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Research Article

Metabolomics using Gas chromatography-mass spectrometry and antibacterial activity of nine *Ocimum* taxa of Dakshin Dinajpur district, West Bengal, India

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Abstract
Ocimum traditionally known as Holy Basil or Tulsi is an available herb plenty across the country. Traditionally, it is used against a number of human diseases. In this present study, the metabolites present in the ethanolic extracts of nine *Ocimum* taxa, *O. tenuiflorum* L. (Green and purple type) two morphotypes of *O. basilicum* L., (Babu and Marua tulsi) two morphotypes of *O. gratissimum* L. (Ram and Ajowan tulsi) and each one from *O. americanum* L. (Bon tulsi), *O.* × *africanum* Lour. (Lebu tulsi), and *O. kilimandscharicum* Guerke.grown naturally in Dakshin Dinajpur district, West Bengal, India were identified using Gas chromatography–mass spectrometry (GC-MS). Among the identified metabolites, carbohydrates, aliphatic alcohols, aliphatic acids, fused ring aromatic hydrocarbon, amino acids, phenolic compounds, quinone, steroids, terpenoids and vitamin E were the chief constituents. The occurrence of these metabolites describes the high biological activity of *Ocimum* species. Furthermore, *in vitro* antibacterial activities were also identified against four bacterial strains, *Staphylococcus aureus* (MTCC 96), *Bacillus cereus* (MTCC 1305), *Proteus vulgaris* (MTCC 1771) and *Escherichia coli* (MTCC 2939). Tested bacterial strains were taken from the Institute of Microbial Technology, Chandigarh, India The results indicated that the ethanolic extracts of all the nine *Ocimum* taxa have satisfactory minimum inhibitory concentration (MIC) values against the tested microorganisms. *O. tenuiflorum* has the highest (91.03 %) metabolic content whereas *O.* × *africanum* has the minimum. This would definitely serve as a scientific basis of the traditional use of basil against human ailments.

Keywords: Antibacterial activity, Ethanolic extracts, GC-MS, MIC, *Ocimum*

INTRODUCTION

The use of Basil (Tulsi) against human diseases traced back from the period of Rigveda (*ca*. 1500 BC) and has mythological significance in Hindu rituals. *Ocimum* is one of the most versatile genuses of medicinal and strongly aromatic plants available plenty in the tropical and subtropical region of Asia and central South America, comprises annual or perennial, much branched herb and shrub, native to the subtropical region of Asia (Labra *et al*., 2004). So far in India about nine species of *Ocimum* have been reported (Rana amd Blazquez, 2015). Traditionally *Ocimum* species have been used for the treatment of common cold and cough, fever,

bronchitis, asthma, tuberculosis, earache, dysentery, ringworm infection and memory enhancer (Vidyarthi *et al*., 2013; Chowdhury *et al*., 2016, Chowdhury *et al*., 2019) and are well documented in Ayurveda. Reports have claimed that *Ocimum* extracts have antibacterial, antifungal (Vieira *et al*., 2014; Chaturvedi *et al*., 2018; Chintaluri and Komarraju, 2019) insecticidal and most interestingly insect (against mosquitoes) repellent properties (Bhavya *et al*., 2018; Benelli *et al*., 2019; Lim *et al*., 2019). Additionally, *O. sanctum* has adaptogenic, antidiabetic, anticancer and anti-inflammatory, anti-stress, anti-carcinogenic, hepato-protective, radio-protective, neuro-protective, cardio-protective and immunomodulatory effects (De Lima *et al*., 2014; Singh *et al*., 2017; Singh and Chaudhuri, 2018).

Recently, research in *Ocimum* has accelerated after finding its HIV-1 reverse transcriptase inhibitory activity (Sonar *et al*., 2017) and decrease platelets aggregation induced by ADP and thrombin (Tohti *et al*., 2006). Fresh or dried Basil leaves can be used as a spice and in different culinary applications. It is an important source of essential oils which are broadly used by perfume and cosmetic industries like body spray, hair dressings, soaps, dental creams, mouth washes, flavouring beverages, food preservative and in pharmaceuticals. It is also a very good source of proteins, carbohydrates, minerals, fat, fiber, mucilage, pigments and moderately high concentration of vitamins such as A, C, E and K (Singh and Chaudhuri, 2018; Zahran *et al*., 2020).

Plants' extracts have always been a better choice against human ailments. However, the advent of modern synthetic drugs has superseded the place. The indiscriminate use of antimicrobial agents has resulted in the emergence of a number of drug-resistant bacteria and fungi. Advanced antimicrobial agents with novel mode of biochemical action must be developed to overcome the increasing risk of resistant pathogenic microbes. As natural products have an imprint of biological structural space, they may be the best alternative in future against synthetic drugs. Frequent studies have revealed that different *Ocimum* essential oils are active against several bacteria as well as against yeasts and fungi (Stanojevic *et al*. 2017; Chintaluri and Komarraju, 2019; Vieira *et al*., 2014; Mohr *et al*., 2017). Diets rich in selected natural antioxidants such as polyphenols, flavonoids, vitamin C and E are related to reducing cardiovascular risk, other chronic diseases and certain types of cancer. This leads to the revival of interest to intake plants-based dietary supplement which serves as an alternative source of vitamins, minerals and natural antioxidants. In addition, people nowadays prefer organic cultivation and natural food additives, hence naturally derived antimicrobial agents such as Basil are becoming more important in antimicrobial packaging as they present a perceived lower risk to consumers (Sappakul *et al*., 2003; Nguefack *et al*., 2009).

Most of the earlier workers from India and abroad have shown the variation of metabolites (phenylpropanoids and terpenes) of different *Ocimum* species and their varieties (Mondello *et al*., 2002; Padalia and Verma, 2011; Verma *et al*., 2013; Verma *et al*., 2016). This may lead to exclusion or increase/decrease in the quantity of some important molecules such as carbohydrates, amino acids and vitamins in the extracts of the natural population of *Ocimum* species under the influence of local ecology. A complete study of the metabolites of Basil extracts from its natural habitat under a given ecological condition and their antimicrobial activities are therefore an unrest demand in the contemporary literature. The present work is reporting the detail identification and quantitation of metabolites present in the ethanolic extracts of *Ocimum* using GC-MS and antibacterial activities (MIC values) of nine species grown naturally in Dakshin Dinajpur district, West Bengal, India.

MATERIALS AND METHODS

Plant materials

After an extensive survey, the fresh mature leaves of nine taxa of tulsi (*Ocimum* sp.) were collected from various places of Dakshin Dinajpur, West Bengal, India, during flowering stage in August-September, 2019. Two morphotypes of *O. tenuiflorum* L. (Green and purple type) two morphotypes of *O. basilicum* L. (/Babu and Marua tulsi) two morphotypes of *O. gratissimum* L. (Ram and Ajowan tulsi) and one each from *O. americanum* L. (Bon tulsi), *O.* × *africanum* Lour. (Lebu tulsi), and *O. kilimandscharicum* Guerke. (Karpur tulsi) were taken for this study. All the specimens were identified by the Botanical Survey of India, Kolkata. Voucher specimens of each taxa was submitted in the North Bengal University herbarium at Department of Botany, West Bengal, India.

Preparation of plant ethanolic extracts

The harvested leaves of different samples were dried under shade and ground to powder in a grinder. Extraction was performed at room temperature in closed vials using ethanol as the solvent for 7 days. Ethanol was recovered under reduced pressure by a rotary evaporator (Buchi RotavaporR-3; Buchi Labortechnik AG, Flawil, Switzerland) at 45 °C. Yellow-greenish ethanolic extracts were lyophilized and kept in vials at 4 °C.

Gas chromatograph - Mass spectrometry (GC-MS) analysis

The chemical compositions of the nine ethanolic extracts of *Ocimum* taxa were identified using GC–MS followed by NIST library search. Each sample was lyophilised and suspended in 100 mL of methanol. 100 µL of this diluted sample was completely dried by pass-

ing nitrogen gas. All samples were derivatised using 30 µL pyridine and 50 µL BSTFA:TMCS (99:1) and incubated for 60 min at 60 °C. GC analyses were carried out on the derivatised samples in an Agilent system 7890A equipped with a DB 5 MS capillary column (30 mL x 0.25 mm ID x 0.25 µm film thicknesses dimension). The helium flow rate was maintained at 1.0 mL/min. The initial column temperature was maintained at 70 °C with 2 min hold time. Then ramp the temperature to 150 °C at the rate of 5 °C/min and again to 280 °C at the rate of 3 °C/min with 2 min hold time and finally to 320 °C temperature at the rate of 10 °C with 3 min hold time. 1.0 µL of the sample was subjected to GC-MS using the split mode (split ratio 10:1). The GC-MS analysis was done on the Agilent system 5975CMSD (Mass selective detector). Ionization for MS was Electron Impact Ionization with ionization energy of 70 eV and mass analyzer was single quadrupole. Mass spectra scan range was from 30 *m/z* – 600 *m/z* with +ve polarity. The interface temperature was set at 310 ^{o}C, source temperature at 250 ^{o}C and quadruple temperature at 150 ^{o}C. Prior to the acquisition, MSD was auto tuned with FC43 (perfluorotributylamine). The GC-MS data was deconvoluted using AMDIS v2.7 (Automated Mass Spectral Deconvolution and Identification System) software. AMDIS extracts spectral data from co-eluting compounds by performing noise reduction and background subtraction. The deconvoluted spectra were then compared with the spectra available in the National Institute Standard and Technology (NIST 2011) library to identify the compounds. Relative percent of metabolites in a mixture was calculated by dividing peak area of the metabolite to the total peak area of all metabolites and multiply the result by 100.

Test microorganisms

The antibacterial activity was evaluated against four microorganisms including Gram-positive *Bacillus cereus* (MTCC 1305), *Staphylococcus aureus* (MTCC 96) and Gram-negative *Proteus vulgaris* (MTCC 1771) and *Escherichia coli* (MTCC 2939). All the bacterial strains were obtained from the Institute of Microbial Technology, Chandigarh, India. Bacterial strains were maintained on nutrient agar (HiMedia, Mumbai, India) slants and the cultures were stored at 4 °C with a subculture period of 30 days.

Determination of antibacterial activity by disc diffusion method

In vitro antibacterial activity of ethanolic extracts of nine *Ocimum* taxa were studied against two Gram-positive and two Gram-negative bacterial strains by agar disc diffusion method according to the protocol by National Committee for Clinical Laboratory Standards, 1997 (Patel 2016). The nutrient agar (HiMedia Laboratories Limited, Mumbai, India) was autoclaved at 121 °C and 1 atm for 30 minutes. The sterile nutrient media was kept away maintaining the temperature at 45-50 °C, after that 100 µL of bacterial suspension containing 10^8 colony-forming units (CFU)/mL were mixed with sterile liquid nutrient agar and poured into the sterile petri dishes. Upon solidification of the media, filter discs (5 mm diameter) were individually soaked with different concentration (25, 50, 75 and 100 mg/mL) of each *Ocimum* extract and placed on the solidified nutrient agar media plates. The different concentrations were made by dissolving the lyophilized extracts in 10% dimethyl sulfoxide (DMSO). Throughout the experiment, solvent control (DMSO) was used that showed no inhibition. The plates were incubated for 24 hours at 37 °C. The diameter of the zone of inhibition (including disc diameter of 5 mm) was measured with a scale. Each experiment was done thrice to minimize any experimental error and the mean values were taken.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was measured by Broth Micro dilution susceptibility method. A serial dilution of plant extracts was made in Nutrient broth medium. Then 1 mL of standard (0.5 McFarland) bacterial suspension was inoculated into each of these tubes. A similar nutrient broth tube without plant extract was also inoculated and used as a negative control. The tubes were incubated at 37 °C for 24 hours. The lowest concentration of plant extract, which inhibited bacterial growth was considered as minimum inhibitory concentration. Final confirmation was done by streaking on nutrient agar medium.

RESULTS AND DISCUSSION

Chemical composition

A detail analyses of GC-MS data (Fig. 1; Table 1) showed that in the ethanolic extracts of nine naturally grown *Ocimum* taxa, carbohydrates were the major components for *O. tenuiflorum* (Krishna tulsi), *O. americanum* (Bon tulsi), *O. gratissimum* (Ram tulsi), *O. basilicum* (Marua tulsi) and *O.* × *africanum* (Lebu tulsi) representing 91.03%, 77.71%, 51.31%, 43.06% and 38.77% of the total metabolites present in the extracts respectively. α-D-glucose was found to be the highest carbohydrate in *O.* × *africanum* and *O. americanum* with natural abundance of 17.23% and 55.32% respectively whereas in Krishna tulsi (*O. tenuiflorum*) fructose was the main carbohydrate with 27.24% natural abundance. However *O. gratissimum* (Ram tulsi) and *O. basilicum* (Marua tulsi) had β-D-glucose 17.76% and 26.98% respectively. This anomeric difference in the carbohydrate content was remarkable, it might be due to any dissimilarity in biological structure space at enzymes from where it is being synthesized. The higher

Table 1. Relative quantitation of metabolites present in different *Ocimum* taxa from GC-MS analyses.

Compounds	Relative percentage composition								
	OG	OG1	OxA	OA	OB	OB1	OTP	OTG	OK
D-Allofuranose	-	-	-	-	-	-	0.41	-	-
β-D-allopyranose	-	-	-	0.24	-	-	-	-	-
Arabinitol	-	-	0.01	0.04	2.22	-	-	0.01	-
D-Arabinopyranose	-	-	-	-	-	-	0.06	-	-
L-(-)-Arabitol	-	-	-	-	-	-	0.68	-	-
Fructose	5.08	3.47	3.56	3.65	-	4.2	27.24	-	-
Furanone	-	-	-	-	-	-	0.03	-	-
D-galactose	-	-	-	-	-	0.56	0.04	-	2.7
D-gluconic acid	-	-	-	0.2	-	-	0.36	-	-
α-D-Glucose	-	-	17.23	55.32	-	-	22.15	0.02	-
β-D-glucose	26.98	16.14	4.23	4.29	-	17.76	2.65	0.73	2.78
D-Mannitol	-	-	-	0.49	-	-	-	-	1.53
α-D-(+)-Mannose	-	-	13.75	0.5	-	14	0.24	0.22	-
Myo-Inositol	0.01	-	-	0.3	-	0.18	1.16	-	-
D-Psicose	-	-	-	4.04	-	-	-	-	-
D-Sorbitol	-	-	-	-	-	-	0.55	-	-
L-(-)-Sorbose	5.9	-	-	6.91	-	-	9.1	-	-
Sucrose	-	-	-	-	-	1	5.65	-	-
α-D-(-)-Tagatose	-	3.46	-	-	-	4.95	19.02	-	-
D-(+)-Talofuranose	-	-	-	-	-	-	0.37	-	-
α-D-(+)-Talose	13.35	-	-	-	-	0.14	1.31	-	-
L-Threitol	-	0.34	-	1.73	-	-	-	-	0.21
Xylitol	-	-	-	-	-	0.26	-	-	1.75
L-Alanine	-	-	-	-	-	-	0.25	-	-
L-Norleucine	-	-	-	-	-	-	0.01	-	-
L-Proline	-	-	-	0.74	-	-	1.23	-	-
L-Homoproline	-	-	-	-	-	-	-	0.05	-
L-Threonic acid	-	-	-	-	-	-	0.14	-	-
L-Threonine	-	-	-	-	-	-	0.08	-	-
L-Valine	-	-	-	0.08	-	-	-	-	-
Glycerol	0.48	0.07	10.87	7.36	15.42	7.17	5.45	21.84	53.29
Hexadecenol	7.1	-	10.06	3.87	12.76	4.62	-	9.94	6.3
Meso-Erythritol	-	-	0.74	-	5.27	0.48	-	0.14	5.13
Acetic acid	-	-	-	-	-	0.74	-	-	-
Butanedioic acid	0.72	0.3	-	0.36	-	-	0.27	0.09	-
Butanoic acid	-	-	-	-	-	-	0.03	-	-
Dodecanoic acid	-	-	-	-	-	0.37	-	-	-
Eicosanoic acid	-	-	-	0.04	-	-	-	0.93	-
Hexadecanoic acid	10.37	6.87	6.8	0.39	29.64	13.46	0.34	15.01	0.69
Linoleic acid	0.13	-	2.14	-	2.39	-	-	2.56	0.45
α-Linolenic acid	0.94	7.05	14.47	2.94	12.64	3.80	-	12.63	7.78
Octadecanoic acid	1.92	0.48	2.49	1.55	6.26	2.83	0.07	8.62	2.08
n-Pentadecanoic acid	-	-	-	-	-	0.12	-	-	-
Propanedioic acid	-	-	-	-	-	-	0.11	-	-

Contd……..

Table 1. Contd……..

Propanoic acid	0.04	1.77	1.77	1.05	1.93	0.02	0.5	0.05	1.42
Benzoic acid	-	-	-	0.14	-	0.19	0.01	-	-
Cinnamic acid	0.21	-	-	-	-	-	-	-	-
Naphthalene	-	-	-	0.07	-	-	-	-	-
Tert-Butylhydroquinone	-	0.09	-	-	-	-	-	-	-
Caffeic acid	-	0.21	-	-	-	-	-	-	-
Catechol	-	-	-	-	-	-	0.08	-	-
Eugenol	12.46	-	-	-	-	-	-	8.61	-
Methyl eugenol	-	-	-	-	-	15.53	-	-	-
Vanillin	0.71	-	-	-	-	-	-	-	-
α-Tocopherol	2.39	1.4	0.4	0.16	-	1.01	-	0.5	0.44
Ergosterol	-	-	0.88	-	-	0.65	-	1.26	-
Stigmasterol	0.88	2.29	1.51	-	-	0.79	-	3.59	-
β-Sitosterol	2.7	3.36	2.5	0.99	3.62	2.55	-	4.53	6.31
α-Amyrin	1.42	-	-	-	-	0.79	-	-	-
Carvacrol	-	0.54	-	-	-	-	-	-	-
Caryophyllene oxide	1.36	-	-	-	-	-	-	0.48	-
β-Elemene	-	-	-	-	-	-	-	1.15	-
Germacrene D	-	-	-	-	-	-	-	0.01	-
Norpinene	-	-	-	-	-	0.01	-	-	-
Phytol	-	14.68	2.94	-	-	0.54	-	-	-
α-Selinene	-	-	-	-	-	-	-	0.43	-
β-Selinene	-	-	-	-	-	-	-	0.64	-
Squalene (Precursor)	1.69	4.78	-	-	-	-	-	-	-
Tau-Cadinol	-	-	-	-	-	0.36	-	-	-
Thymol	-	29.8	0.02	-	-	-	-	0.69	-
Others	3.16	2.91	3.79	2.55	7.83	0.91	0.43	5.28	7.14

OG- *O. gratissimum* (Ram tulsi), OG_1- *O. gratissimum* (Ajowan tulsi), O×A- *O.* × *africanum* (Lebu tulsi), OA- *O. americanum* (Bon tulsi), OB- *O. basilica* (Babu tulsi), OB_1- *O. basilicum* (Marua tulsi), OTP- *O. tenuiflorum* (Krishna tulsi), OTG- *O. tenuiflorum* (Radha tulsi), OK- *O. kilimandschericum* (Karpur tulsi).

percentage of carbohydrate showed the high food value of *Ocimum* species.

Hexadecanoic acid generally known as the palmitic acid is an antioxidant and it has selective antitumor activity (Pereira *et al*., 2014; Ravi and Krishnan, 2017). The present study showd a higher abundance of palmitic acid (29.64%) in Babu tulsi indicating that this extract might be used directly to suppress tumor cells growth. α-linolenic acid has been reported to have anti inflammatory effects (Solomon *et al*., 2012; Pauls *et al*., 2018). Our this finding ascribes the local use of Tulsi leaves to treat inflammation. From GC-MS analysis it was found that all the nine *Ocimum* extracts contained fatty acids in higher proportion signifying the ability of *Ocimum* species to reduce blood cholesterol level and serves as an antioxidant as also reported earlier (Mensink *et al*., 2003; Suanarunsawat *et al*., 2009; Chao *et al*., 2016).

In the present investigation, we found the presence of some amino acids like L-alanine, L-proline, L-norleucine, L-threonine, L-threonic acid, L-valine and L-Homoproline. *O. americanum* contained 0.74% L-proline and 0.08% L-valine. *O. tenuiflorum* contained only 0.05% pipecolic acid. *O. tenuiflorum* contained 0.24% L-alanine, 1.23% L-proline, 0.01% L-norleucine, 0.14% L-threonic acid and 0.08% L-threonine. Since amino acids are the building blocks of proteins, the occurrence of amino acids with higher yields indicated the presence of proteins in Tulsi. Tulsi might, therefore, be considered as a promising source of plant derived proteins.

Sterols were also found in the ethanolic extracts of different species. *O.* × *africanum* was found to contain 0.88% ergosterol, 1.51% stigmasterol, and 2.50% β-sitosterol. But *O. americanum*, *O. kilimandschericum* and *O. basilicum* (Babu tulsi) all three had only β-

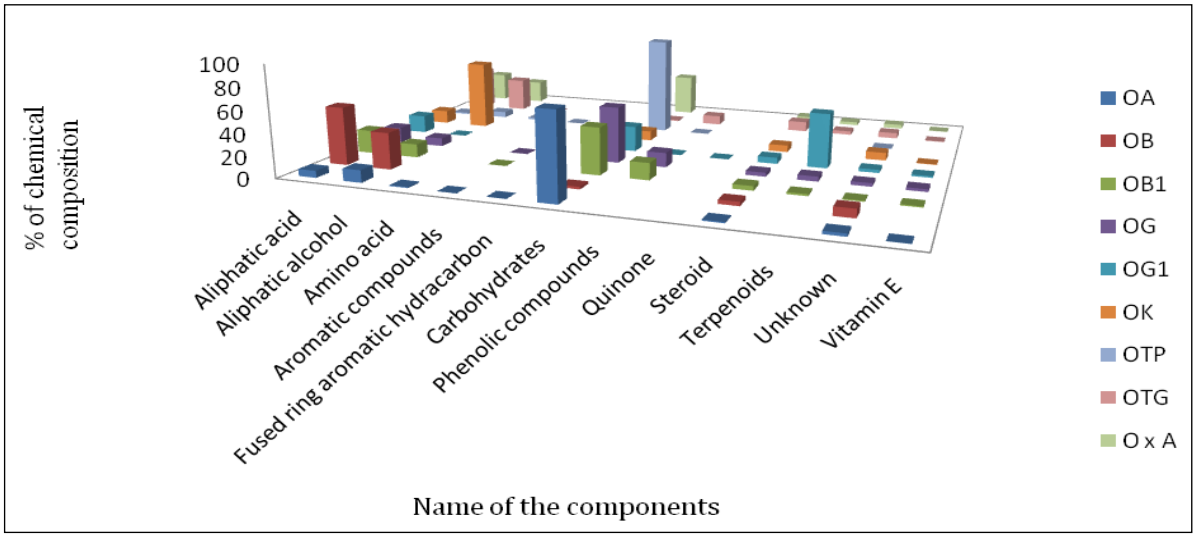

Fig. 1. *Abundance of different metabolites in the ethanolic extracts of nine Ocimum taxa (OA- O. americanum (Bon tulsi), OB- O. basilicum (Babu tulsi), OB1- O. basilicum (Marua tulsi), OG- O. gratissimum (Ram tulsi), OG1- O. gratissimum (Ajoyan tulsi), OK- O. kilimandschericum (Karpur tulsi), OTP- O. tenuiflorum (Krishna tulsi), OTG- O. tenuiflorum (Radha tulsi), OxA- O. x africanum (Lebu tulsi).*

sitosterol as 0.99 %, 6.31% and 3.62% respectively. *O. basilicum* (Marua tulsi) and *O. tenuiflorum* (Radha tulsi) had ergosterol (0.65%, 1.26%), stigmasterol (0.79%, 3.59%) and β-sitosterol (2.55%, 4.53%). Stigmasterol (0.88%) and β-sitosterol (2.70%) were the main constituents of steroid in *O. gratissimum* (Ram tulsi). *O. gratissimum* (Ajowan tulsi) contained β-sitosterol (3.36%) and stigmasterol (2.29%). Among these sterols especially, stigmasterol has proven anti-poisonous/anti-venom effects (Gomes *et al*., 2007; Shabbir *et al*., 2014). The present study thus provide a scientific base to the folk medicine practice of using Tulsi leaves against poisonous insects/strings.

Vitamin E (α-Tocopherol) was found to be present in all the *Ocimum* taxa studied except in *O. tenuiflorum* (Krishna tulsi) and *O. basilicum* (Babu tulsi). It was observed that *O. gratissimum* (Ram tulsi) contained maximum amount of α-tocopherol (2.39%) followed by *O. gratissimum* (Ajowan tulsi) (1.40%), *O. basilicum* (Marua tulsi) (1.01%), *O. tenuiflorum* (Radha tulsi) (0.50%), *O. kilimandschericum* (0.44%), *O.* × *africanum* (0.40%) and *O. americanum* (0.16%). The presence of vitamin E signified the use of Tulsi extracts in medicines/cosmetics to treat skin diseases. α-Tocopherol is the most biologically active form of vitamin E and is the second most common form of vitamin E in the diet. It is a fat soluble antioxidant. Thus continuous use of Tulsi extracts may, therefore, lower the risk of pancreatitis, leukaemia and various forms of skincancers by scavenging the unwanted free radicals (Lai *et al*., 2014; Hu *et al*., 2015 Elsy *et al*., 2017).

A total of 12 terpenoids were identified in the nine *Ocimum* ethanolic extracts. These terpenoids were also responsible for aroma in different Tulsi species in conjugation with naphthalene. In *O.* × *africanum*, *O. basilicum* (Marua tulsi), *O. tenuiflorum* (Radha tulsi), *O. gratissimum* (Ram tulsi) and *O. gratissimum* (Ajowan tulsi) have 2.96%, 1.7%, 3.41%, 4.48% and 49.8% terpenoids respectively. Among them, phytol, α-amyrin, β-elemene, squalene (triterpene precursor) and thymol exhibit maximum abundance of 2.94%, 0.79%, 1.15%, 1.69% and 29.8% respectively. Another interesting terpenoid found in *O. tenuiflorum* (Radha tulsi) and *O. gratissimum* (Ram tulsi) was caryophyllene that has wide antimicrobial effects (Dahham *et al*., 2015). Ram tulsi also contained 1.69% squalene and 1.42% α-amyrin. The co-occurrence of squalene hydrocarbon and α-amyrin confirmed the biosynthetic pathway of pentacyclic triterpenoids such as α-amyrin from squalene. The contemporary medicinal chemistry research is full of anti tumour activities and enzymatic activities of pentacyclic triterpenoids (Mandal *et al*., 2012). Presence of α-amyrin in Ram and Marua tulsi indicates the anti tumour activities of Tulsi extracts. The occurrence of different terpenoids in tulsi extract explains the traditional use of tulsi against bacterial/fungal infections, cough and cold, bronchitis and in asthma. Phenolic compounds such as caffeic acid, catechol, vanillin, eugenol and methyl eugenol were found in the different studied *Ocimum* species. These naturally occurring phenols attribute to the high anti oxidant activities of tulsi.

Antimicrobial activity

The antibacterial activities of nine ethanolic *Ocimum* extracts determined against four bacterial strains by Disc diffusion method are given in Table 2. The ethanolic extracts of *Ocimum* under study showed promising results against all the bacterial strains. From the inhibitory values, it was clear that *O. americanum* (Bon

Table 2. Zone of inhibition (mm) study of ethanolic extracts of nine *ocimum* taxa against different four bacterial strains.

Ocimum sp.	*S. aureus*				*B. cereus*				*P. vulgaris*				*E. coli*			
	Concentration (mg/mL)				Concentration (mg/mL)				Concentration (mg/mL)				Concentration (mg/mL)			
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
	Inhibition zone (mm)															
OA	-	-	-	7	-	-	6	8	-	7	8	10	-	6	7	9
OB	-	-	7	8	-	-	-	7	-	-	-	8	-	-	-	7
OB1	-	-	7	8	-	-	-	8	-	-	7	9	-	6	8	9
OG	-	6	7	9	-	-	-	8	-	-	-	7	-	-	7	9
OG1	-	6	8	9	-	-	7	9	-	-	-	8	6	7	8	10
OK	-	-	-	7	-	7	9	10	-	-	7	9	-	6	7	9
OTP	-	7	8	10	-	-	8	10	-	-	7	9	6	7	9	11
OTG	-	-	7	9	-	7	8	9	-	-	-	8	-	6	8	9
O×A	-	6	8	10	-	-	7	8	-	6	8	10	-	-	7	9

OA- *O. americanum* (Bon tulsi), OB- *O. basilicum* (Babu tulsi), OB_1- *O. basilicum* (Marua tulsi), OG- *O. gratissimum* (Ram tulsi), OG_1- *O. gratissimum* (Ajowan tulsi), OK- *O. kilimandschericum* (Karpur tulsi), OTP- *O. tenuiflorum* (Krishna tulsi), OTG- *O. tenuiflorum* (Radha tulsi), O×A- *O.* × *africanum* (Lebu tulsi).

Table 3. Antibacterial activity of nine *ocimum* taxa against four bacterial strains with MIC (mg/ml) values.

Ocimum sp.	*S. aureus*	*B. cereus*	*P. vulgaris*	*E. coli*
	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)
OA	100	75	50	50
OB	75	100	100	100
OB1	75	100	75	50
OG	50	100	100	75
OG1	50	75	100	25
OK	100	50	75	50
OTP	50	75	75	25
OTG	75	50	100	50
O×A	50	75	50	75

OA- *O. americanum* (Bon tulsi), OB- *O. basilicum* (Babu tulsi), OB_1- *O. basilicum* (Marua tulsi), OG- *O. gratissimum* (Ram tulsi), OG_1- *O. gratissimum* (Ajowan tulsi), OK- *O. kilimandschericum* (Karpur tulsi), OTP- *O. tenuiflorum* (Krishna tulsi), OTG- *O. tenuiflorum* (Radha tulsi), O×A- *O.* × *africanum* (Lebu tulsi).

tulsi) was most effective against *P. vulgaris* and *E. coli*. Interestingly, *O. gratissimum* (Ajowan tulsi) and *O. tenuiflorum* (Krishna tulsi) showed maximum inhibition against *E. coli* with 25 mg/mL MIC values (Table 3). Against *B. cereus*, *O. kilimandschericum* (Karpur tulsi) and *O. tenuiflorum* (Radha tulsi) showed most effective inhibition having 50 mg/mL MIC values. *O.* × *africanum* was found to be the most effective (MIC 50 mg/mL) against *P. vulgaris* and *S. aureus*, on the other hand, *O. gratissimum* (Ram tulsi) was most effective (MIC 50 mg/mL) against *S. aureus* only. *E. coli*, *P. vulgaris* and *S. aureus* was most susceptible to *O. basilicum* (Marua tulsi) at 50 mg/mL and 75 mg/mL MIC respectively, whereas *O. basilicum* (Babu tulsi) was found to show highest value of MIC of 75 mg/mL against *S. aureus*. The presence of different terpenoids and fatty acids such as caryophyllene, thymol, b-elemene, linolenic acid and higher proportion of eugenol and methyl eugenol might be the chief cause for the higher activity of different *Ocimum* species (Singh *et al*., 2005). These antibacterial results implied the significance of determining the biological efficacy of same plant cultivated under varied conditions in a different region. Although the exact mechanisms behind the antimicrobial efficacy of these *Ocimum* extracts are not certain, there are few scientific proposals available in contemporary literature. Complete destruction of cell membrane opens out the bacterial intracellular materials such as protein, lipids and nucleic acids (Oyedemi *et al*., 2009). Notably, the lipophilic terpenoids and phenylpropanoids may induce cell membrane damage, thereby bringing equilibrium of inorganic ions (Trombetta *et al*., 2005; Chouhan *et al*., 2017). An advantage of using plants' extracts against pathogenic bacteria as against to specific antibacterial drug is the incredibly lesser risk of developing resistance since the extracts are a mixture of various an-

timicrobial compounds having different mechanism (Bakkali *et al*., 2008; Rahman and Kang, 2009).

Conclusion

The metabolites from the ethanolic extracts of dried leaves of nine *Ocimum* taxa were separated and identified using GC-MS. In this analysis, a number of compounds were identified such as carbohydrates, amino acids, alcohols, aliphatic acids, aromatic hydrocarbons, phenolic compounds, quinone, steroids, terpenoids, vitamin E *etc*. All the nine different *Ocimum* taxa showed promising antibacterial effects as shown by the MIC values. Notably, *O*. × *africanum* showed 50 mg/mL MIC values against *P*. *vulgaris* and *S*. *aureus* in the antibacterial study.The high carbohydrate content, as revealed from the GC-MS study proves the beneficial food value of *Ocimum* species. The study would have a high impact to gain a detail scientific knowledge about the different naturally growing *Ocimum* species and their chemical constituents with significant antibacterial efficacies.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Bhavya, M.L., Chandu, A.G.S. and Devi, S.S. (2018). *Ocimum tenuiflorum* oil, a potential insecticide against rice weevil with anti-acetylcholinesterase activity. *Ind. Crop Prod.* 126, 434-439. https://doi.org/10.1016/j.indcrop.2018.10.043.
2. Benelli, G., Pavela, R., Maggi, F., Wandjou, J.G.N., Koné-Bamba, D., Sagratini, G., Vittori, S. and Caprioli, G. (2019). Insecticidal activity of the essential oil and polar extracts from *Ocimum gratissimum* grown in Ivory Coast: Efficacy on insect pests and vectors and impact on non-target species. *Ind. Crop Prod.* 132, 377-385. https://doi.org/10.1016/j.indcrop.2019.02.047.
3. Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. (2008). Biological effects of essential oils – a review. *Food Chem. Toxicol*. 46, 446-75. https://doi.org/10.1016/j.fct.2007.09.106.
4. Chowdhury, T., Mandal, A., Jana, A.K., Roy, S.C.and De Sarker, D. (2016). Study of phyto-sociology and ecology of naturally growing *Ocimum* species with their conservational strategies in Dakshin Dinajpur district of West Bengal. *Acta Ecol. Sin.* 36, 483-491. https://doi.org/10.1016/j.chnaes.2016.08.003.
5. Chowdhury, T., Roy, S.C.and De Sarker, D. (2019). Ethnobotanical study of Dakshin Dinajpur district of West Bengal, In: Martinez, J.L., MunÞoz-Acevedo, A., Mahendra Rai, M., (Eds.), Ethnobotany: Local Knowledge and Traditions. CRC Press, New Delhi, India, p132–157. https://doi.org/10.1201/9780429424069.
6. Chaturvedi, T., Kumar, A., Kumar, A., Verma, R.S., Padalia, R.C., Sundaresan, V., Chauhan, A., Saikia, D., Singh, V.R.and Venkatesha, K.T. (2018). Chemical composition, genetic diversity, antibacterial, antifungal and antioxidant activities of camphor-basil (*Ocimum kilimandscharicum* Guerke). *Ind. Crop Prod.* 118, 246-258. https://doi.org/10.1016/j.indcrop.2018.03.050.
7. Chintaluri, A.K. and Komarraju, A.L. (2019). TLC-Bioautographic Detection of Antimicrobial Compounds in Essential Oils of Selected *Ocimum* Species. *J. Essent. Oil Bear. Pl.* 22, 187-199. https://doi.org/10.1080/097206 0X.2019.1602082.
8. Chao, P.Y., Lin, J.A., Ting, W.J., Lee, H.H., Hsieh, K., Chiu, Y.W., Lai, T.J., Hwang, J.M., Liu, J.R.and Huang, C.Y. (2016). *Ocimum gratissmum* aqueous extract reduces plasma lipid in hypercholesterol-fed hamsters. *Int. J. Med. Sci.* 13(11), 819-824. https://doi.org/10.7150/ijms.16474.
9. Chouhan, S., Sharma, K.and Guleria, S. (2017). Antimicrobial activity of some essential oils—present status and future perspectives. Medicines, 4(3), 58. https://doi.org/10.3390/medicines4030058.
10. De Lima, V.T., Vieira, M.C., Kassuya, C.A.L., Cardoso, C.A.L., Alves, J.M., Foglio, M.A., de Carvalho, J.E.and Formagio, A.S.N. (2014). Chemical composition and free radical-scavenging, anticancer and anti-inflammatory activities of the essential oil from *Ocimum kilimandscharicum. Phytomedicine,* 21(11), 1298-1302. https://doi.org/10.1016/j.phymed.2014.07.004.
11. Dahham, S.S., Tabana, Y.M., Iqbal, M.A., Ahamed, M.B., Ezzat, M.O., Majid, A.S. and Majid, A.M. (2015). The Anticancer, Antioxidant and Antimicrobial Properties of the Sesquiterpene β-Caryophyllene from the Essential Oil of *Aquilaria crassna*. *Molecules*, 20, 11808-11829. https://doi:10.3390/molecules200711808.
12. Elsy, B., Khan, A. A. and Maheshwari, V. (2017). Vitamin E isoforms (d α-tocopherol and δ-tocotrienol) promote healing of secondary skin wounds in diabetic rats. *J. Anat. Soc. India.* 66, S14. https://doi.org/10.1016/j.jasi.2017.08.047.
13. Gomes, A., Saha, A., Chatterjee, I. and Chakravarty, A.K. (2007). Viper and cobra venom neutralization by β-sitosterol and stigmasterol isolated from the root extract of Pluchea indica Less. (Asteraceae). *Phyomedicine,* 14, 637-43. https://doi.org/10.1016/j.phymed.2006.12.020.
14. Hu, F., Wu, Z., Li, G., Teng, C., Liu, Y., Wang, F., Zhao, Y. and Pang, D. (2015). The plasma level of retinol, vitamins A, C and α-tocopherol could reduce breast cancer risk? A meta-analysis and meta-regression. *J. Cancer Res. Clin. Oncol.* 141(4), 601-614. https://doi.org/10.1007/s00432-014-1852-7.
15. Labra, M., Miele, M., Ledda, B., Grassi, F., Mazzei, M.and Sala, F. (2004). Morphological characterization, essential

oil composition and DNA genotyping of *Ocimum basilicum* L. cultivars. *Plant Sci.,* 167, 725-731. http://doi:10.1016/j.plantsci.2004.04.026.

16. Lim, V., Mohd Narawi, M., Chiu, H.I., Tung, W.H., Tan, J.J. and Lee, C.K. (2019). Selected essential oils as repellents against *Aedes aegypti*: validation of the bioconstituents using gas chromatography. *J. Essent. Oil Bear. Pl.* 22(4), 1058-1073. https://doi.org/10.1080/0972060X.2019.1661796.
17. Lai, G.Y., Weinstein, S.J., Albanes, D., Taylor, P.R., Virtamo, J., McGlynn, K.A. and Freedman, N.D. (2014). Association of serum α-tocopherol, β-carotene, and retinol with liver cancer incidence and chronic liver disease mortality. *Br. J. Cancer.* 111, 2163-2171. https://doi.org/10.1038/bjc.2014.365.
18. Mohr, F.B.M., Lermen, C., Gazim, Z.C., Gonçalves, J.E.and Alberton, O. (2017). Antifungal activity, yield, and composition of *Ocimum gratissimum* essential oil. Genet. *Mol. Res.* 16, 1-10. https://doi.org/10.4238/gmr16019542.
19. Mondello, L., Zappia, G., Cotroneo, A., Bonaccorsi, I., Chowdhury, J.U., Yusuf, M. and Dugo, G. (2002). Studies on the essential oil bearing plants of Bangladesh, Part VIII. Composition of some *Ocimum* oils *O. basilicum* L. var. *purpurascens*, *O. sanctum* L. green, *O.sanctum* L. purple, *O. americanum* L. citral type, *O. americanum* L. camphor type. *Flav. Fragr. J*. 17, 335-40. https://doi.org/10.1002/ffj.1108.
20. Mensink, R.P., Zock, P.L., Kester, A.D.and Katan, M.B. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am. J. Clin. Nutr.* 77(5), 1146-1155. https://doi.org/10.1093/ajcn/77.5.1146.
21. Mandal, A., Ghosh, S., Bothra, A.K., Nanda, A.K.and Ghosh, P. (2012). Synthesis of friedelan triterpenoid analogs with DNA topoisomerase IIa inhibitory activity and their molecular docking studies. *Eur. J. Med. Chem*. 54, 137-43. https://doi.org/10.1016/j.ejmech.2012.04.037.
22. Nguefack, J., Dongmo, J.L., Dakole, C.D., Leth, V., Vismer, H.F., Torp, J., Guemdjom, E.F.N., Mbeffo, M., Tamgue, O., Fotio, D. and Zollo, P.A. (2009). Food preservative potential of essential oils and fractions from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* against mycotoxigenic fungi. *Int. J. Food Microbiol.* 131(2-3), 151-156. https://doi.org/10.1016/j.ijfoodmicro.2009.02.009.
23. Oyedemi, S.O., Okoh, A.I., Mabinya, L.V., Pirochenva, G. and Afolayan, A.J. (2009). The proposed mechanism of bactericidal action of eugenol, □-terpineol and g-terpinene against *Listeria monocytogenes*, *Strepto-coccus pyogenes*, *Proteus vulgaris* and *Escherichia coli*. *Afr. J. Biotechnol.* 8, 1280-86.
24. Padalia R.C.and Verma R.S. (2011). Comparative volatile oil composition of four *Ocimum* species from northern India. *Nat. Prod. Res.* 25, 569-75. https://doi.org/10.1080/14786419.2010.482936.
25. Patel, J.B. (2017). Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute, Wayne.
26. Pereira, D. M., Correia-da-Silva, G., Valentão, P., Teixeira, N.and Andrade, P. B. (2014). Palmitic acid and ergosta -7, 22-dien-3-ol contribute to the apoptotic effect and cell cycle arrest of an extract from *Marthasterias glacialis* L. in neuroblastoma cells. *Mar. Drugs.* 12(1), 54-68. https://doi.org/10.3390/md12010054.
27. Pauls, S.D., Rodway, L.A., Winter, T., Taylor, C.G., Zahradka, P.and Aukema, H.M. (2018). Anti-inflammatory effects of α-linolenic acid in M1-like macrophages are associated with enhanced production of oxylipins from α-linolenic and linoleic acid. *J. Nutr. Biochem.* 57, 121-129. https://doi.org/10.1016/j.jnutbio.2018.03.020
28. Rana, V.S.and Blazquez, M.A. (2015). Essential oil composition of the aerial parts of five *Ocimum* species from Western India. *J. Essent. Oil Bear Pl.* 18, 1234-1241. https://doi.org/10.1080/0972060X.2014.931255.
29. Ravi, L.and Krishnan, K. (2017). Cytotoxic potential of N-hexadecanoic acid extracted from *Kigelia pinnata* leaves. *Asian J. Cell Biol.* 12, 20-27. https://doi.org/10.3923/ajcb.2017.20.27.
30. Rahman, A.and Kang, S.C. (2009). Inhibition of food borne pathogens and spoiling bacteria by essential oil and extracts of *Erigeron ramosus* (Walt.) B.S.P. *J. Food. Safety.* 29, 176-89. https://doi.org/10.1111/j.1745-4565.2009.00149.x.
31. Singh, V., Krishan, P.and Shri, R. (2017). Neuroprotective effects of *Ocimum kilimandscharicum* leaf extract against ischemia-reperfusion induced cerebral injury in mice. *J. Neurol. Sci.* 381, 997-998. https://doi.org/10.1016/j.jns.2017.08.2814.
32. Singh, D.and Chaudhuri, P.K. (2018). A review on phytochemical and pharmacological properties of Holy basil (*Ocimum sanctum* L.). *Ind. Crop Prod.* 118, 367-382. https://doi.org/10.1016/j.indcrop.2018.03.048.
33. Sonar, V.P., Corona, A., Distinto, S., Maccioni, E., Meleddu, R., Fois, B., Floris, C., Malpure, N.V., Alcaro, S., Tramontano, E. and Cottiglia, F. (2017). Natural product-inspired esters and amides of ferulic and caffeic acid as dual inhibitors of HIV-1 reverse transcriptase. *Eur. J. Med. Chem.* 130, 248-60. https://doi.org/10.1016/j.ejmech.2017.02.054.
34. Stanojevic, L.P., Marjanovic-Balaban, Z.R., Kalaba, V.D., Stanojevic, J.S., Cvetkovic, D.J.and Cakic, M.D. (2017). Chemical composition, antioxidant and antimicrobial activity of basil (*Ocimum basilicum* L.) essential oil. *J. Essent. Oil Bear Pl.* 20(6), 1557-1569. https://doi.org/10.1016/j.foodchem.2007.12.010.
35. Suppakul, P., Miltz, J., Sonneveld, K.and Bigger, S.W. (2003). Antimicrobial properties of basil and its possible application in food packaging. *J. Agric. Food Chem.* 51, 3197-07. https://dx.doi.org/10.1021/jf021038t.
36. Solomon, A., Shmueli, O., Harari, R., Ovadia, H.and Erdinest, N. (2012). Anti-inflammatory Effects of Alpha Linolenic Acid on Human Corneal Epithelial Cells. *Invest Ophthalmol. Vis. Sci.* 53(14), 4194-4194. https://dx.doi.org/10.1167/iovs.12-9724.
37. Suanarunsawat, T., Ayutthaya, W.D.N., Songsa, T. and Rattanamahaphoom, J. (2009). Anti-lipidemic actions of essential oil extracted from *Ocimum sanctum* L. leaves in rats fed with high cholesterol diet. *J. Appl. Biomed.* 7, 45-53. https://dx.doi.org/10.3164/jcbn.09-52.
38. Shabbir, A., Shahzad, M., Masci, P.and Gobe, G.C. (2014). Protective activity of medicinal plants and their isolated compounds against the toxic effects from the venom of Naja (cobra) species. *J. Ethnopharmacol.* 157:

222-227. https://dx.doi.org/10.1016/j.jep.2014.09.039.
39. Singh, S., Malhotra, M.and Majumda, D.K. (2005). Antibacterial activity of *Ocimum sanctum* L. fixed oil. *Indian J. Exp. Biol*. 43, 835-837.
40. Tohti, I., Tursun, M., Umar, A., Turdi, S., Imin, H.and Moore, N. (2006). Aqueous extracts of *Ocimum basilicum* L. (sweet basil) decrease platelet aggregation induced by ADP and thrombin *in vitro* and rats arterio–venous shunt thrombosis *in vivo*. *Thromb. Res.* 118(6), 733-739. https://dx.doi.org/10.1016/j.thromres.2005.12.011.
41. Trombetta, D., Castelli, F., Sarpietro, M.G., Venuti, V., Cristani, M., Daniele, C., Saija, A., Mazzanti, G. and Bisignano, G. (2005). Mechanisms of antibacterial action of three monoterpenes. *Antimicrob. Agents Chemother.* 49 (6), 2474-2478. https://dx.doi.org/10.1128/AAC.49.6.2474-2478.2005.
42. Vidyarthi, S., Samant, S.S.and Sharma, P. (2013). Traditional and indigenous uses of medicinal plants by local residents in Himachal Pradesh North Western Himalaya, India. *Int. J. Biodivers. Sci. Ecosyst. Serv. Manag.* 9, 185-200. https://doi.org/10.1080/21513732.2013.823113.
43. Vieira, P.R., de Morais, S.M., Bezerra, F.H., Ferreira, P.A.T., Oliveira, Í.R.and Silva, M.G.V. (2014). Chemical composition and antifungal activity of essential oils from *Ocimum* species. *Ind. Crop. Prod.* 55, 267-27. https://doi.org/10.1016/j.indcrop.2015.07.004.
44. Verma, R.S., Padalia, R.C., Chauhan, A.and Thul, S.T. (2013). Exploring compositional diversity in the essential oils of 34 *Ocimum* taxa from Indian flora. *Ind. Crops Prod*. 45, 7-19. https://doi.org/10.1016/j.indcrop.2012.12.005.
45. Verma, R.S., Kumar, A., Mishra, P., Kuppusamy, B., Padalia, R.C.and Sundaresan, V. (2016). Essential oil composition of four *Ocimum* spp. from the Peninsular India. *J. Essent. Oil Res.* 28, 35-41. https://doi.org/10.1080/10412905.2015.1076742.
46. Zahran, E.M., Abdelmohsen, U.R., Khalil, H.E., Desoukey, S.Y., Fouad, M.A. and Kamel, M.S. (2020). Diversity, phytochemical and medicinal potential of the genus *Ocimum* L. (Lamiaceae). *Phytochem. Rev.* 19(17), 1-47. https://doi.org/10.1007/s11101-020-09690-9.