Variable Secondary Metabolite Profiles Across Cultivars of *Curcuma longa* L. and *C. aromatica* Salisb.

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**Background:** *Curcuma* spp. (Zingiberaceae) are used as a spice and coloring agent. Their rhizomes and essential oils are known for medicinal properties, besides their use in the flavoring and cosmetic industry. Most of these biological activities were attributed to volatile and nonvolatile secondary metabolites present in the rhizomes of *Curcuma* spp. The metabolite variations among the species and even cultivars need to be established for optimized use of *Curcuma* spp.

**Objectives:** We compared the phytochemical profiles of rhizomes and their essential oils to establish the variability among seven cultivars: five of *Curcuma longa* L. (Alleppey Supreme, Duggirala Red, Prathibha, Salem, Suguna) and two of *C. aromatica* Salisb. (Kasturi Araku, Kasturi Avidi). The GC-MS and LC-MS-based analyses were employed to profile secondary metabolites of these selected cultivars.

**Methods:** Rhizomes of *Curcuma* spp. were subjected to hydro-distillation to collect essential oil and analyzed by GC-MS. The methanol extracts of fresh rhizomes were subjected to LC-MS analyses. The compounds were identified by using the relevant MS library databases as many compounds as possible.

**Results:** The essential oil content of the cultivars was in the range of 0.74–1.62%. Several compounds were detected from the essential oils and rhizome extracts by GC-MS and LC-MS, respectively. Of these, 28 compounds (13 from GCMS and 15 from LCMS) were common in all seven cultivars, e.g., α-thujene, and diarylheptanoids like curcumin. Furthermore, a total of 39 new compounds were identified from *C. longa* L. and/or *C. aromatica* Salisb., most of them being cultivar-specific. Of these compounds, 35 were detected by GC-MS analyses of essential oils, 1,2-cyclohexanediol, 1-methyl-4-(1-methylethyl)-, and santolina alcohol, to name a few. The other four compounds were detected by LC-MS of the methanolic extracts of the rhizomes, e.g., kaempferol-3,7-O-dimethyl ether and 5,7,8-trihydroxy-2′,5′-dimethoxy-3′,4′-methylene dioxyisoflavanone.

**Abbreviations:** CU, curcumin; cvs, cultivars; DMC, demethoxy curcumin; BDMC, bisdemethoxycurcumin.
INTRODUCTION

Turmeric (Curcuma longa L.) is a perennial rhizomatous herb that belongs to the family Zingiberaceae (Prasath et al., 2018). It has been used traditionally in India for its medicinal value and as a spice (Srinivasan et al., 2004; Aggarwal et al., 2007; Esatbeyoglu et al., 2012). In Ayurvedic medicine, turmeric is used internally (as a stomachic, tonic, and blood purifier) or externally (prevention and treatment of skin diseases) (Gounder and Lingamallu, 2012). Turmeric was scientifically validated for several pharmacological benefits, including antioxidant, anti-inflammatory, and chemoprotective properties (Miquel et al., 2002; Krup et al., 2013; Kanase and Khan, 2018; Umar et al., 2020). The rhizomes of turmeric are enriched with several bioactive metabolites, though the attention was mostly on curcuminoids. Besides curcumin (a curcuminoid), the essential oil of C. longa L. showed antimicrobial activity and ability to suppress aflatoxin production (Ferreira et al., 2013).

Out of 110 species of genus Curcuma, only ∼20 species were used so far for phytochemical studies (Nahar and Sarker, 2007). Curcuma longa L. is popularly known as turmeric, while C. aromatica Salisb. and C. caesia Roxb. are known as wild turmeric and black turmeric, respectively. C. longa L. and a few other species, including C. aromatica Salisb., produce curcumin, a yellow colored curcuminoid. So far, at least 235 compounds, primarily phenolics, terpenoids, and alkaloids, were identified from Curcuma spp. (Li et al., 2011). About 70 varieties of C. longa L. are cultivated in India (Sasikumar, 2005; Parthasarathy and Chempakam, 2008), but very few are chemically profiled.

The essential oil of Curcuma spp. is used in traditional medicine for many ailments (Dosoky and Setzer, 2018). The volatile component of C. longa’s rhizome is responsible for its aromatic flavor and odor (Gounder and Lingamallu, 2012). Its essential oil is considered safe for human use (Tisserand and Young, 2013). The oils of C. longa L. and C. aromatica Salisb. have applications in the food and pharmaceutical industries due to their antioxidant, antibacterial, and anti-inflammatory properties (Dosoky and Setzer, 2018). The essential oil also improved the bioavailability of curcumin, thereby its bioactivity (Shishu and Maheshwari, 2010). Preliminarily clinical trials indicated that the essential oil from C. longa L. and C. aromatica Salisb. was helpful against cancer, asthma, and other ailments (Cheng et al., 1999; Joshi et al., 2003; Li Y. et al., 2009). Thus, there is a need to identify high-yielding cultivars containing curcuminoids and essential oil.

Since the pharmacological properties of Curcuma spp. are dependent on their chemical profiles, studies on the chemical constituents of turmeric/wild turmeric and their essential oils gained significance. Thin-layer chromatography (TLC) is one of the methods employed to quantify curcumin (Setyaningsih et al., 2016) and other curcuminoids (Phathanawasin et al., 2009) in Curcuma longa L. A few different techniques used were HPTLC (Pathania et al., 2006; Paramasivam et al., 2009), nuclear magnetic resonance (NMR) spectroscopy (Li W. et al., 2009), and the HPLC method (Kulyal et al., 2016).

Our present study on metabolite profiles would pave the way for metabolomics by providing the identity of several metabolites. Metabolomics is a practical approach for the comprehensive profiling and comparison of metabolites in plant systems (De Vos et al., 2007). It is crucial for quality evaluation and scientific validation of medicinal plants and their products (Mukherjee et al., 2016). Mainly information on secondary metabolites of medicinal plants/spices is of great importance in health, food, and nutrition sectors, due to the antioxidant nature, color, or flavor of these secondary compounds (Beekwilder et al., 2005; Dixon et al., 2006; Hall, 2006). The quality of turmeric and other spices depends on factors, such as cultivation, collection, storage, milling, and processing, apart from genetics and adulteration issues. Therefore, metabolomics provides a practical approach for quality control (Mukherjee et al., 2016; Tetali et al., 2021).

Over the past decade, several methods suitable for large-scale analysis of metabolites in plant extracts were developed (Dixon et al., 2006; Hall, 2006). However, to date, no single analytical method can successfully detect the entire metabolome of higher plants, especially of medicinal and aromatic plants, as they are highly rich in chemically diverse metabolites (Tetali et al., 2021). The GC-MS and LC-MS techniques mutually complement each other in unraveling secondary metabolomes comprising a wide range of volatile and nonvolatile compounds. These compounds belonged to terpenes, phenolic acids, phenylpropanoids, saponins, alkaloids, polyamines, and their derivatives (Huhman and Sumner, 2002; Moco et al., 2006).

Essential oils from different Curcuma species, including C. longa L. and C. aromatica Salisb., were studied for their chemical constituents (Choudhury et al., 1996; Angel et al., 2014; Nampoothiri et al., 2015; Dosoky and Setzer, 2018) to establish their variability. Variation in the volatile compositions of Curcuma spp. such as C. longa L. and C. zedoaria, was done using GC-MS (Dosoky et al., 2019). A combination of GC-MS and LC-MS techniques was used for metabolite analysis of C. domestica L. (C. longa L.) (Herebian et al., 2009). In the present study, the volatile (essential oil) and nonvolatile (total extract) components of the fresh rhizome of the seven cultivars of Curcuma spp. were analyzed by the GC-MS and LC-MS techniques. The present study is the first report revealing such detailed...
TABLE 1 | Essential oil content and total number of compounds detected by GC-MS in the rhizomes of Curcuma species.

| Sl. No. | Cultivar (species) | Essential oil (%) | Identified (Reported in Curcuma spp. or another plant species) | Unidentified |
|---------|-------------------|-------------------|---------------------------------------------------------------|--------------|
| 1       | Alleppey Supreme  | 1.42              | 31                                                            | 58           |
|         | (C. longa L.)     |                   |                                                               | 111          |
| 2       | Duggirala Red     | 0.74              | 36                                                            | 56           |
|         | (C. longa L.)     |                   |                                                               | 108          |
| 3       | Prathibha         | 1.20              | 44                                                            | 60           |
|         | (C. longa L.)     |                   |                                                               | 96           |
| 4       | Salem             | 1.00              | 30                                                            | 39           |
|         | (C. longa L.)     |                   |                                                               | 131          |
| 5       | Suguna            | 0.80              | 35                                                            | 51           |
|         | (C. longa L.)     |                   |                                                               | 114          |
| 6       | Kasturi Avidi     | 0.78              | 29                                                            | 64           |
|         | (C. aromatica Salisb.) |         |                                                               | 107          |
| 7       | Kasturi Araku     | 1.62              | 31                                                            | 60           |
|         | (C. aromatica Salisb.) |         |                                                               | 109          |

metabolite profiles of the selected cultivars to the best of our knowledge. These cultivars, except Alleppey Supreme, are typically cultivated in Telangana and Andhra Pradesh, and these states are among the largest producers of turmeric in India (Parthasarathy and Chempakam, 2008). Most of the studies worldwide on Curcuma spp., for their curative properties, were with C. longa L., followed by C. aromatica Salisb, C. aeruginosa Roxb. (Simoh and Zainal, 2015), and C. kwangsiensis S. K. Lee & C. F. Liang (Zeng et al., 2009). Several cultivars exist within these species, which vary in their chemical profiles. The present article is the first attempt to characterize both volatile (essential oil) and nonvolatile (crude extract) components of fresh rhizomes of seven cultivars of Curcuma spp. by the GC-MS and LC-MS techniques. Our results using GC-MS and LC-MS analyses revealed high variability in their metabolite profiles of seven cultivars of genus Curcuma. We emphasize that such an approach could be exploited to distinguish cultivars for a specific application based on their metabolite profile.

MATERIALS AND METHODS

Materials and Reagents

LC-MS grade methanol, water, and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA, United States). Ammonium formate, formic acid, 4-fluoro-4′-hydroxy benzophenone (97%), and n-hexane were from Sigma-Aldrich, India. Anhydrous sodium sulfate (99.99%) was from Merck Millipore, India.

Fresh rhizomes of four cultivars of Curcuma longa L. (Duggirala Red, Prathibha, Salem, and Suguna) and two cultivars of C. aromatica Salisb. (Kasturi Araku and Kasturi Avidi) were collected from Turmeric Research Station, Kammarpally, Telangana State, India. Alleppey Supreme cultivar of C. longa L. was from the Indian Institute of Spices Research, Marikunnu (IISR) Kozhikode, Kerala, India. The mature rhizome samples were collected during the postharvest season of turmeric (May–Jun) in 2011 and 2012 and cryopreserved at −80°C until extraction and analysis.

Isolation of Essential Oil by Hydrodistillation for GC-MS Analysis

50 g each of fresh turmeric rhizome of five cultivars of C. longa L. cvs. Alleppey Supreme, Duggirala Red, Prathibha, Salem, Suguna, and two cultivars of C. aromatica Salisb. cvs. Kasturi Araku and Kasturi Avidi were taken out from a −80°C freezer, made into pieces, and ground in a pestle with a mortar to a fine powder under liquid nitrogen. The powder was subjected to hydrodistillation in a Clevenger-type apparatus for 7 h. The essential oil obtained after distillation was dried over anhydrous sodium sulfate and kept at −80°C until GC-MS analysis.

GC-MS Running Conditions and Metabolite Identification

The chemical composition of the Curcuma spp. essential oil was analyzed by the GC-MS technique using Agilent 7890 A gas chromatograph coupled with a Leco Pegasus HT TOF mass spectrometer equipped with a 29.8 m × 320 μm HP-5MS 5% phenyl methyl siloxane capillary column with 0.25 μm film thickness. The oven temperature was programmed at 65°C for 2 min and then increased from 65 to 90°C at 5°C/min (held for 3 min). Then the temperature was increased from 90 to 103°C (held for 3 min) and from 103 to 150°C (held for 15 min) at 20°C/min and 8°C/min, respectively. The temperature was raised finally from 150 to 280°C at 20°C/min. The injector, interphase, and ion source were maintained at 250°C, 280°C, and 250°C, respectively. The detector voltage was 1500 V. A solvent delay of 2 min was selected. One microliter (diluted with n-hexane; 1:10) of essential oil sample was injected into the GC-MS system using split mode (50:1). Helium was used as a carrier gas at a flow rate of 1 ml/min. GC-MS data were measured at 70 eV; mass scan 40–1000 amu.

The compounds were identified by comparing their mass spectra with the data available in the literature, National
Institute of Standards Technology NIST, and Leco-Fiehn Rtx5 libraries. The compounds originated from the GC-MS data file were identified by matching most resembling spectra with the NIST library. Each search produced a hit list of compounds according to match factor or similarity with the library spectra. All the compounds showing similarity more than 70% with the

![Representative TIC chromatograms from GC-MS of essential oil from cultivars (A) Alleppey Supreme, (B) Duggirala Red, (C) Prathibha, (D) Salem, (E) Suguna of Curcuma longa L. and cvs. (F) Kasturi Araku, (G) Kasturi Avidi of C. aromatica Salisb.](image-url)
TABLE 2: Cultivar-specific compounds identified, in one of the seven cultivars of Curcuma longa L. or C. aromatica Salisb. by GC-MS in the essential oil from rhizomes. The structures of the compounds (serial numbers from 1 to 23) are given in Figure 2 (panel numbers: 1–23), and this is the first report of these compounds from the genus Curcuma L. These compounds, however, were reported from genus other than Curcuma L. The compounds from serial numbers 24 to 41 are already reported in Curcuma species. Structures for few compounds (serial numbers 24–30) are given in Figure 3 (panel numbers: 1–7). Abbreviations used: AS, Alleppey Supreme; DR, Duggirala Red; PR, Prathibha; SA, Salem; SU, Suguna; KAr, Kasturi Araku; KAv, Kasturi Avidi.

| Sl. No. | Compound name | Cultivar | RT (Min) | Area/abundance | Formula | Mass (Mass fragmentations) | Class of compound | Reported from plant species | References |
|--------|----------------|----------|----------|----------------|---------|---------------------------|------------------|-----------------------------|------------|
| 1 | 1,2-Cyclohexanediol, 1-methyl-4-(1-methylthyl)- | AS | 6.38 | 958955880 | C12H20O2 | 172.146, 43, 71, 111, 154 | Monoterpenoid | Citrus medica L. Leaf and peel essential oil | Bhuiyan et al. (2009) |
| 2 | Trans-trans-Octa-2,4-dienyl acetate | AS | 8.22 | 8498 | C10H16O2 | 168.115, 43, 77, 79 | Dienen acetate | Kaempferia galanga L. Dried rhizomes | Othman et al. (2006) |
| 3 | Phenol, 2-methoxy-3-(2-propenyl)- | AS | 17.05 | 200016 | C10H12O2 | 164.083, 77, 131 | Phenolic monoterpenoid | Dalbergia stevensoni Standl. Wood extracts | Jiang et al. (2018) |
| 4 | 3-Isopropyl-4-methyl-1-pentyn-3-ol | DR | 13.59 | 805101 | C9H16O | 140.120, 43, 97 | Alcohol constituent | Anethum sowa Roxb. ex, Fleming | Saleh-e-in et al. (2010) |
| 5 | 5,9-Tetradecadiyne | DR | 19.45 | 11283632 | C14H22 | 190.172 | Unsaturated hydrocarbon | Ferula ves:nertensis Coss. & Durieu ex Trab. | Zellagui et al. (2012) |
| 6 | Naphthalene, 5-butyl-1,2,3,4-tetrahydro- | DR | 20.62 | 1290163 | C14H20 | 188.156, 91, 145 | Tetralin | Meconopsis punicea Maxim. and M. delavayi (Franch.) Franch. Ex Prain, essential oil | Yuan et al. (2003) |
| 7 | Santolinal alcohol | DR | 23.63 | 841206 | C10H18O | 188.156 | Tertiary alcohol | Achillea filipendulina L., aerial part | Sharopov and Setzer (2010) |
| 8 | 2-Pentanone, 4-mercapto-4-methyl- | PR | 5.32 | 1330293 | C6H12OS | 132.060 | Ketone | Camellia sinensis (L.) Kuntze | Kumazawa et al. (2005) |
| 9 | 8-Methylene-3-oxatricyclo[5.2.0.0(2,4)]nonane- | PR | 11.72 | 25554 | C9H12O | 136.08, 121, 43, 55 | Alcohol constituent | Abies alba Mill. | Wang et al. (2005) |
| 10 | 7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11 tetramethyl- | PR | 19.16 | 21297286 | C15H24O | 188.156, 91, 145 | Triterpenoid | Parkia speciosa Hassk. | Salman et al. (2006) |
| 11 | Bicyclo(2.2.1)hept-2-ene, 2,3-dimethyl- | PR | 19.41 | 23461594 | C9H14 | 122.109 | Alcohol constituent | Linderag aggregata (Sims) Kosterm., essential oil | Hong (2011) |
| 12 | 1H-3a,7-methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3à,3aá,7á,8aà)] | PR | 19.69 | 1483176 | C15H24 | 204.187, 93, 119, 161 | Sesquiterpene | Lindera aggregata (Sims) Kosterm., essential oil | Yang et al. (2009) |
| 13 | Cholesta-8,24-dien-3-ol, 4-methyl-, [3ß(3a,7,8a)] | PR | 21.56 | 84886714 | C20H40O | 398.354, 69, 105 | Triterpenoid | Parkia speciosa Hassk. seed | Salman et al. (2006) |
| 14 | 4-Ethylphenoylethylamine | PR | 25.88 | 507899185 | C10H15N | 149.120 | Amines | Psidium guajava L. stem bark essential oil | Fasola et al. (2011) |
| 15 | Cyclohexan-2-methyl-5-(1-methylthiyl)- | PR | 26.57 | 3966291 | C9H12O | 154.135, 67, 107, 136 | Monoterpenoid | Mentha spicata L. aerial parts | Mohammed et al. (2017) |
| 16 | Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylthiyl)- | PR | 26.59 | 26170712 | C16H18S4 | 192.187, 107, 149 | Monoterpenoid | Rhanterium adpressum Coss. & Durieu Aerial parts | Kala et al. (2009) |
| 17 | 5,8,11,14-Eicosatetraenoic acid, phenylmethyl ester, (all-Z)- | SA | 5.39 | 15244294 | C20H30O2 | 394.287, 67, 91, 205 | Fatty acid | Ficus hispida L. Fresh male and female receptive figs, leaves | Sathyabalan et al. (2014) |
| 18 | 11-Dodecen-2-one | SA | 36.83 | 511982 | C10H22O | 182.167, 43, 124, 182 | Ketone | Coriandrum sativum L., leaf oil | Song et al. (2001) |
| 19 | E-11-Tetradecenoic acid | SA | 37.15 | 335669 | C10H20O2 | 226.193, 41, 56, 69 | Fatty acid | Alpinia speciosa (J.C. Wendl) K. Schum | Bhuiyan et al. (2009) |
| 20 | 2-Nonen-4-yn-1-ol, (Z)- | SU | 10.68 | 287984 | C9H16O | 154.135, 41, 67 | Alcohol | Psidium guajava L. stem bark essential oil | Ho (2010) |
Cultivar-specific compounds identified, in one of the seven cultivars of Curcuma longa L. or C. aromatica Salisb. by GC-MS in the essential oil from rhizomes. The structures of the compounds (serial numbers from 1 to 23) are given in Figure 2 (panel numbers: 1–23), and this is the first report of these compounds from the genus Curcuma L. These compounds, however, were reported from genus other than Curcuma L. The compounds from serial numbers 24 to 41 are already reported in Curcuma species. Structures for few compounds (serial numbers 24–30) are given in Figure 3 (panel numbers: 1–7).

| Sl. No. | Compound name | Cultivar | RT (Min) | Area/abundance | Formula | Mass (amu) | Class of compound | Reported from plant species | References |
|--------|---------------|----------|----------|----------------|---------|------------|-------------------|----------------------------|------------|
| 21     | 3-Cyclohexen-1-one, 3,5,5-trimethyl- | SU       | 21.81    | 10709364      | C9H14O  | 95, 138    | Cyclohexene       | Seeds and leaves            | D’Auria et al. (2006) |
| 22     | 6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl- | SU       | 23.27    | 18320904      | C15H24O | 96, 138    | Sesquerpenoid      | Dried saffron               | Venkataramani and Chinagounder (2012) |
| 23     | 3-Octen-5-yne, 2,7-dimethyl-, (Z)- | KAv      | 7.91     | 132889991     | C10H16  | 93, 121, 136 | Monoterpene       | Litsea glutinosa (Lour.) C.B. Rob Fruit oil | Chowdhury et al. (2008b) |
| 24     | Aromadendrene | PR       | 21.68    | 32839807      | C10H16  | 204.187    | Hydrocarbon        | Curcuma aromatica Salisb., rhizome | Hong et al. (2014) |
| 25     | Isoborneol    | PR       | 10.16    | 931077        | C10H18O | 154.135    | Monoterpenoid      | Curcuma aromatica Salisb., rhizome | Sasikumar (2005) |
| 26     | β-Elemene     | PR       | 17.87    | 8265459       | C15H24  | 204.187    | Sesquerpenoid      | Curcuma longa L., rhizome Essential oil | Ma and Gang (2006) |
| 27     | α-Santalone   | PR       | 19.15    | 167463111     | C15H24  | 204.187    | Sesquerpenoid      | Curcuma longa L., rhizome Essential oil | Chowdhury et al. (2008a) |
| 28     | 2-Tridecanone | PR       | 36.82    | 145399        | C18H36  | 180.182    | Ketone             | Curcuma albedora Thwaites, rhizome Essential oil | Herath et al. (2017) |
| 29     | Nonanoic acid | SU       | 37.82    | 1054190       | C9H18O2 | 158.130    | Fatty acid         | Curcuma longa L., rhizome Essential oil | Nieman et al. (2012) |
| 30     | Eucalyptol    | KAr      | 6.34     | 73923505      | C10H16O | 154.135    | Monoterpene        | Curcuma longa L., rhizome Essential oil | Chowdhury et al. (2008a) |
| 31     | Carvacrol     | AS       | 15.46    | 800060        | C10H16O | 150.104    | Monoterpene        | Curcuma longa L., rhizome Essential oil | Awasthi and Dixit (2009) |
| 32     | endo-Borneol  | PR       | 25.95    | 762477        | C10H18O | 154.135    | Monoterpene        | Curcuma longa L., rhizome Essential oil | Chowdhury et al. (2008a) |
| 33     | 1,3,5-Cycloheptatriene, 3,7,7-trimethyl- | KAv      | 22.26    | 44959671      | C10H16  | 134.109,120 | Cyclic hydrocarbon | Curcuma longa L., rhizome Essential oil | Chowdhury et al. (2008a) |
| 34     | p-Cymen-8-ol  | KAr      | 11.08    | 28783214      | C10H16O | 150.104    | Monoterpenoid      | Curcuma longa L., rhizome Essential oil | Chowdhury et al. (2008a) |
| 35     | Camphor       | PR       | 9.68     | 7983536       | C10H16O | 152.120    | Terpenoid ketone   | Curcuma longa L., rhizome Essential oil | Leela et al. (2002) |
| 36     | α-Bisabolol   | PR       | 22.60    | 26108003      | C15H24O | 222.198    | Sesquerpenoid      | Curcuma longa L., rhizome Essential oil | Chowdhury et al. (2008a) |
| 37     | α-Elemone     | PR       | 22.99    | 5796701       | C15H24O | 218.167    | Sesquerpenoid      | Curcuma longa L., rhizome Essential oil | Singh et al. (2010) |
| 38     | Caryophyllene oxide | PR | 21.85    | 84236617     | C10H16O | 220.182    | Sesquerpenoid oxide | Curcuma longa L., rhizome Essential oil | Chowdhury et al. (2008a) |
| 39     | Citral        | KAr      | 10.81    | 4942325       | C10H16O | 152.120    | Monoterpene        | Curcuma longa L., rhizome Essential oil | Chowdhury et al. (2008a) |
| 40     | Neoisolongifolene, 8,9-dehydro- | KAr    | 20.92    | 956644398     | C10H16  | 204.187    | Bicyclic hydrocarbon | Curcuma longa L., rhizome Essential oil | Chowdhury et al. (2008a) |
| 41     | Sabinene hydrate | KAv  | 19.81    | 30005835      | C10H18O | 154.135    | Monoterpenoid      | Zingiber officinalis Roscoe, rhizome essential oil | Koo and Gang (2012) |
FIGURE 2 | Structures of first-time reported (total 39) from the genus Curcuma, identified from cultivars of Curcuma longa L. and C. aromatica Salisb. detected in essential oil (panel numbers 1–23 and 24–35 corresponding to serial numbers 1–23 and 1–12 of Tables 2, 3 respectively) and rhizome extracts (panel numbers 36 and 37–39 corresponding to serial numbers 1 and 1–3 of Tables 6, 7 respectively) by GC-MS and LC-MS, respectively. Details of all these compounds are given in Supplementary Tables S3, S4.

Continued
NIST library were selected by the software (Software: Version 4.22 optimized for Pegasus®). Software searches (identifies) compound from their mass spectra and includes MS interpretation programs for analyzing mass spectra based on chemical structure, molecular formula, isotopic pattern, etc. The similarity of 70% or above between the m/z values of the compound detected in the respective cultivar and the MS-libraries’ mass fragmentation pattern was considered as identification. Furthermore, mass spectra of all compounds were also matched with ranges available as per their CAS number. Compounds for which the CAS number was not generated, the PubChem CID was used. Compounds below the similarity level of 70% were not considered and grouped as unknown. The data obtained with the samples collected in 2012 are presented in this article.

Preparation of Rhizome Extracts for LC-MS Analysis

Samples for LC-MS analysis were prepared by grinding the fresh rhizome to a fine powder in a mortar and pestle under liquid nitrogen. 1 g of the rhizome powder was suspended in 2 ml of MeOH (LC-MS grade). The samples were sonicated for 30 min and centrifuged for 25 min at 1500 rpm, and the supernatants were separated by filtering through a 0.45-μm Nylon filter disk. These extracts were freshly prepared for the analysis. A 200 μl aliquot of the extract was diluted quantitatively with internal standard (IS) 200 μl 4-fluoro-4′-hydroxy benzophenone solution. It was prepared freshly for each analysis by dissolving in methanol for a final concentration of 0.58 mg/ml. The samples were subjected to LC-MS analysis for the complete metabolite profile. The data obtained with the samples collected in 2012 were presented in this article.

LC-MS/MS Conditions and Metabolite Identification

LC-MS analyses of the crude extract of fresh rhizome of Curcuma spp. were performed according to Jiang et al. (2006) using Agilent 6520 Accurate Q-TOF (Agilent Santa Clara, CA), and the column used was Zorbax Eclipse XDB-C 18, 4.6 × 50 mm, 1.8 μ; Mobile phase: A) buffer (5 mM ammonium formate, 0.1% formic acid, in deionized and distilled H2O) and B) acetonitrile; gradient (in buffer A): 0–2 min, 5% B; 2–5 min, 5–100% B; 57–60 min, 100% B; 60–65 min, 100–5% B; flow rate: 0.25 ml/min; temperature, 40°C; injection volume 5 μl. For the MS detection, Agilent MSD-Trap-SL was equipped with electrospray ionization (ESI) interface as the ion source. The acquisition parameters for the negative mode were: drying N2 temperature, 350°C, 8 l/min; nebulizer pressure 40 psi; HV capillary 4000 V; skimmer 65.0 V; spray voltage: 4 kV; scan rate 1.4. We analyzed the results in both the positive and the negative ion mode acquired by Agilent TOF/Q-TOF mass spectrometry and full MS scan, in the form of total ion current (TIC) chromatogram, and the metabolites were identified based on their MS/MS spectra and fragmentation rules reported previously (Jiang et al., 2006).

RESULTS

Essential Oil Content

The oil was obtained by hydro-distillation, in a Cleverenger-type apparatus, of the fresh rhizomes of five cultivars (Alleppey Supreme, Duggirala Red, Prathibha, Salem, and Suguna) of Curcuma longa L. and two cultivars (Kasturi Araku and Kasturi Avidi) of C. aromatica Salisb. The yield of essential oil from the seven cultivars was in the range of 0.74–1.62% on a fresh weight basis, with the highest yield of 1.62% in cv. Kasturi Avidi (C. aromatica Salisb.) followed by cv. Alleppey Supreme (C. longa L.) with an amount of 1.42% and the lowest yield of 0.74% in cv. Duggirala Red (C. longa L.). The essential oil yields from the other five rhizomes were in between these values (Table 1). The oil yields of C. longa L. varieties were higher than those of C. aromatica Salisb.

GC-MS Analysis of Essential Oil

Essential oils of seven cultivars of Curcuma spp. were subjected to GC-MS analysis, and the results from one of such studies for each cultivar are presented in this article. The representative TIC chromatograms of these cultivars are shown in Figure 1. Several compounds were detected in each cultivar’s essential oil (Table 1). Only a few of the identified compounds were confirmed based on their match with the compound profiles found in the NIST database and Leco-Fiehn Rtx5 library. Up to 44 compounds were identified from the five cvs. of C. longa L. and 31 compounds from two cvs. of C. aromatica Salisb. (Table 1). Altogether 80 compounds were grouped into three categories: cultivar-specific (41), present in more than one cultivar (26), and common in all seven cultivars (13). These 41 cultivar-specific compounds were detected in the essential oil of one of the cultivars of C. longa L. or C. aromatica Salisb. (Table 2). The essential oil of C. longa L. cv. Prathibha had the highest number of cultivar-specific compounds, whereas C. aromatica Salisb. had the highest number of mass fragmentation patterns.
### TABLE 3

Compounds detected in more than one cultivar of *C. longa* L. and *C. aromatica* Salisb. identified by GCMS in the essential oil from rhizomes. The structures of the compounds from serial numbers 1–12 are given in Figure 2 (panel numbers: 24–35), and the compounds with Sl.No. 13–18 are shown in Figure 3, with corresponding panel numbers: 8–13 respectively. Abbreviations used: AS, Alleppey Supreme; DR, Duggirala Red; PR, Prathibha; SA, Salem; SU, Suguna; KAR, Kasturi Araku; KAV, Kasturi Avidi.

| Sl. No. | Compound name | Cultivar | RT (Min) | Area/abundance | Formula | Mass (m/z) | Mass fragment ions | Class of compound | Reported from plant species | References |
|---------|---------------|----------|----------|----------------|---------|------------|-------------------|-------------------|-----------------------------|------------|
| 1       | 1,3,5-Cycloheptatriene | AS, PR, SA, SU, KAR, KAV | 2.16 | 6243556 | 1,3,5-C6H8 | 92.0626 | 65,91 | Closed ring organic compound | *Cereogia wooldi* Schltr | Meng et al. (2010) |
| 2       | Bicyclo(3.1.0)hexane, 4-methyl-1-(1-methyl) | DR, PR, SA, SU, KAR, KAV | 5.70 | 89775296 | C10H16 | 136.1252 | 41, 77, 93 | Monoterpenes | *Zingiber officinale* Roscoe | Tang et al. (2012) |
| 3       | Bicyclo(3.2.0)oct-2-ene, 3-methyl-4-methylene- | DR, KAR, KAV | 9.35 | 559966 | C10H16 | 134.1096 | 91, 105, 134 | Monoterpenes | *Sesel duxialium* C.B. Clarke | Mohiuddin et al. (2012) |
| 4       | Oxirane, 2-(hexyn-1-yl)-3-methoxymethylene- | DR, KAR, KAV | 9.75 | 71555 | C10H14O2 | 166.0994 | 79, 110 | Cyclic ether and epoxide | *Hypotis spicigera* Lam | Ladan et al. (2011) |
| 5       | Bergamotol, Z-α-trans- | AS, SA | 20.90 | 3926774 | C15H24O | 220.182 | 91, 93, 119, 187 | Sesquiterpene | *Aristolochia chinensis* (Panigrahi) Press | Kumar et al. (2019) |
| 6       | (1,3-Dimethyl-2-methylene-cyclopropyl) methanol | AS, DR, SA, SU, KAR, KAV | 21.05 | 2503789 | C10H16 | 140.1201 | 67, 77, 94, 109 | Alcohol | *Hypotis spicigera* Lam | Ladan et al. (2011) |
| 7       | 12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-[1R*,3E,7E,11R*]- | DR, KAR, KAV | 21.67 | 2130448 | C15H24O | 220.1827 | 67, 96, 109, 128 | Epoxide | *Eugenia Caryophyllus* (Spredy.) Bullock & S.G. Harrison | Mani and Boominathan (2011) |
| 8       | Isolongifolene, 4,5,9,10-dehydro- | AS, DR, SA, SU, KAR, KAV | 22.10 | 350003 | C15H20 | 200.1565 | 77, 91, 143, 157, 185 | Polycyclic hydrocarbon | *Cymbopogon citratus* (DC.) Stapf | Tajdin (2012) |
| 9       | Z,Z,Z-4,6,9-Nonadecatriene | DR, KAR, KAV | 22.33 | 16921575 | C34H19 | 262.2661 | 79, 93 | Hydrocarbon | *Papaver somniferum* L. | Kumaravel el al. (2019) |
| 10      | 6-β-Tolyll-2-methyl-2-heptanol | AS, SU, KAR, KAV | 22.98 | 6040656 | C15H22O | 218.182 | 91, 119, 202 | Aromatic alcohol | *Zingiber officinale* Roscoe | Choudhari and Kereppa (2013) |
| 11      | 6-Tridecen-4-yne, (Z)- | DR, KAR, KAV | 23.14 | 1539997 | C13H22 | 178.172 | 43, 79, 94 | Hydrocarbon | *Ambrosia trifida* L. | Wang et al. (2005) |
| 12      | 1,4-Cyclohexadiene, 1-methyl- | AS, PR, SA, SU, KAR, KAV | 4.56 | 930505 | C7H10 | 94.0783 | 55, 79, 94 | Monoterpenes | *Curcuma longa* L. | Usman et al. (2012) |
| 13      | α-Phellandrene | AS, DR, SA, SU, KAR, KAV | 5.72 | 26453570 | C10H16 | 136.125 | 77, 93 | Monoterpenes | *Curcuma longa* L. | Choudhury et al. (2008a) |
| 14      | Limonene | AS, SU, KAR, KAV | 6.27 | 20198837 | C10H16 | 136.125 | 68, 93 | Monoterpenes | *Curcuma longa* L. | Singh et al. (2010) |
| 15      | α-Terpinenol | AS, PR, SA, SU, KAR, KAV | 11.25 | 35019844 | C10H16O | 154.135 | 59 | Monoterpenoid | *Curcuma longa* L. | Gopalan et al. (2000) |
| 16      | β-Sesquiphellandren | AS, DR, KAR, KAV | 20.73 | 96269070 | C10H16 | 204.187 | 43, 79, 94 | Monoterpenes | *Curcuma longa* L. | Choudhury et al. (2008a) |
| 17      | Nerolidol | DR, KAR, KAV | 21.79 | 15626126 | C10H16O | 222.198 | 69, 93 | Sesquiterpene | *Curcuma longa* L. | Awasti and Dixit (2009) |
| 18      | Bicyclo(4.1.0)hept-2-ene, 3,7,7-trimethyl- | DR, KAR, KAV | 5.63 | 227551 | C10H16 | 136.125 | 93, 121 | Monoterpenes | *Curcuma longa* L. | Choudhury et al. (2008b) |
| 19      | α-Terpine | AS, DR, SA, SU, KAR, KAV | 6.00 | 28366949 | C10H16 | 136.125 | 93, 121, 136 | Monoterpenes | *Curcuma longa* L. | Choudhury et al. (2008a) |
| 20      | cis-Ocimene | DR, KAR, KAV | 6.74 | 466438 | C10H16 | 136.125 | 41, 93 | Monoterpenes | *Curcuma longa* L. | Usman et al. (2009) |
| 21      | γ-Terpine | AS, DR, SA, KAR, KAV | 7.01 | 26650014 | C10H16 | 136.125 | 93, 119, 136 | Monoterpenes | *Curcuma longa* L. | Usman et al. (2009) |
| 22      | Linalool | AS, DR, SU, KAR, KAV | 8.15 | 500594 | C10H16O | 154.135 | 71, 93, 121 | Alcohol | *Curcuma longa* L. | Leela et al. (2002) |

(Continued on following page)
TABLE 3 | (Continued) Compounds detected in more than one cultivar of *C. longa* L. and *C. aromatica* Salisb. identified by GC-MS in the essential oil from rhizomes. The structures of the compounds from serial numbers 1–12 are given in Figure 2 (panel numbers: 24–35), and the compounds with Sl.No. 13–18 are shown in Figure 3, with corresponding panel numbers: 8–13 respectively. Abbreviations used: AS, Alleppey Supreme; DR, Duggirala Red; PR, Prathibha; SA, Salem; SU, Suguna; KAr, Kasturi Araku; KAv, Kasturi Avidi.

| Sl No. | Compound name | Cultivar | RT (Min) | Area/abundance | Formula | Mass | Mass fragment ions | Class of compound | Reported from plant species | References |
|-------|---------------|----------|----------|----------------|---------|------|-------------------|---------------------|--------------------------------|-------------|
| 24    | Terpinene-4-ol | AS, DR, PR, SA, SU | 10.83 | 2557284 | C_{10}H_{18}O | 154.135 | 73, 94, 154 | Monoterpene | *Curcuma longa* L. | Singh et al. (2010) |
| 25    | cis-α-A Bisabolene | PR, KAr | 20.40 | 11244271 | C_{15}H_{24} | 204.187 | 67, 93, 161, 204 | Sesquiterpene | *Curcuma longa* L. | Chowdhury et al. (2008a) |
| 26    | Ar-Tumerone | DR, SA, KAv | 25.87 | 328137262 | C_{15}H_{22}O | 216.151 | 83, 119, 173, 216 | Sesquiterpene | *Curcuma longa* L. | Chowdhury et al. (2008a) |

TABLE 4 | Compounds common in the seven cultivars of *Curcuma* spp detected by GC-MS in essential oil obtained from rhizomes. The structures of the two compounds with serial numbers 10 and 13 are given in Figure 3 (panel numbers: 14–15 respectively).

| Sl No. | Compound name | RT (Min) | Area/abundance | Formula | Mass | Mass fragment ions | Class of compound | Reported from plant species | References |
|-------|---------------|----------|----------------|---------|------|-------------------|---------------------|--------------------------------|-------------|
| 1     | α-Thujene     | 4.13 | 9407392 | C_{10}H_{16} | 136.125 | 93, 136 | Monoterpenoid | *Curcuma longa* L. | Raina et al. (2005) |
| 2     | 1s-α-Pinene   | 4.27 | 122103688 | C_{10}H_{16} | 136.125 | 39, 41, 93 | Monoterpenoid | *Curcuma longa* L. | Singh et al. (2002) |
| 3     | Sabinene      | 5.05 | 3901899 | C_{10}H_{16} | 136.125 | 93, 136 | Monoterpenoid | *Curcuma longa* L., leaves | Behura et al. (2002) |
| 4     | β or m-Cymene | 6.20 | 338719033 | C_{10}H_{14} | 134.109 | 65, 91, 119 | Aromatic hydrocarbon | *Curcuma longa* L. | Singh et al. (2002) |
| 5     | Terpinolene   | 7.82 | 225168296 | C_{10}H_{16} | 136.125 | 93, 121 | Monoterpenoid | *Curcuma longa* L. | Leela et al. (2002) |
| 6     | trans-α-Bergamotene | 18.86 | 2365810 | C_{15}H_{24} | 204.187 | 69, 93, 119, 161 | Sesquiterpene | *Curcuma longa* L., leaves | Behura et al. (2002) |
| 7     | α-Caryophyllene | 19.23 | 6097261 | C_{10}H_{16} | 136.125 | 93, 121 | Sesquiterpene | *Curcuma longa* L. | Raina et al. (2005) |
| 8     | trans-β-Farnesene | 19.37 | 211225438 | C_{15}H_{24} | 204.187 | 69, 93, 133 | Sesquiterpene | *Curcuma longa* L. | Singh et al. (2002) |
| 9     | Ar-Curcumene  | 19.88 | 482956678 | C_{15}H_{22} | 202.172 | 132, 202 | Sesquiterpene | *Curcuma longa* L. | Singh et al. (2002) |
| 10    | α-Zingiberene | 20.25 | 1117738917 | C_{15}H_{24} | 204.187 | 69, 93, 119, 204 | Sesquiterpene | *Curcuma longa* L. | Chowdhury et al. (2008a) |
| 11    | Tumerone      | 22.16 | 67277186 | C_{15}H_{22}O | 218.167 | 83, 157 | Sesquiterpene | *Curcuma longa* L. | Singh et al. (2002) |
| 12    | Curlone       | 27.34 | 436064258 | C_{15}H_{22}O | 218.167 | 83, 120, 218 | Sesquiterpene | *Curcuma longa* L. | Leela et al. (2002) |
| 13    | 2-Heptadecanone | 39.17 | 152911 | C_{17}H_{34}O | 254.261 | 43, 55, 71, 125 | Ketone | *Curcuma angustifolia* Roxb | Srivastava et al. (2006) |
FIGURE 4 | Structure of few selected cultivar-specific (panels 3, 5–11 corresponding to serial numbers 2, 3–9 of Table 6) compounds already reported from genus Curcuma in methanolic extract from rhizomes by LCMS analysis of seven cultivars of Curcuma spp.: five of Curcuma longa L. (cvs. Alleppey Supreme, Duggirala Red, Prathibha, Salem, and Suguna) and two of C. aromatica Salisb. (cvs. Kasturi Araku and Kasturi Avidi). (1) Kaempferol-3-rhamnoside, (2) 3-acetyl coumarin, (3) luteolin-7-O-glucoside, (4) turmeronol, (5) 1,7-bis(4-hydroxy-3,5-dimethoxyphenyl)-1,6-heptadiene-3,5-dione, (6) 1,7-bis(3,4-dimethoxyphenyl)-1,6-heptadiene-3,5-dione, (7) (6S)-2-methyl-6-[1R,5S)-(4-methene-5-hydroxyl-2-cyclohexen)-2-hepten-4-one, (8) 1,7-bis(4-hydroxyphenyl)-3,5-heptanediol, (9) 1,7-bis(3,5-diethyl-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione, (10) 1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-1,4-pentadiene-3-one, (11) (-)-(12E,2S,3S,4R, 5R, 9S, 11S, 15R)-3,15-dibenzoyloxy-5,6-epoxylathyri-12-en-14-one, (12) 7-(3,4-dimethoxyphenyl)-5-hydroxy-1-phenyl-(1E)-1-heptene, (13) 1-(3,4-dihydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione, (14) 1-(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one, (15) 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one, (16) 1-heptene-3,5-dione, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one.
TABLE 5 | Total number of compounds detected by LC-MS from the rhizome extract of *C. longa* L. and *C. aromatica* Salisb.

| Sl. No. | Cultivar (Species) | Total number of Metabolites detected | Metabolites identified | Unknown Metabolites |
|---------|-------------------|-------------------------------------|------------------------|-------------------|
| 1       | Alleppey Supreme  (C. longa L.) | 86                                  | 43                     | 43                |
| 2       | Duggirala Red (C. longa L.) | 107                                 | 23                     | 84                |
| 3       | Prathibha (C. longa L.) | 60                                  | 28                     | 32                |
| 4       | Salem (C. longa L.) | 91                                  | 28                     | 63                |
| 5       | Suguna (C. longa L.) | 96                                  | 30                     | 66                |
| 6       | Kasturi Avidi (C. aromatica Salisb.) | 90                                  | 30                     | 60                |
| 7       | Kasturi Araku (C. aromatica Salisb.) | 92                                  | 29                     | 63                |

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Curcuma *longa* quite helpful. TIC chromatograms of all seven cultivars of *Curcuma longa* L. were presented in Supplementary Figure S1B for negative mode and in Supplementary Figure S2 for positive mode. A typical LC-MS analysis of methanolic extracts from the rhizomes of *C. longa* L. cv. Alleppey Supreme revealed the presence of up to 86 compounds. Out of these, 43 were identified, and the remaining 43 compounds remained unknown. The (-) ESI-LC-MS detected 30 known compounds, and the (+) ESI-LC-MS detected 23 known compounds with an overlap of 10 compounds, detected by both negative and positive ion modes. A similar assessment of data was done with all seven cultivars of *Curcuma* spp. (Table 5). Altogether 62 compounds were identified, as presented in Supplementary Table S2. These compounds were grouped into three categories: cultivar-specific, detected in more than one cultivar, and common. There were 23 cultivar-specific compounds present in any one cultivar of *C. longa* L. or *C. aromatica* Salisb. (Table 6). 24 compounds were present in more than one cultivar of *C. longa* L. and/or *C. aromatica* Salisb. (Table 7). The remaining 15 were common in all seven cultivars (Table 8). Of these 15 common compounds found in the LC-MS/MS chromatograms, only one was a “Bisabolane” sesquiterpene (Parthasarathy et al., 2009) and all other 14 were diarylheptanoids. These were identified based on the MS/MS spectra reported by Jiang et al. (2006), including curcumin (CU), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). Among the other diarylheptanoids, 1-[(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one; 1,5-bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one, etc., were common in all the cultivars of *C. longa* L. and *C. aromatica* Salisb. (Table 8). The structure of the five common compounds was given in Figure 4 (panels 13, 14–15, 16, and 17 corresponding to serial numbers 10, 5–6, 15, and 1, respectively, of Table 8). In addition to diarylheptanoids, several other classes (phenolic acids, flavonoids, ketonic sesquiterpenes, and fatty acid derivatives) were also detected in the turmeric rhizomes.

**LC-MS Analysis of Methanol Extracts**

Methanolic extracts of rhizomes from the seven cultivars of *Curcuma* spp. were subjected to LC-MS analysis. The results from one of such analyses for each cultivar are presented in this article. The use of "positive" and "negative" modes of LC-MS was quite helpful. TIC chromatograms of all seven cultivars of *Curcuma longa* L. and *C. aromatica* Salisb. were shown in Supplementary Figure S1A for negative mode and Supplementary Figure S1B for positive mode.

A typical LC-MS analysis of methanolic extracts from rhizomes of *C. longa* L. cv. Alleppey Supreme revealed the presence of up to 86 compounds. Out of these, 43 were identified, and the remaining 43 compounds remained unknown. The (-) ESI-LC-MS detected 30 known compounds, and the (+) ESI-LC-MS detected 23 known compounds with an overlap of 10 compounds, detected by both negative and positive ion modes. A similar assessment of data was done with all seven cultivars of *Curcuma* spp. (Table 5). Altogether 62 compounds were identified, as presented in Supplementary Table S2. These compounds were grouped into three categories: cultivar-specific, detected in more than one cultivar, and common. There were 23 cultivar-specific compounds present in any one cultivar of *C. longa* L. or *C. aromatica* Salisb. (Table 6). 24 compounds were present in more than one cultivar of *C. longa* L. and/or *C. aromatica* Salisb. (Table 7). The remaining 15 were common in all seven cultivars (Table 8). Of these 15 common compounds found in the LC-MS/MS chromatograms, only one was a “Bisabolane” sesquiterpene (Parthasarathy et al., 2009) and all other 14 were diarylheptanoids. These were identified based on the MS/MS spectra reported by Jiang et al. (2006), including curcumin (CU), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). Among the other diarylheptanoids, 1-[(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one; 1,5-bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one, etc., were common in all the cultivars of *C. longa* L. and *C. aromatica* Salisb. (Table 8). The structure of the five common compounds was given in Figure 4 (panels 13, 14–15, 16, and 17 corresponding to serial numbers 10, 5–6, 15, and 1, respectively, of Table 8). In addition to diarylheptanoids, several other classes (phenolic acids, flavonoids, ketonic sesquiterpenes, and fatty acid derivatives) were also detected in the turmeric rhizomes.

**Compounds Reported First Time From the Genus Curcuma Using GC-MS and LC-MS Analysis**

A total of 39 compounds were detected (Figure 2) for the first time from the genus *Curcuma*. Out of these, 35 and 4 compounds were identified respectively in the essential oils and whole rhizome extracts of *C. longa* L. and *C. aromatica* Salisb. by the GC-MS and LC-MS techniques. Details of the compounds, including the class of compound, molecular weight, are given in Tables 2, 3, 5, and 6; structures of all these compounds are shown in Figure 2 (panels: 1–39). The MS and MS/MS spectra of these compounds are presented in Supplementary Figure S2 (panels: 1–39). These compounds were reported earlier from plants belonging to any genus other than *Curcuma*, and this is the first report from genus...
### TABLE 6 | Cultivar-specific compounds identified by LC-MS in the rhizome extracts from one of the seven cultivars of Curcuma longa L. and C. aromatica Salisb. The structure of the compound with the serial number “1” is given in Figure 2 (panel number: 36) and compounds with Sl. Nos. 2–9 are given in Figure 4 with the corresponding panel nos. 3, 5–11, respectively. Abbreviations used: AS, Alleppey Supreme; DR, Duggirala Red; PR, Prathibha; SA, Salem; SU, Suguna; KAr, Kasturi Araku; KAv, Kasturi Avidi.

| Sl. No. | Compound name | Cultivar | RT (Min) | Area/abundance | Formula | Mass (m/z) | Mass fragment ions | Class of compound | Reported from plant species | References |
|--------|---------------|----------|----------|----------------|---------|------------|-------------------|-------------------|-----------------------------|-------------|
| 1      | Kaempferol-3,7-O-dimethyl ether | AS       | 12.77    | 25537          | C_{11}H_{14}O_{6} | 313.0721 M-H | 106; 123; 152; 153 | Flavonoid | Lumnitzera racemosa Willd and Artemisia vulgaris L. | Nikolova (2006); DeSouza et al. (2010) |
| 2      | Luteolin-7-O-glucoside | AS       | 42.2     | 5324           | C_{21}H_{20}O_{11} | 447.2733 M-H | 110; 185; 279; 280 | Flavonoid | Curcuma Zedoaria (Christm.) Roscoe | Mass bank ACCESSION: TY00-0145; Rahmatullah et al. (2012) |
| 3      | 1,7-Bis(4-hydroxy-3,5-dimethoxyphenyl)-1,6-heptadiene-3,5-dione | PR       | 32.9     | 186331         | C_{23}H_{24}O_{8} | 428.2 M-H | 109; 123; 137; 159; 191; 209 | Diarylheptanoid | Curcuma longa L. | Chen et al. (2006) |
| 4      | 1,7-Bis(3,4-dimethoxy phenyl)-1,6-heptadiene-3,5-dione | PR       | 38.3     | 335360         | C_{23}H_{24}O_{6} | 396.2 M-H | 105; 107; 119; 123; 137; 145; 155; 195 | Diarylheptanoid | Curcuma longa L. | Li et al. (2006) |
| 5      | 1,7-Bis(4-hydroxyphenyl)-3,5-heptanediol | KAr      | 22.8     | 8120           | C_{19}H_{24}O_{4} | 316.1 M-H | 106; 107; 119; 120; 121; 147; 148 | Diarylheptanoid | Curcuma longa L. | Li et al. (2011) |
| 6      | 1,7-Bis(3,5-diethyl-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione | KAr      | 60.6     | 129145         | C_{27}H_{32}O_{4} | 420.3 M-H | 106; 107; 119; 120; 121; 147; 148 | Diarylheptanoid | Curcuma longa L. | Ma and Gang (2006) |
| 7      | 1,7-Bis(3,5-diethyl-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione | KAv      | 28.8     | 1418           | C_{19}H_{20}O_{4} | 312.1 M-H | 144; 145; 146; 147; 171; 172; 175; 187; 197; 201; 209; 211; 213; 223; 237; 239; 241; 323 | Aromatic | Curcuma longa L. | Li et al. (2011) |
| 8      | 1-(4-Hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-1,4-pentadiene-3-one | KAv      | 28.8     | 8009           | C_{18}H_{18}O_{4} | 296.1 M-H | 105; 109; 117; 119; 133; 145; 159; 161; 171; 173; 181; 185; 1207; 209; 223; 233; 239; 251; 279 | Diarylheptanoid | Curcuma longa L. | Park and Kim (2002) |
| 9      | (-)-(12E,2S,3S,4R,5R,6R,9S,11S,15R)-3,15-Dibenzoyloxy-5,6-epoxylathyr-12-en-14-one | KAv      | 39.8     | 5683           | C_{34}H_{38}O_{6} | 542.2 M-H | 119; 145; 183; 211; 212; 237 | Diterpenoid | Euphorbia microtactis | Tan et al. (2011) |
| 10     | 5'-methoxycurcumin | PR       | 35.56    | 388655         | C_{22}H_{22}O_{7} | 398.1 M-H | 117; 119; 129; 137; 145; 149; 161; 175; 207 | Diarylheptanoid | Curcuma longa L. | Ravindran (2000) |
| 11     | Methyl-7-methoxycurcumin,4- | SU       | 34.4     | 67927          | C_{11}H_{14}O_{6} | 190.1953 M-H | 115; 116; 117; 119; 120 | Coumarin | none | NIST CAS register No. 2555–28–4 |
| 12     | Hydroferulic acid | KAr      | 16.8     | 13992          | C_{12}H_{14}O_{6} | 195.10 M-H | 109; 121; 122 | Phenolic acid | Curcuma longa L. | Ma and Gang (2006) |
| 13     | 1,2,3,4-Tetraphenylbutane-2,3-diol | KAr      | 11.1     | 3926           | C_{18}H_{22}O_{2} | 394.1 M-H | 112; 129; 133; 180; 207; 243; 247; 263; 269 | Aliphatic diol | none | Pubchem Compound ID: 344369 |
| 14     | 4-Hepten-3-one, 5-hydroxy-1,7-bis(4-hydroxyphenyl)- | PR       | 25.2     | 1418           | C_{10}H_{18}O_{2} | 312.1 M-H | 118; 119; 120; 146; 161 | Diarylheptanoid | Curcuma longa L. | Jang et al. (2006) |
| 15     | 5,7-Dihydrouracil-2-(4-hydroxyphenyl)-chroman-4-one | PR       | 26.8     | 12833          | C_{15}H_{14}O_{4} | 272.0 M-H | 107; 119; 120 | Phenolic acid | none | Chromadex |
| 16     | Tetradecanoic acid/myristic acid | AS       | 29.47    | 2381           | C_{14}H_{28}O_{2} | 228.0 M-H | 128; 130; 143; 155; 158; 182; 183; 184; 210 | Fatty acid | none | NIST CAS 544–63–8 |
| 17     | 1-(4-Hydroxy-3-methoxyphenyl)-7-(4-hydroxy-3,5-dimethoxyphenyl)-4,6-heptadiene-3-one | PR       | 32.0     | 194388         | C_{22}H_{22}O_{6} | 384.1 M-H and M-H | 150; 151; 158; 165 | Diarylheptanoid | Curcuma longa L. | Jang et al. (2006) |
| 18     | Tumerone | AS       | 35.3     | 6120           | C_{12}H_{20}O | 218.0 M-H | 180 | Bisabolane sesquiterpene | Curcuma longa L. | He (2000) | (Continued on following page) |
Out of the total of 62 compounds detected by LC-MS analyses of rhizome extracts, four compounds were reported for the first time from the Curcuma genus. One was cultivar-specific (Table 6; Sl. Nos. 1; Figure 2, panels: 36), and three were present in more than one cultivar (Table 7; Sl. Nos. 1–3). The structures of these first-time reported compounds are given in Figure 2 (panels: 37–39), and their corresponding mass fragmentation spectra are shown in Supplementary Figure S2 (panels: 36–39).

A comprehensive table each for GCMS (Supplementary Table S3) and LCMS (Supplementary Table S4) shows the details of compound identification methods used in the present study for the first-time reported compounds from genus Curcuma and the previous literature. A list of 80 (GC-MS) and 62 (LC-MS) compounds can be seen in Supplementary Tables S5, S6 respectively.

**DISCUSSION**

In one of our previous studies, we reported that the HPLC method could be a valuable tool to differentiate the cultivars of Curcuma spp. based on their curcuminoids content ratios (Kulyal et al., 2016). Curcuminoids play a significant role in food, cosmetics, and medicinal compounds. But there are several other secondary metabolites such as terpenoids (e.g., mono-, sesqui-, di-, tri-, so on), alkenes, aromatic compounds, flavonoids, coumarins, etc. that are responsible for various biological activities. All these secondary metabolites are present in either the volatile essential oil or the nonvolatile fraction of the Curcuma spp. Employing untargeted metabolomics would be the ideal way to identify as many metabolites as possible. Therefore, in the present study, we analyzed these secondary compounds using GC-MS and LC-MS/MS.

**Versatility of GC-MS and LC-MS Techniques to Identify a Large Number of Metabolites**

GC-MS analysis is an appropriate technique for analyzing volatile compounds, whereas LC-MS is for detecting polar compounds, and thus, these two techniques are mutually complementary to each other. In the present study, several of the volatile compounds present in the cultivars of C. longa L. and C. aromatica Salisb. belonging to mono- and sesquiterpenoids were detected by GC-MS (Tables 2–4). On the other hand, LC-MS analysis detected phenolic (Tables 6–8) compounds, including several diarylheptanoids in the methanolic extracts of both C. longa L. and C. aromatica Salisb. (Figure 4). Electrospray ionization (ESI), coupled with LC/MS/MS, turned out to be a powerful tool in metabolite profiling and metabolomics research. Studies on chemical derivatization and quantification of several metabolites in turmeric powders and fresh rhizome extracts by LC-MS or LC-MS/MS were made. But the rapid screening within the cultivars of C. longa L. of fresh turmeric rhizome has not yet been reported. To the best of our knowledge, we were able to record the presence of several metabolites, which were not reported so far in the C. longa L. and C. aromatica Salisb. (Tables 2, 3, 6, 7, and Figure 2), using the available literature search, Metlin library, mass bank, and NIST library.
TABLE 7 | Compounds identified by LC-MS in rhizome extracts of more than one cultivar of Curcuma longa L. and C. aromatica Salisb. The compounds from serial numbers 1–3 are reported first time from the genus Curcuma, the structures of these compounds along with few others are given in Figure 2 (panels: 37–39; panels 1–2, 4, 12 corresponding to serial numbers 4–5, 6, 7). Abbreviations used: AS, Alleppey Supreme; DR, Duggirala Red; PR, Prathibha; SA, Salem; SU, Suguna; KAr, Kasturi Araku; KAv, Kasturi Avidi.

| Sl. No. | Compound name | Cultivar | RT (Min) | Area/abundance | Formula | Mass (m/z) | Mass fragment ions | Class of compound | Reported from plant species | References |
|--------|---------------|----------|----------|----------------|---------|-----------|-------------------|-------------------|-----------------------------|------------|
| 1      | 5,7,8-Trihydroxy-2',5'-dimethoxy-3',4'-methylene dioxyisoflavone | AS, DR, KAv | 2.2      | 51978          | C_{18}H_{18}O_{9} | 377.1059 | M-H 101; 102; 113; 119; 161; 163; 228; 336 | Flavonoid | Terminalia ivorensis A. Chev | Ogundare and Olajuyigbe (2012) |
| 2      | Chavicol      | AS, SU   | 38.83    | 55821          | C_{23}H_{20}O_{5} | 356.1319 | M-H 102; 115 | Terpenoid | Piper betle L. | NIST CAS No: 501–92–8 |
| 3      | Kaempferol-3-O-rutinoside-7-O-glucoside | PR, SA | 33.2     | 3752            | C_{33}H_{40}O_{20} | 755.2655 | M-H 135; 161; 176; 176; 191; 439; 579; 755; 756 | Flavonoid | Lycopersicon esculentum Mill | Le Gall et al. (2003) |
| 4      | Kaempferol-3-rhamnoside | AS, DR | 10.76    | 5367            | C_{21}H_{20}O_{10} | 431.9911 | M-H 125; 142; 146; 150 | Flavonoid | Curcuma xanthorrhiza Roxb | Ruslay et al. (2007) |
| 5      | 3-Acetyl coumarin | AS, PR, SA, SU, KAv | 34.65    | 289624         | C_{11}H_{8}O_{3} | 188.0534 | 115; 116; 117; 118 | Coumarin | None | Pub Chem ID 24852845 |
| 6      | Turmeronol    | AS, DR, SU | 25.36    | 87260          | C_{15}H_{20}O_{2} | 232.1436 | 103; 104; 105 | Bisabolane sesquiterpene | Curcuma longa L. | Ma and Gang (2006) |
| 7      | 7-(3,4-Dihydroxyphenyl)-5-hydroxy-1-phenyl-(1E)-1-heptene | AS, KAv | 35.99    | 16570          | C_{19}H_{22}O_{3} | 298.1 | M-H 119; 133; 143 | Diarylheptanoid | Curcuma xanthorrhiza Roxb | Suriyasakul et al. (1994) |
| 8      | 1,7-Diphenyl-1,6-heptadiene-3,5-dione | AS, SA | 2.49     | 9697            | C_{17}H_{18}O_{2} | 276.0 | 115 | Diarylheptanoid | Synthesized the compound | NIST CAS 3160–35–8 |
| 9      | 1-Hepten-3-one, 5-hydroxy-1,7-bis(3,4-dihydroxyphenyl)- | PR, SA, SU, KAr, KAv | 21.2     | 1660            | C_{19}H_{20}O_{6} | 344.1 | 107; 121; 134; 135; 136; 159; 161; 162; 177 | Diarylheptanoid | Alnus japonica (Thunb.) Steud | Sati et al. (2011) |
| 10     | 4-(p-Hydroxyphenyl)-3-buten-2-one | AS, DR, SA, KAv | 22.16    | 18641          | C_{17}H_{22}O_{2} | 162.0 | 117; 118 | Flavonoid | None | NIST CAS 3160–35–8 |
| 11     | 5-Hydroxy-7-(4-hydroxyphenyl)(-1E)-1-heptene | AS, DR, PR, SA, KAv | 23.11    | 6169            | C_{16}H_{22}O_{4} | 328.1 | 107; 119; 133; 134; 135; 143; 159; 158; 161; 162; 177 | Diarylheptanoid | Curcuma xanthorrhiza Roxb | Suriyasakul et al. (1994) |
| 12     | 1-(4-Hydroxy-3-methoxyphenyl)-7-(4-hydroxy-3,5-dimethoxyphenyl)-4,6-heptadiene-3-one | AS, DR, KAr, KAv | 25.8     | 6408            | C_{19}H_{22}O_{6} | 384.1 | 133; 134; 147; 148; 150; 151; 158; 162; 175; 176; 186; 187; 188; 189; 203; 204; 232 | Diarylheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 13     | 1,5-Bis(3,4-methylenedioxyphenyl)-1,4-pentadien-3-one | SU, KAv | 26.0     | 9161            | C_{19}H_{20}O_{6} | 322.0 | 115; 119; 121; 133; 143; 143; 237; 247; 263; 275 | Diarylheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 14     | 1-Hydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-6-heptan-3,5-dione | AS, KAv | 26.7     | 45589          | C_{20}H_{20}O_{7} | 372.1 | 103; 117; 131; 137; 143; 145; 149; 163; 177 | Diarylheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 15     | 1,7-Bis(4-hydroxyphenyl)-1-heptene-3,5-dione | AS, DR, PR, SA, KAv | 27.4     | 8495            | C_{19}H_{18}O_{4} | 310.1 | 117; 118; 119; 145; 146; 161; 175; 176 | Diarylheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 16     | 1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one | AS, DR, PR, SA, KAv | 30.0     | 7240            | C_{19}H_{16}O_{3} | 292.1 | 115; 117; 119; 120; 143; 145 | Diarylheptanoid | Curcuma longa L. | Li et al. (2011) |
| 17     | 7-(4-Hydroxy-3-methoxyphenyl)-1-(4-hydroxy phenyl)-4,6-heptadien-3-one | AS, KAv | 31.8     | 138675          | C_{20}H_{20}O_{4} | 324.1 | 107; 117; 119; 120; 122; 123; 131; 135; 137; 145; 146; 147; 149; 163; 195; 223 | Diarylheptanoid | Curcuma longa L. | Jiang et al. (2006) |

(Continued on following page)
TABLE 7 | Continued

| Sl. No. | Compound name | Cultivar | RT (min) | Areal abundance (m/z) | Formula | Mass fragment ions | Class of compound | Reported from plant species | References |
|--------|---------------|----------|----------|----------------------|---------|-------------------|-------------------|--------------------------|------------|
| 19     | Coumaran      | AS, DR, SU | 34.36    | 6563 C 8H8O 120.0    | 116.117, 118.119 | Compound | Coumarin            | None | Chromadex         |
| 20     | 5,7-Dihydroxy-4-methylcoumarin | AS, SA, SU | 34.57    | 92188 C 10H8O4 192.17 | 113, 115, 116, 117, 118, 119 | Compound | Coumarin            | None | PubChem CID 5354284 |
| 21     | Curcumol      | AS, DR, SU, KAr | 35.82    | 4972 C 25H28O8 456.3 | 280, 281, 286, 289 | Compound | Diarylheptanoid     | Synthesised the compound | Hahm et al. (2002) |
| 22     | Tumorone      | AS, DR, SU, KAr | 35.67    | 34556 C 15H22O2 234.1 | 105, 107, 109, 117, 123, 125 | Compound | Sesquiterpene       | Curcuma longa L. | Valeton & Zijp (2009) |
| 23     | Oleic acid    | SA, KAr   | 60.2     | 15974 C 18H32O2 282.1 | 168, 257 | Fatty acid         | Oleic acid | None | Ma and Gang (2006) |

**Cultivar Variability Based on Secondary Metabolites**

Based on the presence or absence of metabolites identified by GC-MS and LC-MS analyses, there was a need to authenticate cultivar variability. Thus, the metabolite library can be constructed based on the cultivar-specific and compounds found in more than one cultivar. There are very few reports on cultivar-specific secondary metabolite variation. Out of a total of 142 compounds identified by both GC-MS and LC-MS, only 28 compounds (13 from GCMS and 15 from LCMS) were common (Tables 4, 8) present in all the cultivars of *C. longa* L. and *C. aromatica* Salisb. Ten of 13 common compounds (GCMS) were reported earlier from *C. longa* L. rhizome. Two compounds, namely sabine and α-caryophyllene, were reported from the rhizomes of *C. aromatica*. The remaining one compound, i.e., 2-heptadecanone, was detected for the first time from these two Curcuma species. This compound was earlier reported in the essential oil of *Curcuma angustifolia* Roxb. rhizome (Srivastava et al., 2006).

As per our analyses, 64 compounds (Tables 2, 6) out of 142 compounds were cultivar-specific. Of these 64 compounds, 41 were identified in essential oils by GC-MS (e.g., carvacol, endo-borneol) and 23 (e.g., tumerone, methyl-7-methoxyguaramin, 4-) in fresh rhizome extracts (LC-MS) of any one of the cultivars of *C. longa* L. or *C. aromatica* Salisb. In addition, 50 compounds (Tables 3, 7) were identified to be present in some of the cultivars, present in more than one cultivar but not common to all the cultivars of *C. longa* L. and *C. aromatica* Salisb. Out of these 50 compounds, 26 were identified in essential oils through GC-MS. For example, 1,3,5-cycloheptatriene was detected in all six cultivars except cv. Duggirala Red, whereas 12-oxabicyclo(9.1.0)dodeca-3,7-diene, 1,3,5-cycloheptatriene was detected in all six cultivars except cv. Duggirala Red and Kasturi Araku. The rest 24 compounds were detected in rhizome extracts by LC-MS (e.g., chavicol detected in cvs. Alleppey Supreme and Suguna). The present extensive analyses of both essential oils and whole rhizome secondary metabolome of seven cultivars of *C. longa* L. and *C. aromatica* Salisb. established cultivar variability. Variability of the compounds within or/and in between the cultivars of *C. longa* L. and *C. aromatica* Salisb. will give a better understanding of their selection. The current study will help select cultivars for use in pharmacology or the food industry.

**Discovery of First-Time Reported Metabolites in *C. longa* L. and *C. aromatica* Salisb.**

In the present study, as many as 142 compounds were identified in the essential oils and rhizome extracts of *C. longa* L. and *C. aromatica* Salisb. Out of these, 39 compounds were identified for the first time in the genus *Curcuma*. However, these compounds were found in other plant genera. The structures of these compounds are shown in Figure 2, and corresponding details, including the class of compound, molecular weight, are given in Tables 2, 3, 6, and 7. As an example, cv. Alleppey Supreme of *C. longa* L. showed three cultivar-specific compounds. Among these, 1,2-cyclohexanediol, 1-methyl-4-(1-methylethyl)- (oxygenated
TABLE 8 | Compounds commonly detected by LC-MS analysis of rhizomes extract of all seven cultivars of Curcuma spp.: five of Curcuma longa L. (cvs. Alleppey Supreme, Duggirala Red, Prathibha, Salem, and Suguna) and two of C. aromatica Salisb. (cvs. Kasturi Araku, Kasturi Avidi). The structures for the compounds in the serial numbers 10, 5, 6, 15, and 1 are given in Figure 4 (panels 13, 14, 15, 16, and 17 respectively).

| Sl. No. | Compound name | RT (Min) | Area/abundance | Formula | Mass (m/z) | Mass fragment ions | Class of compound | Reported from plant species | References |
|---------|---------------|----------|----------------|---------|------------|-------------------|-------------------|---------------------------|------------|
| 1       | 1,5-Bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one | 23.5 | 3535 | C_{19}H_{18}O_{5} | 325.1245 | 117; 118; 119; 120; 135; 143; 145; 146; 159; 161; 187 | Diaryheptanoid | Curcuma longa L. | Li et al. (2011) |
| 2       | Ar-Turmerone | 24.7 | 23545 | C_{15}H_{20}O | 216.1 | 103; 104; 105; 106; 107; 108; 115; 116; 117; 118; 119; 120 | Bisabolane sesquiterpene | Curcuma longa L. | Parthasarathy and Chempakam (2008) |
| 3       | Tetrahydroxybisdemethoxycurcumin | 25.4 | 4773 | C_{19}H_{20}O_{4} | 311.1446 | 117; 118; 119; 120 | Diaryheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 4       | Tetrahydrodimerhoxycurcumin | 25.9 | 7734 | C_{20}H_{22}O_{5} | 341.1272 | 101; 113; 119 | Diaryheptanoid | Piper nigrum L. | Jiang et al. (2006) |
| 5       | 1-(4-Hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one | 26.1 | 76458 | C_{20}H_{18}O_{4} | 321.0938 | 115; 117; 119; 121; 132; 133; 134; 143; 145; 174; 235; 237; 247; 263/264; 274 | Diaryheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 6       | 1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one | 26.3 | 64172 | C_{21}H_{20}O_{5} | 351.1123 | 108; 115; 119; 136; 143; 148; 164; 195; 207; 223; 224; 235; 245; 251; 261; 262; 263; 279; 291; 307 | Diaryheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 7       | Tetrahydroxycurcumin | 26.5 | 81260 | C_{20}H_{22}O_{4} | 371.1686 | 108; 115; 119; 136; 143; 148; 164; 195; 207; 223; 224; 235; 245; 251; 261; 262; 263; 279; 291; 307 | Diaryheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 8       | 1-(4-Hydroxy-3-methoxyphenyl)-7-(4-hydroxy-3,5-dimethoxyphenyl)-1,4,6-heptatrien-3-one | 26.8 | 58571 | C_{22}H_{22}O_{6} | 382.1 | 149; 159; 173; 197; 208; 211; 221; 233; 237; 239; 249; 261; 267; 277; 289; 293; 295; 305; 309 | Diaryheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 9       | 1,6-Heptadiene-3,5-dione, 1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one | 31.6 | 5753 | C_{19}H_{18}O_{5} | 324.1 | 134; 135; 136; 143 | Diaryheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 10      | 1,3,4-Dihydroxyphenyl-1,4-di(3,5-dimethoxyphenyl)-1,6-diene-3,5-dione | 32.3 | 16375 | C_{20}H_{18}O_{5} | 353.1212 | 134; 135; 136; 150 | Diaryheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 11      | Bisdemethoxycurcumin | 34.5 | 1675644 | C_{19}H_{18}O_{4} | 307.1132 | 117; 119; 120; 143; 145 | Diaryheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 12      | Demethoxycurcumin | 35.4 | 1302410 | C_{20}H_{18}O_{5} | 337.1251 | 117; 119; 120; 132; 134; 143; 145; 158 | Diaryheptanoid | Curcuma longa L. | Jiang et al. (2006) |
| 13      | Dihydrocurcumin | 35.8 | 5151 | C_{21}H_{22}O_{5} | 369.1533 | 160; 175; 201 | Diaryheptanoid | Curcuma longa L. | Jiang et al. (2006) |

(Continued on following page)
alcoholic monoterpenoid) was earlier reported from leaf and peel essential oil of *Citrus medica* L. (Rutaceae). This compound is used as a flavoring agent (Bhuiyan et al., 2009); trans, trans-octa-2,4-dienyl acetate, present in common Malaysian *Kaempferia galanga* L. (Zingiberaceae), was used for its food-flavoring property (Othman et al., 2006). Phenol, 2-methoxy-3-(2-propenyl)-, an allyl chain-substituted guaiacol was reported from rosewood extracts (Jiang et al., 2018).

The following four compounds were identified from cv. Duggirala Red (*C. longa* L.): 3-isopropyl-4-methyl-1-pentyn-3-ol (alcohol constituent) containing leaf and stem of *Anethum sowa* Roxb. ex, Fleming, used for flavoring of food, beverages and also for many medical preparations (Saleh-e-in et al., 2010); 5,9-tetradecadiyne (unsaturated hydrocarbon) was found to be a major component of *Ferula vescentriensis* Coss. & Durieu ex Trab. leaf essential oil (Zellagui et al., 2012); naphthalene, 5-buty1-1,2,3,4-tetrahydro- (tetralin type of compounds) found in the essential oil of *Meconopsis punicea* Maxim. and *M.delavayi* (Franch.) Franch. Ex Prain (Slavík and Slavíková; Yuan et al., 2003); and santolina alcohol was reported from plant *Achillea filipendulina* Lam. (Sharopov and Setzer, 2010). A total of nine cultivar-specific compounds were detected in cv. Prathibha (*C. longa* L.) and the examples of these compounds and their source plants, respectively, are 7-tetracyclo[6.2.1.0(3,8)0(3,9)]undecan-4,4,11,11-tetramethyl- in *Cyperus articulatus* L. (Metuge et al., 2014); bicyclo(2.2.1)hept-2-ene, 2,3-dimethyl- in *Abies alba* Mill. (Yang et al., 2009). Cultivar-specific compounds of three each were detected in cvs. Salem (*C. longa* L.) and Suguna (*C. longa* L.) (Table 2). In *C. aromatica* Salisb., only one cultivar-specific compound was detected in cv. Kasturi Avidi, i.e., 3-octen-5-yne, 2,7-dimethyl-, (Z)-, and this compound was earlier reported from the medicinally important *Litsea glutinosa* (Lour.) C.B. Rob. fruit essential oil (Chowdhury et al., 2008a).

Some of the compounds identified in our study were present in more than one cultivar. For example, 6-(p-tolyl)-2-methyl-2-heptenol (Table 3) was detected in three cvs.: Alleppey supreme, Suguna of *C. longa* L., and Kasturi Avidi of *C. aromatica* Salisb. This compound was earlier reported from *Zingiber officinale* Roscoe (Zingiberaceae), used as a spice, food products, and beverages (Choudhari and Kareppa, 2013).

Limitations and Strengths of the Present Study

There is significant variability within and between the cultivars of *C. longa* L. and *C. aromatica* Salisb, which can be exploited to differentiate the cultivars of *Curcuma* spp. The feasibility of studies without using any standard compounds was pointed out by Núñez et al. (2020). Similarly, reference compounds were not used in our study to derive arithmetic indices under the experimental conditions. Despite the dilution made in the essential oil sample before injecting into the GC-MS system, the sample was still too concentrated. The high concentration of oil might have restricted the resolution due to overloading the detector. This could be the reason that we could not identify several compounds. We would ensure the further dilution of the oil sample in our future studies. However, the technology employed, GC-TOFMS and LC-QTOFMS, and MS-spectral database/literature search enabled us to establish the cultivar variability of *Curcuma* spp. The detailed information on the metabolite variability within or/and between the cultivars of *C. longa* L. and *C. aromatica* Salisb. may assist us in selecting the cultivars for a specific purpose, like culinary use, coloring, or pharmacological purpose. The studies such as the present one can help to select cultivars, particularly for use in pharmacology or the food industry. Metabolite variability poses a challenge in the use of turmeric in therapy. The practitioners need to be quite careful and use the identified cultivar and avoid mix-up. The caution applies to commercial/industrial use. Once standardized, the protocol should ensure the use of a specific cultivar. Our GC-MS and LC-MS-based metabolite identification is distinct from chemophenetic studies but is a complementary approach to characterize the *Curcuma* metabolome.

Importance of *Curcuma* spp. Metabolites for Human Health

Curcuminoids (CU, DMC, and BDMC) were identified as the main bioactive compounds of genus *Curcuma* and proved to have a broad spectrum of biological activities based on pharmacological studies. However, rhizomes and their essential oils of *Curcuma* spp. contained several other

| Sl. No. | Compound Name | RT (Min) | Area/Abundance | Formula | Mass (m/z) | Class of Compound | Reported from Plant Species | References |
|-------|---------------|---------|----------------|---------|------------|-------------------|-----------------------------|------------|
| 14    | Curcumin      | 36.2    | 1503497        | C21H20O6 | 367.1374   | Diaryheptanoid    | Curcuma longa L.            | Jiang et al. (2008) |
| 15    | 1-Heptene-3,5-dione, 1,7-bis-(4-hydroxy-3-methoxyphenyl)- | 43.2 | 5078           | C20H16O6  | 339.1473 | Diaryheptanoid    | Curcuma longa L.            | Jiang et al. (2008) |

**Table 8** (Continued) Compounds commonly detected by LC-MS analysis of rhizomes extract of all seven cultivars of *Curcuma* spp.: five of *Curcuma longa* L. (cvs. Alleppey Supreme, Duggirala Red, Prathibha, Salem, and Suguna) and two of *C. aromatica* Salisb. (cvs. Kasturi Araku, Kasturi Avidi). The structures for the compounds in the serial numbers 10, 5, 6, 15, and 1 are given in Figure 4 (panels 13, 14, 15, 16, and 17 respectively).
TABLE 9 | Pharmacological activity of metabolites identified, other than major curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxy curcumin), in C. longa L. and C. aromatica Salisb.

| Sl. No. | Compound name | Source plant | Tested Compound/essential oil/extract | Pharmacological activity/health benefit of the compound or compound containing plant product | References |
|---------|---------------|--------------|----------------------------------------|-----------------------------------------------|-------------|
| 1       | Carvacrol     | Curcuma longa L. | Compound                                 | Antibacterial                                | Sunares et al. (2015) |
| 2       | p-Cymene      | Curcuma longa L. | Compound                                 | Antioxidant                                  | De Oliveira et al. (2015) |
| 3       | Eucalyptol    | Curcuma longa L. | Compound                                 | Anti-inflammatory, anticancer, and antimicrobial effects | Marchese et al. (2017) |
| 4       | α-Pinenol     | Curcuma longa L. | Compound                                 | Anti-inflammatory and chondroprotective       | Murata et al. (2013); Islam et al. (2014); De Oliveira et al. (2012) |
| 5       | α-Terpinol    | Curcuma longa L. | Compound                                 | Anti-inflammatory                            | De Oliveira et al. (2012) |
| 6       | Terpinolene   | Curcuma longa L. | Compound                                 | Anticancer                                   | Okumura et al. (2012) |
| 7       | 2-Heptadecanone | Curcuma angustifolia Roxb | Dried rhizome essential oil | As a coolant, demulcent | Srivastava et al. (2006) |
| 8       | Santolina alcohol | Achillea filipendulina Lam | Aerial part essential oil | Traditional herbal medicine | Sharopov and Setzer (2010) |
| 9       | Cyclohexanol, 2-methyl-5-(1-methylethenyl)- | Mentha spicata L. | Aerial parts essential oil | Antifungal | Mohammed et al. (2017) |
| 10      | Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl)- | Rhanterium adpressum Coss. & Durieu | Aerial parts essential oil | Antifungal | Kala et al. (2009) |
| 11      | 4-Ethylphenethylamine | Psidium guajava L. | Stem bark essential oil | Antioxidant | Fasola et al., (2011) |
| 12      | 5,9-Tetradecadiylne | Ferula vescentensis Coss. & Durieu ex Trab | Leaves essential oil | Antibacterial | Zellagui et al. (2012) |
| 13      | E-11-Tetradecenoic acid | Coriandrum sativum L. | Leaf essential oil | Spices, flavoring agent, antimicrobial | Bhuilyan et al. (2009) |
| 14      | 6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl- | Hipptage benghalensis (L.) Kurz | Leaf essential oil | Treatment of skin diseases, cough, asthma, leprosy | Venkataranamini and Chinnagounder (2012) |
| 15      | Chavicol       | Piper betle L. | Leaf oil | Antifungal, antiseptic, and anthelmintic Antibiotic | Niagori et al. (2011) |
| 16      | 1,2-Cyclohexanediol, 1-methyl-4-(1-methylethyl)- | Citrus medica L. | Leaf and peel essential oil | Flavored of food and beverages, antimicrobial, antioxidant | Bhuilyan et al. (2009) |
| 17      | 3-Isopropyl-4-methyl-1-pentyn-3-ol | Anethum sowa Roxb. ex Fleming | Leaf and stem essential oil | Seasonal scavenging activity | Saleh-e-in et al. (2010) |
| 18      | Bicyclo(2.2.1)hept-2-ene, 2,3-dimethyl- | Abies alba Mill | Leaf and twig essential oil | As a food and herbal medicine, mosquito larvicidal activity | Ho (2010) |
| 19      | 2-Norien-4-yn-1-ol, (Z)- | Alpinia speciosa (J.C. Wendl.) K. Schum | Seeds and leaves essential oil | As a food and herbal medicine, mosquito larvicidal activity | Wang et al. (2005) |
| 20      | 8-Methylene-3-octatrienoic acid | Schisandra chinensis (Turcz.) Baill | Dried fruit essential oil | Antioxidant | Wang et al. (2005) |
| 21      | 3-Cyclohexen-1-one, 3,5,5-trimethyl- | Crocus sativus L. | Dried saffron essential oil | Antitumor | Othman et al. (2006) |
| 22      | Ar-Tumerone a-Tumerone β-Tumerone a-Santalayne | Curcuma longa L. | Phyzome essential oil | Antioxidant | Singh et al. (2010) |
| 23      | 7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl- | Oryxus articulatus L. | Roots/rhizome essential oil | Anti-onchocera activity | Metuge et al. (2014) |
| 24      | Cholesta-8,24-dien-3-ol, 4-methyl- | Pankia speciosa Hassk. | Seed essential oil | High nutritional and medicinal value | Salman et al. (2006) |
| 25      | 3-Octen-5-yn, 2,7-dimethyl-, (Z)- | Lizuca glutinosus (Lour.) C.B. Rob | Fruit essential oil | Antirheumatic | Chowdhury et al. (2008b) |
| 26      | 5,8,11,14-Eicosatetraenoic acid, phenylmethyl ester, (all-2)- | Petiveria alliacea L. | Whole plant essential oil | Used as folk medicine to enhance memory and in treatment of common cold, flu, other viral, or bacterial infections | Sathyabalan et al. (2014) |
| 27      | Naphthalene, 5-buty1-2,3,4-tetrahydro- | Mecanopsis punicea Maxim. and M. delavayi (Franch.) Franch. Ex Prian | Whole plant essential oil | As a traditional medicinal plant for anti-inflammatory and analgesic activity | Yuan et al. (2003) |
| 28      | 1H-3a,7-methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(2a,3a,7a,8a)]-trans, trans-Octa-2,4-dienyl acetate | Lindera aggregata (Simiu) Kosterm | Root/tubers essential oil | Treatment of decubitus ulcer | Hong (2011) |
| 29      | | Kaempferia galanga L. | Dried rhizomes | Spice, food-flavoring agent | Othman et al. (2006) |

(Continued on following page)
bioactive (volatile and nonvolatile) compounds. A summary of the pharmacological studies with the metabolites detected in the present study is given in Table 9. Some of the studies demonstrated therapeutic activity with the isolated metabolites, e.g., carvacrol (Suntres et al., 2015), p-cymene (De Oliveira et al., 2015), which are commonly found in essential oils of Curcuma spp. A few other reports correlated anti-inflammatory and antioxidant properties of C. longa L. essential oil with its chemical components ar-tumerone, a-santalene (Singh et al., 2010) (Table 9). Several compounds detected in the present study in the essential oil or rhizome extracts of C. longa L. or C. aromatic Salisb. were also found in the essential oil of other medicinal plants, traditionally used for their health benefits. The examples of such compounds are 5,9-tetradecadiyne, a cultivar-specific compound of Duggirala Red (C. longa L.), earlier reported in Ferula vesceritensis Coss. & Durieu ex Trab. leaf essential oil, exhibiting antibacterial activity; 3-octen-5-yn-1, 2,7-dimethyl-, (Z)-, a hydrocarbon monoterpene, identified from cv. Kasturi Avidi (C. aromatic Salisb.) was earlier reported from fruit essential oil of the medicinally important plant, Litsea glutinosa (Lour.) C.B. Rob. (Chowdhury et al., 2008a). We suggest that the medicinal use of the genus Curcuma can be not only species but also cultivar-specific.

### CONCLUDING REMARKS

Essential oils from spices and aromatic plants are enriched with bioactive metabolites, easily isolated and used, unlike the difficulties encountered with synthetic chemical products. The low mammalian toxicity and biodegradable nature of the natural secondary products provide an attractive option to develop them also for crop protection. Metabolomics is a practical and dynamic approach to make a comprehensive study. Both GC-MS and LC-MS techniques should be used to characterize the metabolite profiles of as many cultivars as possible for building a reference library. Preparative LC can be helpful to collect individual metabolite fractions and establish their identity. Several metabolites detected in 7 selected cultivars of Curcuma spp. by GC-MS and LC-MS analyses are reported first time in Curcuma spp. We suggest that the seven Indian cultivars of Curcuma spp. employed in our study can be used as sources of such compounds. High-throughput analysis of cultivar-specific and first-time detected compounds in the present study may lead to new drug candidates. The metabolites validated for their medicinal or other users can be quantified using simple techniques such as HPLC or TLC to ensure their presence in the herbal preparations.

### DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ebi.ac.uk/metabolights/MTBLS2790.

### AUTHOR CONTRIBUTIONS

ST and AR planned and designed all the experiments. PK and PKK did the experiments and preliminary data interpretation related to GC-MS and LC-MS/MS, respectively. PK, ST, and AR re-analyzed the data and wrote the draft of the manuscript. SA prepared tables and reference search. AA drew the figures and helped in editing the manuscript. All the authors read and approved the final version of the manuscript.
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**SUPPLEMENTARY MATERIAL**
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.659546/full#supplementary-material

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**Supplementary Figure 1** (A) Representative TIC chromatograms from negative ion (-) ESI-HPLC from cultivars (A) Alleppey Supreme, (B) Duggirala Red, (C) Prathibha, (D) Salem, (E) Suguna of Curcuma longa L. and cvs. (F) Kasturi Araku, (G) Kasturi Avidi of C. aromatica Salisb. Peak labelled IS represents internal standard. (B) Representative TIC chromatograms from positive ion (+) ESI-HPLC from cultivars (A) Alleppey Supreme, (B) Duggirala Red, (C) Prathibha, (D) Salem, (E) Suguna of Curcuma longa L. and cvs. (F) Kasturi Araku, (G) Kasturi Avidi of C. aromatica Salisb. Peak labelled IS represents internal standard.

**Supplementary Table 1** A list of secondary metabolites identified by GC-MS in the essential oil from the rhizomes of five cvs. of C. longa L. and two cvs. of C. aromatica Salisb. Abbreviations: AS, Alleppey Supreme; DR, Duggirala Red; PR, Prathibha; SA, Salem; SU, Suguna; KAr, Kasturi Araku; KAv, Kasturi Avidi.

**Supplementary Table 2** A list of secondary metabolites identified by LC-MS in the rhizome extracts of five cvs. of C. longa L. and two cvs. of C. aromatica Salisb. Abbreviations used: AS, Alleppey Supreme; DR, Duggirala Red; PR, Prathibha; SA, Salem; SU, Suguna; KAr, Kasturi Araku; KAv, Kasturi Avidi.

**Supplementary Table 3** Identification parameters of first-time reported compounds by GC-MS in the essential oil from the seven cultivars of Curcuma spp. along with the methods used in previous literature.

**Supplementary Table 4** Identification parameters of first-time reported compounds by LC-MS in the rhizome extracts of genus Curcuma along with the methods used in related previous literature.

**Supplementary Table 5** The list of 80 compounds identified by GCMS analysis.

**Supplementary Table 6** The list of 62 compounds identified by LCMS analysis.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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