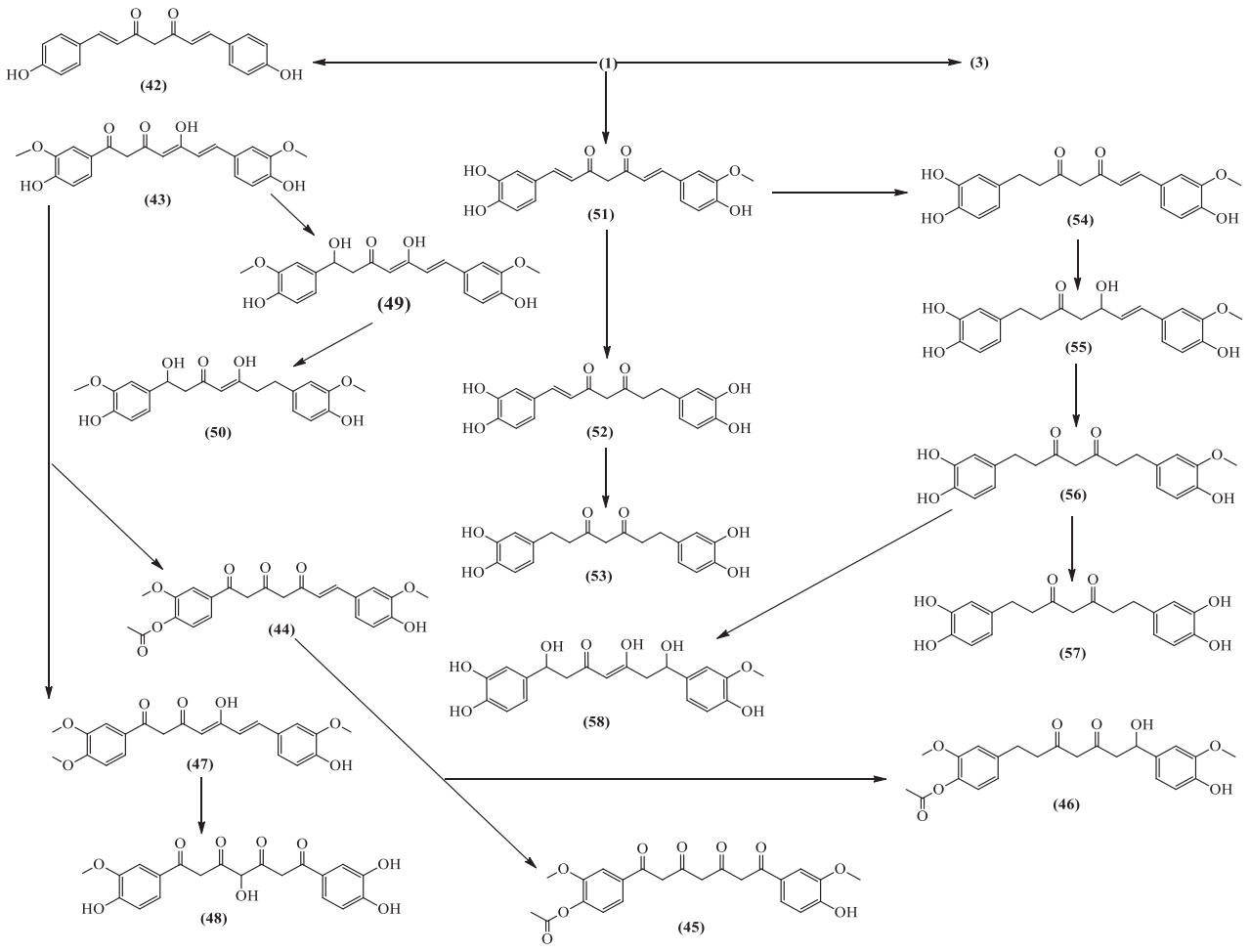
Lou et al. (2015) proposed a strategy for rapid analysis of the metabolic profile of curcumin in human colonic flora using ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry



Scheme 8. Biotransformation of curcumin by an *in vitro* faecal model.



Scheme 9. Biotransformation of curcumin by (i) the gut microbiota of mice and (ii) *B. blautia* sp.



Scheme 10. Biotransformation of curcumin in human colonic flora.

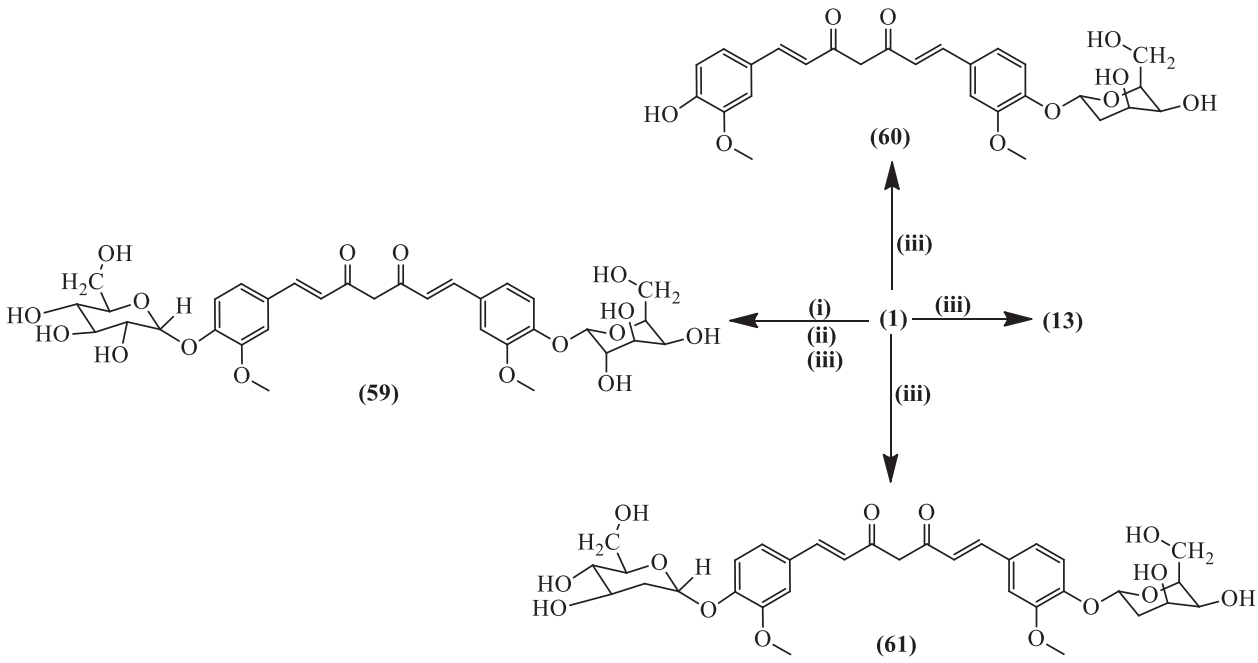
(UPLC-Q-TOF MS). A total of 23 curcumin metabolites were detected and identified *in vitro* (Scheme 10). The reactions that took place to form these metabolites were demethoxylation, reduction, hydroxylation, methylation, acetylation, and demethylation. The removal of dimethoxy groups could be seen in the formation of metabolite **42**. Meanwhile, reduction processes involving the reduction of alkene and ketone functionalities could be seen in the formation of metabolites **3**, **43 − 53**. Hydroxylation could be observed in the formation of metabolites to **43 − 45**, **54 − 56**, and **58**. The methylation of the phenolic group reaction only occurred for the formation of metabolite **47** from **43**. Other than that, demethylation could be identified in the formation of metabolites **51**, **56**, and **57**. Acetylation also occurred for the formation of metabolite **44** from **43.**

3.3. Biotransformation of curcumin by enzymes

Both biotransformation of curcumin **(1)** by amyloglucosidase from *Rhizopus* sp. (Vijayakumar and Divakar 2005) and β-glucuronidase from almonds (Prasad et al. 2014) yielded curcumin-bis-α-*D*-glucoside **(59)**. Both biotransformation routes afforded the glycosylation of the hydroxyl group at diphenyl rings (Scheme 11). Gurung et al. (2017) investigated the glycosylation of curcumin transformed by OPME consisting of UMP kinase, acetate kinase, UDP-α-*D*-glucose synthase, phosphomannomutase, *N*-acetyl-*D*-glucosamine kinase, glycosyltransferase, UDP-α-*D*-glucose, and UDP-α-*D*-2-deoxyglucose catalysts. However, the glycosylated metabolites produced were mainly due to the enzymatic reaction of UDP-α-*D*-glucose and UDP-α-*D*-2-deoxyglucose. Based on the result, the biotransformation of curcumin **(1)** to its glycosylated metabolites yielded curcumin 4′-*O*-β-glucoside **(13)**, curcumin 4′,4″-di-*O*-β-glucoside **(59)**, curcumin 4′-*O*-β-2-deoxyglucoside **(60)**, and curcumin 4′,4″-di-*O*-β-2-deoxyglucoside **(61)**

4. Structure–activity relationship of curcumin analogues

The SAR is the relationship between a chemical structure and its biological activity that enables the



Scheme 11. Biotransformation of curcumin by (i) amyloglucosidase, (ii) β-glucuronidase, and (iii) OPME.

determination of certain functional groups or structural moieties responsible for evoking a target for biological activity. The effect or potency of certain bioactive compounds can be altered by changing the chemical substructure. The SAR can be correlated either through qualitative or quantitative analysis. A qualitative SAR is an overview or prediction made based on the association between the bioactive compound substructure and the potential to exhibit a certain biological property effect, whereas a quantitative SAR is the prediction based on a regression equation or a mathematical model developed from known activities and structures of the bioactive compounds. The biotransformation of curcumin **(1)** afforded many analogues; thus, a comprehensive understanding of the SAR is needed to compare their effectiveness with original substrates. Curcumin **(1)** and its analogues have received immense attention due to their bioactivities, such as antioxidant, anticancer, and anti-inflammatory; hence, many scientific works, both *in vitro* and *in vivo*, have been reported. However, to date, only qualitative SAR studies have been reported for the biotransformation of curcumin **(1)** by fungi, gut microbiota, or enzymes. The presence of several moieties in the structure of curcumin **(1)** as shown in Figure 3, including phenolic, conjugated keto-enol, and β-diketone, is responsible for the SAR of the bioactivities reported.

4.1. Antioxidant activity

An antioxidant is defined as a substance that inhibits or delays the oxidation of biologically relevant molecules, either by specifically quenching free radicals or chelating redox metals (Flora et al. 2015). Younis et al. compared the *in vitro* antioxidant activities of curcumin **(1)** and its three biotransformation metabolites (i.e. vanillin **(9)**, didesmethylcurcumin **(10)**, and curcumin-4′-*O*-β-*D*-glucoside **(11)**) using the DPPH radical scavenging method. The results implied that metabolites **10** and **11** had enhanced antioxidant activities compared to curcumin **(1)** (Younis et al. 2016). The higher number of phenolic moieties and the addition of glucose molecules in metabolites **10** and **11**, respectively, serve as additional hydrogen donors, thus enhancing their ability to scavenge the DPPH radicals compared to curcumin **(1)**. Curcumin **(1)** and its reduced analogue, tetrahydrocurcumin **(2)**, also possessed good antioxidant activity. However, curcumin **(1)** may display pro-oxidant effect at higher dosages due to its ability to generate reactive oxygen species (ROS) through the modification of enzyme activity in contrary to tetrahydrocurcumin **(2)**. Due to the lack of conjugated bonds, tetrahydrocurcumin **(2)** does not generate ROS (Lai et al. 2020). The antioxidant activity of tetrahydrocurcumin **(2)** is also more significant than curcumin **(1)** against DPPH and hydroxyl radicals (Morales et al. 2015), superoxide anion radical

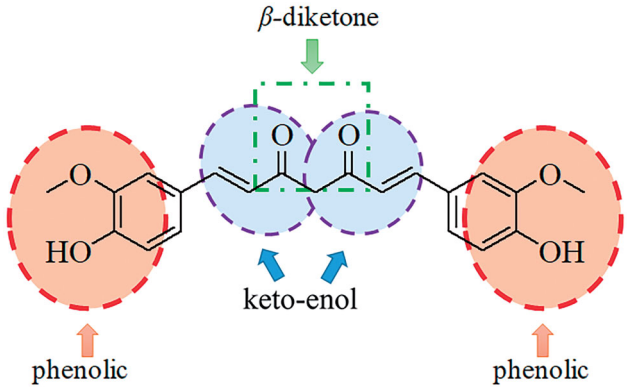


Figure 3. The moieties responsible for the SAR of curcumin.

scavenging (Suzuki et al. 2005), lipid peroxidation, and red blood cell haemolysis (Somparn et al. 2007).

Okada et al. reported the differences of the antioxidant effect between curcumin **(1)** and tetrahydrocurcumin **(2)** through an *in vivo* study by performing diet on mice. Tetrahydrocurcumin **(2)** can influence the release of antioxidant enzymes, such as glutathione peroxidase, glutathione-*S*-transferase, NADPH, and quinone reductase better than or comparable with curcumin **(1)** (Okada et al. 2001). Another *in vivo* antioxidant study in streptozotocin-nicotinamide-induced diabetic rats also revealed the ability of tetrahydrocurcumin **(2)** as an antioxidant by significantly enhancing the enzymatic activity, including superoxide dismutase, catalase, glutathione peroxidase, glutathione-*S*-transferase, reduced glutathione, as well as vitamin C and E. In addition, the formation of thiobarbituric acid reactive substances and hydroperoxides decreased significantly (Murugan and Pari 2006). The antioxidant activity of both curcumin **(1)** and tetrahydrocurcumin **(2)** was attributed to their phenolic groups and keto-enol moiety (Lai et al. 2020). The oxidative cleavage of C-C bond between the diketone moiety produced ortho-methoxy phenol as a product, which also acted as an antioxidant (Lai et al. 2020; Pandey et al. 2020).

The antioxidant activities of curcumin **(1)** and tetrahydrocurcumin **(2)** are also greatly affected by its solubility. Curcumin **(1)** showed better antioxidant potency than tetrahydrocurcumin **(2)** for gamma radiation-induced lipid peroxidation due to its higher lipid solubility. In contrast, tetrahydrocurcumin **(2)** showed higher antioxidant activities for N_2O triggered HO-induced lipid peroxidation due to its higher water solubility than curcumin **(1)** (Aggarwal et al. 2014). Similarly, hexahydrocurcumin **(3)**, another reduced metabolite, showed stronger activity than curcumin **(1)**, as proven through DPPH scavenging assay, lipid peroxidation, and red blood cell haemolysis assay (Huang et al. 2018). Oxidative stress resulting from an imbalance between free radical generation and reduced activity of antioxidants has been suggested as a contributing factor in the development and complication of many fatal and non-communicable diseases. Owing to their antioxidant activities, tetrahydrocurcumin **(2)** showed a positive outcome in the *in vivo* studies of antidiabetic (Murugan and Pari 2006), anti-inflammatory (Kim et al. 2021), anti-hypertension, and cardioprotective (Nakmareong et al. 2012), while hexahydrocurcumin **(3)** exhibited improvement in memory impairment (Jearjaroen et al. 2021) and enhanced the inhibitory effect of 5-fluorouracil on HT-29 colon cancer cells (Srimuangwong et al. 2012).

4.2. Cytotoxic and anticancer activities

The cytotoxic activity of a compound is usually reflected by a disturbance induced in cell cycle distribution (Gyovai et al. 2018). Basically, it is the ability of a compound to inhibit the growth of cells by inducing resistance in their cell cycle advancement. The cytotoxic activities of curcumin **(1)**, tetrahydrocurcumin **(2)**, hexahydrocurcumin **(3)**, 1,7-bis(4-hydroxy-3-methoxyphenyl)heptane-3,5-diol **(4)**, demethoxyhexahydrocurcumin **(7)**, demethoxyoctahydrocurcumin **(8)**, vanillin **(9)**, didesmethylcurcumin **(10)**, and curcumin-4′-*O*-*β*-*D*-glucoside **(11)** against CaCo-2 colorectal cancer cell line were investigated (Younis et al. 2016). The metabolites **2**, **3**, **8**, **10**, and **11** presented higher cytotoxic activities than curcumin **(1)**, which implied that they possessed a better chemotherapeutic role against colorectal cancer (Younis et al. 2016).

Numerous studies have also compared the anticancer potential of curcumin **(1)** with its reduced metabolites (i.e. tetrahydrocurcumin **(2)** and hexahydrocurcumin **(3)**). Several *in vitro* and *in vivo* studies demonstrated that tetrahydrocurcumin **(2)** portrayed better efficacy than curcumin **(1)** in inducing the cell apoptosis hepatocellular carcinoma H-22 ascitic tumour-bearing mice (Liu et al. 2017). It was also proven effective in reducing colorectal carcinogenesis, inhibiting fibrosarcoma metastasis, and increasing leukaemia cell death (Wu et al. 2014). Despite that, tetrahydrocurcumin **(2)** has also been reported to exhibit less potency than curcumin **(1)** in phorbol ester-induced tumour promotion, inducing apoptosis in human leukaemia HL-60 cells, and inhibiting the growth of human breast cancer cells (Aggarwal et al. 2014). These contradictory findings imply that the activity of tetrahydrocurcumin **(2)** is not always superior to curcumin **(1)** for cytotoxic or anticancer activity.

The SAR varied according to the specific studies conducted and the type of cancer cells tested. Thus, the SAR prediction of curcumin and the reduced metabolites cannot be generalized for all cytotoxic activities.

Glycosylation has also been associated with enhanced cytotoxicity and specificity modification of compounds on its target. Four glycosidic metabolites, curcumin 4′-*O*-β-glucoside **(13)**, curcumin 4′,4″-di-*O*-β-glucoside **(59)**, curcumin 4′-*O*-β-2-deoxyglucoside **(60)**, and curcumin 4′,4″-di-*O*-β-2-deoxyglucoside **(61)** were tested against several cell lines (i.e. gastric carcinoma (AGS), colon carcinoma (HCT116), hepatocarcinoma (HepG2), cervical carcinoma (HeLa), glioblastoma (U87MG), and melanoma (B16F10)). All metabolites **59 − 61** displayed better cytotoxicity activities against curcumin **(1)** (Gurung et al. 2017), confirming the presence of glycosyl groups as the main contributing factor. The monoglycoside metabolite demonstrated better cytotoxic activity than diglycosides. Among the monoglycosides, 2-deoxyglucoside exhibited greater cytotoxic activity than their corresponding oxyform (Gurung et al. 2017).

4.3. Antimicrobial activity

An antimicrobial agent is defined as a natural or synthetic substance that kills or inhibits the growth of microorganisms, such as bacteria, fungi, and algae (Burnett-Boothroyd and McCarthy 2011). Curcumin extract exhibited a broad spectrum of antibacterial activity, especially Gram-positive strains and multiple antibiotic-resistant bacteria (Chattopadhyay et al. 2004). Younis *et al.* investigated the antimicrobial activities of curcumin **(1)** and its biotransformation metabolites of tetrahydrocurcumin (**2**), hexahydrocurcumin (**3**), 1,7-bis(4-hydroxy-3-methoxyphenyl)heptane-3,5-diol (**4**), demethoxyhexahydrocurcumin **(7)**, demethoxyoctahydrocurcumin **(8)**, vanillin **(9)**, didesmethylcurcumin **(10)**, and curcumin-4′-*O*-β-*D*-glucoside **(11)** against *Staphylococcus aureus*, *E. coli*, *Candida albicans*, and *A. niger* through disc diffusion method. Metabolites **2**, **3**, **4**, and **11** exhibited stronger antimicrobial activities, as depicted by a larger inhibition diameter than curcumin **(1)** (Younis et al. 2016).

The antimicrobial activity of curcumin **(1)** and curcumin-bis-β-*D*-glucoside **(59)** was compared using minimum inhibitory concentration (MIC) assay against *S. aureus*, *Bacillus cereus*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *C. albicans*, and *Aspergillus fumigatus*. Metabolite **59** exhibited lower MIC values than curcumin **(1)**, indicating stronger antimicrobial activity. Meanwhile, metabolites curcumin 4′-*O*-β-2-deoxyglucoside **(60)** and curcumin 4′,4″-di-*O*-β-2-deoxyglucoside **(61)** showed higher antimicrobial activities against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *Enterobacter cloaceae subsp. disolvens* compared to curcumin **(1)** (Gurung et al. 2017). The higher polarity of curcumin metabolites contributed by the presence of multiple hydroxyl groups led to increased solubility, which enhanced microbial cellular uptake, reduced metabolism, and better binding to cell components, thus explaining the stronger antimicrobial activity (Prasad et al. 2014). Glycosylate curcumin metabolites exhibited better antimicrobial activity against both Gram-positive and Gram-negative bacteria than the aglycon metabolite. In contrast, a higher number of glucose units did not further enhance the activity. The diglucoside metabolites reduced the antimicrobial potency due to greater bulkiness with the attachment of two sugar units compared to monoglucoside (Gurung et al. 2017).

5. Conclusion and future perspectives

The findings mentioned in this systematic review have given a summary of how the biotransformation of curcumin **(1)** by fungi, gut microbiota, and enzymes is able to produce analogues with higher polarity and aqueous solubility. The most common structural modifications to produce the analogues are the reduction of olefins and ketone to alcohol functionalities, demethylation, hydroxylation, and glycosylation. This review implied biotransformation as a green and economical approach that offers various choices of natural catalysts in a synthetic toolbox for the structural diversification of curcumin. The SAR study also provided insights on the enhancement of various *in vitro* and *in vivo* biological activities by the analogues, many of which are different from curcumin. However, these findings need further validation by animal models or extended *in vivo* efficacy studies to provide additional insights, such as the bioavailability for further development as medicinal agents. Despite that, it is also worth noting that there is an advanced development in the formulation of curcumin through nanotechnology delivery systems, such as liposomes, polymers, conjugates, cyclodextrins, micelles, dendrimers, and nanoparticles (Karthikeyan et al. 2020). This advancement is able to enhance the bioavailability of curcumin, improve its low water solubility and therapeutic efficacy, as well as simultaneously reduce toxic side effects (Lee et al. 2014), hence offering alternative routes to maximise curcumin's pharmacological activity. Nevertheless, this systematic review may unlock a multitude range of

SAR studies to assist in the drug design of curcumin analogues through the biotransformation route.

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