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African and Holy Basil - a review of ethnobotany, phytochemistry, and toxicity of their essential oil: Current trends and prospects for antimicrobial/antiparasitic pharmacology

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KEYWORDS

African Basil (Ocimum gratissimum); Holy Basil (Ocimum sanctum); Ocimum tenuiflorum; Mosquitocidal/anti-malarial; Ethnopharmacology; Essential oil extraction Abstract The increased global health burden and mortality rate due to synthetic drug side effects and microbial resistance requires immediate attention for safer and better agents. This quest has fueled the search for phytotherapeutic alternatives, such as essential oils (EOs). Ocimum (Basil) essential oil has pleiotropic health-promoting potential in the treatment of a variety of diseases. This review focused on the ethnobotany, phytochemicals, antimicrobial properties, and toxicity of African and Holy Basil essential oils. African Basil EOs have been used to treat malaria, typhoid, yellow fever candidiasis, influenza, tooth gargle, sore eyes, and ear infections, among other things. Similarly, Holy Basil is used locally as a remedy for diseases such as colds, coughs, malaria, asthma, genitourinary infections, stomach acidity, diabetes, and influenza. This potency could be attributed to the abundance of phytochemicals in the plants, such as eugenol, linalool, and 1, 8-cineole. Experimental evidence has shown that the phytonutrients found primarily in their EOs have antimicrobial activity against many bacteria, fungi, viruses, and protozoans. This study discusses the multitargeted approach of these compounds in eliminating microorganisms by distorting their cellular architecture, which leads to membrane permeability disruption, denaturation of key proteins for survival, damage to the microbial DNA and replication machinery, and ultimately cell lysis and

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organism death. Their antimicrobial pharmacology invariably positions them as a new, effective, and safer Phyto antimicrobial agent to reduce morbidity and mortality due to microbial resistance.
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1. Introduction

Substances derived from aromatic plants, particularly essential oils (EOs), have been used since antiquity and are currently extensively utilized by the cosmetics, food, and pharmaceutical industries (Chimnoi et al., 2018a; Melo et al., 2019a; Melo et al., 2019; Tran et al., 2018). African Basil (Ocimum gratissimum) and holy Basil (Ocimum sanctum). both members of the Lamiaceae family's Ocimum genus, are rich in EOs (Avetisyan et al., 2017). Ocimum gratissimum, also known as African Basil, clove Basil, and wild Basil, is native to Asia, India, and Africa (Mohr et al., 2017). African Basil is grown in home gardens in South East Asia and Africa and used in cooking local foods/dishes, soups, pasta, jellies, salads, and vinegar (Nweze and Eze, 2009). However, it has recently been commercially cultivated in many parts of the world. Ocimum sanctum is native to Asia and Central and Western Africa (Saharkhiz et al., 2015). It is grown worldwide mainly for religious and therapeutic reasons (Choudhary, 2020). Although both African and Holy Basil is members of the same genus, emerging data indicate significant differences in their morphology and ethnobotany.

African Basil leaves are ovate or ovate-lanceolate in shape, cuneate and decurrent at the base, with a coarsely crenate, serrate margin and glandular trichomes (Gupta et al., 2021; Prabhu et al., 2009a). At the same time, the stem and flowers are dark brown and creamy white, respectively (Rawat et al., 2016). Holy Basil differs from African Basil by having internally glabrous calyces and spreading pedicels, as well as slightly toothed, hairy, sub-ovate, and sub-serrate leaves (Kumar et al., 2021, 2022; Malav et al., 2015; Rawat et al., 2016). The stem and flower colours are purple, green, and purple white, respectively (Rawat et al., 2016). African Basil has been utilized to treat digestive disorders, stomach disorders, malaria, typhoid, and yellow fever (Lal et al., 2021; Mukaila et al., 2021). Based on other reports, African Basil can be used to treat ear infections, tooth decay, sore eyes, fever, convulsions, coughs, measles, and tuberculosis (Bankole et al., 2018; Faustin et al., 2021). Holy Basil is traditionally regarded as a sacred plant and is used in religious rituals (Rupani and Chavez, 2018). Among the ethnobotany applications of Holy Basil are cough, cold, asthma, and kidney stone treatment: tooth, skin, and eve protection: and the prevention of lung disease, heart disease, and cancer (Choudhary, 2020). The health benefits of these two Ocimum species could invariably be anchored on their abundant plant-derived phytochemicals.

Although the phytochemical profile of African and Holy Basil varies depending on geographical origin, cultivar type, drying method, and distillation method, eugenol, 1,8-cineole (Melo et al., 2019b; Mohr et al., 2017), linalool, cis-ocimene (Chimnoi et al., 2018b), thymol, p-cymere (Kpoviessi et al., 2014), and β -caryophyllene are the major constituents found in their EOs (Ashish et al., 2022; Kumar et al., 2010; Srivastava et al., 2021). Eugenol, a phenylpropanoid synthesized via the shikimic acid pathway and the most active component of the essential oil of the Basil genus, has antioxidant, cardioprotective, and antimicrobial properties against a variety of microbes (Srivastava et al., 2021; Wang et al., 2018). In addition, it regulates unsaturated lipid oxidation and free radical scavenging, extending the shelf life of food products (Kumar et al., 2010). Eugenol's antimicrobial action is mediated mainly through inhibition of energy generation and the conversion of cytochrome P-450 into the cytotoxic quinine methide (Saharkhiz et al., 2015). 1,8-cineole is a monoterpenoid with anticancer, antimicrobial, antioxidant, and anti-inflammatory properties (Ashish et al., 2022; Cai et al., 2020; Lal et al., 2022). It induces apoptosis through MAPK-mediated and caspase-dependent pathways, targets L-asparaginase in spoilage organisms and many pathogens, and reduces oxidative stress and inflammatory aberrations through regulation of the nuclear factor erythroid-2-related factor 2 (Nrf2) and nuclear factor-kappa B (NF- κ B) signaling pathways, respectively (Cai et al., 2020). Linalool, a monoterpenoid produced by the mevalonic acid pathway, has antimicrobial and antioxidant properties against various microorganisms (Avetisyan et al., 2017).

Using essential oils as antimicrobial agents may be a permanent solution to the recent global health challenge and cost burden caused by antibiotic and multidrug resistance in clinical practice. Approximately 700,000 deaths are recorded annually due to infections caused by drug-resistant microorganisms. The value is expected to increase to 10 million annually by 2050 if effective proactive measures are not taken (Somtochukwu et al., 2021). In addition, antibiotic resistance tends to increase hospitalization time and patient costs, increasing morbidity and mortality rates (Melo et al., 2019b). The antimicrobial potentials of African and Holy Basil using different analytical models show that their EOs had maximal inhibitory activities against several bacteria, fungi, viruses, protozoa, and mosquito larvae. Moreover, recent studies have shown that the nano-derived formulation or nano-conjugation of their essential oils not only improves their biological activities but as well are advantageous to improving their stability, controlling/sustaining their release over time, and achieving targeted delivery (Badea et al., 2019; Predoi et al., 2018; Raita et al., 2020).

Although several empirical research on African and Holy Basil have been undertaken, there is still a demand for detailed comparative studies of ethnobotany, phytochemistry, antimicrobial pharmacology, and toxicity of their essential oils. This study reviews and presents a comparative overview of the ethnobotanical applications, morphological characteristics of African and Holy Basil, and the phytochemical variation of their essential oils. Moreover, emphasis was placed on examining the antimicrobial pharmacology of their EOs with a special focus on their antibacterial, antifungal, antiviral, anti-protozoan, and mosquitocidal activities comprehensively. Finally, we reviewed studies on their possible toxicities to establish possible acceptable dietary intake and prospects for application in clinical practice and industries.

2. Search strategy

This study retrieved relevant literature by adapting the set down principles of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) protocol. The primary search was performed on Scopus, Science Direct, and PubMed using focused keywords such as Ocimum gratissimum, Ocimum sanctum/tenuiflorum, anti-microbial, anti-fungal, antibacterial, anti-parasitic, anti-helminthic, mosquitocidal, essential oil, and others. Whereas the Google Scholar database was used as a secondary search source. The Boolean connectors AND or OR were applied to achieve a focused search for useful literature for this review. The inclusion criteria include the following; first, the article must be focused on essential oils fraction of either African or Holy Basil plants. Secondly, selected articles must be reported in the English language. And finally, the article should report anti-microbial or antiparasitic bioactivities. Preferences were given to recently published papers (after 2010), and non-focused studies were excluded. A total of 402 works of literature were obtained using the focused keywords and combined with the Boolean

connectors. The duplicates were removed before screening the full text and abstract for eligibility. Finally, a total of 113 works published between 2010 and 2022 were used for the study after excluding works that were too old, not written in English, peer-reviewed, and informative regarding the topic (Fig. 1).

3. Ethnobotanical and morphological variations between African and holy Basils

Ethnobotany studies the direct relationship between plants and man in his culture (Rahman et al., 2019). It refers to the various local uses of plants alongside their medicinal purposes. Plant morphology, on the other hand, refers to the study of plants' physical and external features. It is one of the significant steps in establishing the true identity of plants (Akinyemi and Ayodele, 2020), and it is essential to the taxonomy for phylogenetic studies and understanding plant growth (Heslop-Harrison, 2017). Morphology is also crucial for standardizing crude drugs (Agarwal et al., 2013; Lal et al., 2022). Morphological features include size, arrangement, texture, venation, surface characters, markings, and hardness of plant parts (Agarwal et al., 2013). The morphology of African and Holy Basil helps to distinguish the closely related species from each other so as not to misappropriate their usage.

Ocimum gratissimum and Ocimum sanctum belong to the same botanical family (Lamiaceae; formerly Labiatae), as highlighted in the Taxonomical classification in Table 1. However, despite the similarity in taxonomical classification, variation exists in the ethnobotany and morphology of holy and African Basils. The ethnobotanical uses of both Basils are addressed in the subsequent section.

3.1. Ethnobotanical variation between African and holy Basils

African Basil is used as medicine, food, ritual, chemical, and ornamental plant (Kpètèhoto et al., 2017). It is used in the

 Table 1
 Taxonomical classification of African and Holy Basil.

| Botanical classification | Ocimum gratissimum | Ocimum sanctum |
|-----------------------------|-----------------------|----------------------|
| Kingdom | Plantae | Plantae |
| Subkingdom | Tracheobionta | Tracheobionta |
| Super division | Spermatophyta | Spermatophyta |
| Division | Magnoliophyta | Magnoliophyta |
| Class | Magnoliopsida | Magnoliopsida |
| Subclass | Asteridae | Asteridae |
| Order | Lamiales | Lamiales |
| Family | Lamiaceae | Lamiaceae |
| Genus | Ocimum | Ocimum |
| Species | Gratissimum | O. Sanctum |
| References | (Imosemi, 2020) | (Kumar et al., 2022) |

North East of Brazil for culinary and medicinal purposes. The decoction of its roots is used in tropical forest Brazil as a sedative for children. Due to their essential oil content, flowers and leaves are used in preparing teas and infusions. In combination with Xylopia aethiopica, African Basil is used to preparing potions and teas for women during puerperium (Prabhu et al., 2009b). Half a glass of African Basil leaf juice with salt, taken once daily for five days, treats digestive disorders in the Ibarapa area of Ovo State. Nigeria (Ovelakin et al., 2020). Its leaves also treat stomach problems in Enugu State, Nigeria. It is used to cure the pile by taking its extracted leaves and root of Zanthoxylum xanthoxyloides twice daily for one week. Together with Psidium guajava, African Basil leaves are used for curbing diabetes (Aiyeloja and Bello, 2006). Tincture of O. gratissimum leaves in alcohol treats measles and tuberculosis in Ogun State, Nigeria (Bankole et al., 2018). In Ile-Ife, Osun State, Nigeria, its leaf juice is used to treat fresh wounds, and maceration of the leaf in water is used to cure malaria, typhoid, and yellow fever (Mukaila et al., 2021).

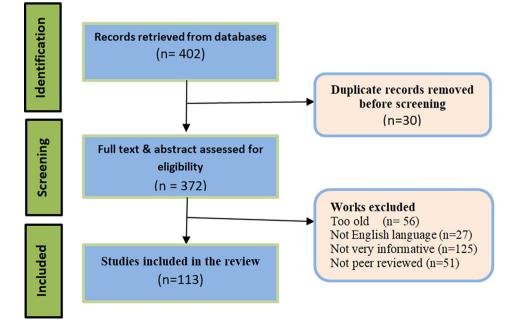


Fig. 1 Flowchart showing the steps of published data selection for inclusion in this study.

The leaf is also applied in treating typhoid, malaria, dysentery, diarrhea, abdominal pain, and nausea in Uromi, Delta State, Nigeria (Igberaese and Ogbole, 2018; Lal et al., 2023). In Kenya and other sub-Saharan African communities, rubbing the leaves between the palms and sniffing is used to cure blocked nostrils. They also use the plant for abdominal pains, ear infections, tooth gargling, sore eyes, fever, convulsions, coughs, barrenness, regulation of menstruation, and to cure prolapse of the rectum. In Benin, African Basil is a flavoring, aids digestion, and combats intestinal worms (Faustin et al., 2021). Decoction and pounding of the leaf stem are also used in Southern Benin to treat candidiasis (Fanou et al., 2020). African Basil is used in Cameroon for treating hemorrhoids, witchcraft problems, incurable wounds, and breast pain (Japhette et al., 2019) and as an insect repeller (Youmsi et al., 2017). The whole African Basil plant is used in India to treat sunstroke, influenza, and headache. In Côte d'Ivoire, the leaves are recommended against sore throat, sinusitis, influenza, colds, coryza, and dizziness. The leafy stem treats hypertension, diabetes, and varicella (Yvette et al., 2014).

Holy Basil and its essential oil are cultivated for religious and medicinal purposes (Choudhary, 2020). It is mainly used as healing remedies, cosmetics, preservatives, and herbal tea preparation. It is considered a sacred plant and may be taken in water as a daily tonic or as part of a religious ritual (Rupani and Chavez, 2018). O. sanctum is used to relieve fear, stress, pain, insect bites, and stings; treatment of cough, cold, asthma, and kidney stones; protection of the teeth, skin, and eyes; prevention of lung disorder, heart disease, and cancer (Choudhary, 2020). The whole plant of Ocimum tenuiflorum is used by the people of Thoppampatti, Tamilnadu, India, for the treatment of cardiopathy, leucoderma, asthma, genitourinary diseases, and ophthalmia (Sivasankari et al., 2014). Specifically, its leaves are used in Mauritius to treat stomach acidity, insomnia, abdominal pain, dark spots, high blood pressure. stress, diabetes, influenza, and flatulence (Mahomoodally, 2014). The paste of its leaves is used to treat scorpion stings and chest pains by the Paliyar tribe in India (Ignacimuthu et al., 2008). The presence of eugenol in its leaves contributes to its medicinal properties as a painkiller and reduction of blood glucose levels in type-2 diabetics (Malav et al., 2015). The juice of the fresh leaves (10 ml) is also taken twice a day to relieve patients suffering from kidney stones. Its leaves are used together with the stem of Tinospora cordifolia to treat fever in Chhattisgarh, India (Pandey, 2021). Its leaves and roots are prepared as juice and used by the Kani tribe in India to treat colds, coughs, and fevers (Ayyanar & Ignacimuthu, 2011; Xavier et al., 2014). Decoction of the leaves and roots supplemented with honey is used twice daily before meals to treat malaria in Jonai, India (Wangpan et al., 2016). Chewing the leaf or its juice is also used for malaria prevention in Odisha, India (Nagendrappa et al., 2013). It is also used in North India as a mosquito repellant (Kantheti and Padma, 2017). In Malaysia, the decoction of the leaves and seeds is also used to cure malaria (Al-adhroey et al., 2010).

3.2. Morphological variation between African and holy Basils

Although Africa and Holy Basil have the same taxonomical family, several distinct morphological variations exist for iden-

tification and survival advantages. Some of these morphological variations between the two plants are summarized in Table 2. African Basil is a shrub, more strongly scented than other Ocimum species, growing up to 102.6 cm in height (Rawat et al., 2016). Its leaves are ovate or ovate-lanceolate, measure up to 10×5 cm, and sub-acuminate to acuminate at the apex, with dots on both sides. They are also cuneate and decurrent at the base, having a coarsely crenate, serrate margin (Prabhu et al., 2009b). There are glandular trichomes on the leaves. The ordinary trichomes are few. Long trichomes - up to 6-celled - are mainly present on the margin, while the 2celled trichomes (short ones) are found majorly on the lamina. Stomata are present on the lower surface but rare or absent on the upper surface of the leaves (Prabhu et al., 2009b). The petioles are up to 6 cm long, and the racemes are up to 18 cm long. Lamina is ovate-lanceolate, 12.4 cm long, and possesses a serrated margin. The peduncles are densely pubescent. The calyx is campanulate, up to 5 mm long, and greenish-white to greenish-yellow in color. When wet, the nutlets are mucilaginous. Seeds are globose and brown, with an average 1000seed weight of 0.9 g. The stem and flower colors are dark brown and creamy white, respectively. The plant height and canopy are 102.6 cm and 787.53 cm², respectively (Fig. 2) (Rawat et al., 2016).

Holy Basil (O. tenuiflorum/sanctum) is an erect, manybranched, about 60 cm in height, aromatic and sweet-scented sub-shrub cultivated in India at a large scale (Baseer and Jain, 2016; Choudhary, 2020). It is readily differentiated from the other Ocimum species by its internally glabrous calyces and spreading pedicels (Malav et al., 2015). The leaves are simple, purple or green, ovate, acute or obtuse, slightly toothed, and possess sub-serrate, entire, or dentate margins. They are hairy and sub-quadrangular, with a dark purple to black color on the outside and a cream color on the inside (Malav et al., 2015; Rawat et al., 2016; Kumar et al., 2022). Both sides of the leaves are pubescent, dotted with minute glands, and have hairy slender petioles. Lamina is sub-ovate, 4.3 cm in length, with a sub-serrate margin (Fig. 2). The inflorescence has purplish white, zygomorphic, and hermaphrodite flowers arranged in elongate racemes in close whorls. Pedicels of the flower are longer than the calyx, and the calyx is ovoid or campanulate, 3-4 mm bilipped (Kumar et al., 2022). The seeds are globose to subglobose, brownish-reddish-yellow, with a shining seed coat that becomes mucilaginous when wet. Seeds are globose and brown, with an average 1000-seed weight of 0.3 g. The stem and flower colors are purple, green, purple, and white. The plant canopy is 692.24 cm^2 (Rawat et al., 2016).

| Table 2 | Some morphological | variations of | African an | d Holy |
|------------|---------------------|---------------|------------|--------|
| Basils (Ra | awat et al., 2016). | | | |

| Morphological character | African Basil | Holy Basil |
|---|------------------|--------------|
| Plant height (cm) | 102.6 | 51.46 |
| Lamina length (cm) | 12.4 | 4.3 |
| Lamina shape | Ovate-lanceolate | Sub-ovate |
| Lamina margin | Serrate | Sub-serrate |
| Flower color | Creamy white | Purple white |
| Stem color | Dark brown | Purple green |
| 1000-seed weight average (g) | 0.9 | 0.3 |
| Plant canopy average (cm ²) | 787.53 | 692.24 |



Fig. 2 A snapshot of African and Holy Basil plants showing some morphological features.

African Basil

Plant

4. Essential oil from African and Holy Basil - extraction and phytochemical composition

Essential oil is one of African and Holy Basil's most valuable components and metabolites, with vast biological activities and industrial usefulness (Kunihiro et al., 2022). Generally, the EO from Africa and Holy Basil are extracted by either hydrodistillation or steam distillation. However, some studies have reported energy-assisted systems, such as microwaveassisted and ultrasound-assisted distillation extraction (Boumahdi et al., 2021; Ghazanfari et al., 2020; Sneha et al., 2022). The nature and composition of EO from different Basil species are strongly affected by the distillation techniques, distillation time, cultivars type, method of drying, ecotypes, and chemotypes (Shiwakoti et al., 2017), in addition to climate, ecological zone, vegetative stage, time of harvesting, genetic results, water, and nutrients availability (Maurya et al., 2022; Melo et al., 2019b; Verma et al., 2011). A substantial number of studies conducted on holy and African Basil essential oils revealed variations in oil from different distillation techniques. Tables 3 and 4 show the major constituents identified from OGEO and OSEO, respectively. The significant compounds out of the 37 constituents identified in SD of OGEO were eugenol (55.6%), cis-ocimene (13.9%), γ-muurolene (11.6%), (Z,E)-α-farnesene (5.6%), αtrans-bergamotene (4.1%),and caryophyllene (2.7%)(Chimnoi et al., 2018b). On the other hand, Melo (Melo et al., 2019b) obtained 19 compounds from HD of OG leaves belonging to several chemotypes, namely phenylpropanoid chemotype (74.83%), oxygenated monoterpenes chemotype (16.09%), hydrocarbon sesquiterpenes chemotype (6.96%), and minor amounts of hydrocarbon monoterpenes chemotype (0.92%) and oxygenated sesquiterpenes chemotype (0.62%) with 74.83 % eugenol, 1,8-Cineole (15.16%), β-Selinene (2.82 %) and (E)-Caryophyllene (2.20 %) being the highest. In the same vein, it was also reported that out of the 30 volatile compounds extracted with HD EG by Mohr (Mohr et al., 2017), oxygenated monoterpenes (72.30 %), sesquiterpene hydrocarbons (10.16 %), phenylpropanoids (7.42 %), oxygenated sesquiterpenes (5.18 %) and monoterpene hydrocarbons (4.82 %) were recorded (Fig. 3). Similarly, a study on the chemical composition of OG grown in Togo extracted with HD recorded monoterpene hydrocarbons (56.21 %), oxygenated monoterpenes (37.85 % and sesquiterpene hydrocarbons (3.80 % with thymol (31.79 %), p-cymene (15.57 %) and γ -terpinene (12.34 %), myrcene (6.94 %) and α -thujene (6.11 %) as the major components (Koba et al., 2009).

Holy Basil Plant

The compound group in EO of holy Basil were predominantly oxygenated monoterpenes (75% in HD; 67% in SD), sesquiterpene hydrocarbon (17% in HD; 27% in SD), and a trace amount of monoterpene hydrocarbons. Specifically, methyl eugenol (68% in HD; 59% in SD), caryophyllene (8% in HD; 13% in SD), and eugenol (7% in HD; 9% in SD) were the major identified compounds in holy Basil EO (Shiwakoti et al., 2017). The high content of EO in SD compared to HD is due to the direct contact of the plant with heat, which easily ruptures the oil gland and higher heat transfer in an index which leads to fast elution of volatiles (Fig. 4) (Shiwakoti et al., 2017).

| Compounds | Phytochemical class | Abundance (%) | Extraction methods | Analytical methods | Molecular formulae | MW (g/mol) | References |
|----------------------------|-----------------------------|------------------|--------------------|-----------------------|--------------------------------------|---------------|---|
| Eugenol | Phenylpropanoid | 7.42–74.83 | SD and HD | GC-MS | $C_{10}H_{12}O_2$ | 164.2 | (Chimnoi et al., 2018b; Melo et al., 2019b; Mohr et al., 201 |
| Linalool | Monoterpenoid. | 0.47-32.95 | SD and HD | GC-MS | $\underline{C_{10}H_{18}O}$ | 154.25 | (Chimnoi et al., 2018b; Kobena et al., 2021b; Melo et al., 2019 Mohr et al., 2017) |
| 1,8-Cineole | Monoterpenoid | 0.1–21.91 | HD and SD | GC-MS | $C_{10}H_{18}O$ | 154.25 | (Koba et al., 2009; Kobenan et al., 2021b; Melo et al., 2019 Mohr et al., 2017) |
| Thymol | Monoterpenoid phenol | 18.7 | SD | GC-MS | $\underline{C_{10}H_{14}O}$ | 150.22 | (Kobenan et al., 2021b) |
| cis-Ocimene | Monoterpenes | 0.43–13.89 | SD and HD | GC-MS | $C_{10}H_{16}$ | 136.23 | (Chimnoi et al., 2018b; Koba et al., 2009; Kobenan et al., 2021b) |
| γ-Muurolene | Sesquiterpenoids. | 11.55 | SD | GC-MS | $C_{15}H_{24}$ | 204.35 | (Chimnoi et al., 2018b) |
| (Z,E)-α-farnesene | sesquiterpene isoprenoid | 5.58 | SD | GC-MS | $C_{15}H_{24}$ | 204.35 | (Chimnoi et al., 2018b) |
| α- Cadinol | sesquiterpenoids | 0.06-5.18 | SD and HD | GC-MS | <u>C₁₅H₂₆O</u> | 222.37 | (Chimnoi et al., 2018b; Mohr et al., 2017) |
| β-Selinene | sesquiterpenoids | 2.82-5.0 | HD | GC-MS | <u>C₁₅H₂₄</u> | 204.35 | (Koba et al., 2009; Kobenan et al., 2021b; Melo et al., 2019 Mohr et al., 2017) |
| Germacrene D | sesquiterpenoids | 0.1–4.76 | SD and HD | GC-MS | <u>C₁₅H₂₄</u> | 204.35 | (Kobenan et al., 2021b; Moh et al., 2017) |
| α-Bergamotene- trans | sesquiterpenoids. | 0.2–4.12 | SD | GC-MS | $C_{15}H_{24}$ | 204.35 | (Chimnoi et al., 2018b; Kober et al., 2021b) |
| α-Terpineol | monoterpenoids | 0.31-3.36 | HD | GC-MS | $\underline{C_{10}H_{18}O}$ | 154.25 | (Koba et al., 2009; Melo et a 2019a) |
| β-Caryophyllene | sesquiterpenoids | 1.68–2.67 | HD and SD | GC-MS | <u>C15H24</u> | 204.35 | (Chimnoi et al., 2018b; Koba et al., 2009; Kobenan et al., 2021b; Melo et al., 2019a; Me et al., 2017) |
| α-Pinene | monoterpenoid | 0.08–2.4 | SD and HD | GC-MS | <u>C₁₀H₁₆</u> | 136.23 | (Koba et al., 2009; Kobenan et al., 2021b; Melo et al., 201 Mohr et al., 2017) |
| trans-Ocimene | Monoterpenoid | 0.10–1.75 | HD and SD | GC-MS | $\underline{C_{10}H_{16}}$ | 136.23 | (Chimnoi et al., 2018b; Koba et al., 2009; Kobenan et al., 2021b; Melo et al., 2019a) |
| α-Copaene | Sesquiterpenes | 0.85-1.22 | SD and HD | GC-MS | <u>C₁₅H₂₄</u> | 204.35 | (Chimnoi et al., 2018b; Koba et al., 2009; Kobenan et al., 2021b) |
| β-Pinene | monoterpenoid | 0.43-1.25 | SD and HD | GC-MS | <u>C₁₀H₁₆</u> | 136.23 | (Koba et al., 2009; Kobenan et al., 2021b; Melo et al., 201 Mohr et al., 2017) |
| Terpinen-4-ol | Monoterpenoid | 0.16-1.13 | HD | GC-MS | <u>C₁₀H₁₈O</u> | 154.25 | (Koba et al., 2009; Melo et a 2019b; Mohr et al., 2017) |
| Sabinene | monoterpenoids | 0.56–1.2 | HD and SD | GC-MS | <u>C₁₀H₁₆</u> | 136.23 | (Koba et al., 2009; Kobenan et al., 2021b; Melo et al., 201 Mohr et al., 2017) |
| α- <i>cis</i> -Bergamotene | monoterpenoids. | 0.03-1.07 | SD and HD | GC-MS | $C_{15}H_{24}$ | 204.35 | (Chimnoi et al., 2018b; Mohr et al., 2017) |
| γ-Cadinene | sesquiterpenoids | 0.04-1.05 | SD | GC-MS | $\underline{C_{15}H_{24}}$ | 204.35 | (Chimnoi et al., 2018b; Mohr et al., 2017) |
| Caryophyllene oxide | sesquiterpenoid oxide | 0.05-1.0 | SD and HD | GC-MS | <u>C15H24O</u> | 220.35 | (Chimnoi et al., 2018b; Kober et al., 2021b; Melo et al., 201 |

*HD - Hydrodistillation, SD - Steam distillation, GC-MS - Gas Chromatography Mass Spectrometry.

Results of several studies have also proved that vegetative stages can influence the constitution of EOs (Melo et al., 2019b). Specifically, (Saharkhiz et al., 2015) realized variations in the prevalence of constituents at the different stages of growth and development from Iran OSEO. From their study, eugenol (37.15 %), 1.8-cineole (26.45 %), and β -Bisabolen (20.99 %) were the most abundant compounds in floral budding (emergence of flower buds), full flowering, and vegetative stages respectively.

Taken together, a study carried out by (Joshi, 2013) to characterize EO from OG and OS showed that the composition of 31 constituents identified in OGEO were 5 phenyl

| Compounds Ocimum sanctum | Phytochemcal Class | Abundance (%) | Extraction method | Analytical methods | Molecular Formulae | MW (g/mol) | References |
|--------------------------------|--------------------------|------------------|----------------------|-----------------------|-----------------------------|---------------|--|
| Methyl eugeno | d phenylpropanoid | 92.4 | SD | GC-MS | $C_{11}H_{14}O_2$ | 178.23 | (Joshi, 2013) |
| Eugenol | phenylpropanoid | 2.4-61.30 | HD and SD | GC-MS | $C_{10}H_{12}O_2$ | 164.2 | (Joshi, 2013; Kumar et al., 2010; Saharkhiz et al., 2015) |
| 1,8-Cineole | Monoterpenoid | 0.01–19.41 | HD and SD | GC-MS | $\underline{C_{10}H_{18}O}$ | 154.25 | (Joshi, 2013; Saharkhiz et al., 2015 |
| β-Caryophyller | ne sesquiterpenoids | 11.89 | HD | GC-MS | $C_{15}H_{24}$ | 204.35 | (Kumar et al., 2010) |
| Germacrene D | sesquiterpenoids | 9.14 | HD | GC-MS | $C_{15}H_{24}$ | 204.35 | (Kumar et al., 2010) |
| β-Elemene | sesquiterpenoids | 0.4–7.7 | HD and SD | GC-MS | $C_{15}H_{24}$ | 204.35 | (Joshi, 2013; Kumar et al., 2010; Saharkhiz et al., 2015) |
| α-Cubebene | sesquiterpenoids. | 2.54 | HD | GC-MS | $C_{15}H_{24}$ | 204.35 | (Kumar et al., 2010) |
| Carvacrol | phenolic monoterpenes | 2.04 | HD | GC-MS | $C_{10}H_{14}O$ | 150.22 | (Kumar et al., 2010) |
| β-Pinene | terpenoid | 0.06-1.53 | HD | GC-MS | $\underline{C_{10}H_{16}}$ | 136.23 | (Kumar et al., 2010; Saharkhiz et al., 2015) |
| β-Selinene | sesquiterpene | 1.34 | HD | GC-MS | $C_{15}H_{24}$ | 204.35 | (Kumar et al., 2010) |
| α-Humulene | sesquiterpene | 0.22–1.29 | HD | GC-MS | $\underline{C_{15}H_{24}}$ | 204.35 | (Kumar et al., 2010; Saharkhiz et al., 2015) |
| α-Pinene | monoterpene | 0.41-0.61 | HD | GC-MS | $\underline{C_{10}H_{16}}$ | 136.23 | (Kumar et al., 2010; Saharkhiz et al., 2015) |
| p-Cymene | monoterpene | 0.83 | HD | GC-MS | $C_{10}H_{14}$ | 134.22 | (Kumar et al., 2010) |
| Sabinene | monoterpenoids | 0.54 | HD | GC-MS | $C_{10}H_{16}$ | 136.23 | (Kumar et al., 2010) |
| γ-Terpinene | monoterpenoids | 0.56 | HD | GC-MS | $C_{10}H_{16}$ | 136.23 | (Kumar et al., 2010) |
| Limonene | monoterpenoids | 0.71 | HD | GC-MS | $C_{10}H_{16}$ | 136.23 | (Kumar et al., 2010) |
| Thymol | monoterpenoid | 0.82 | HD | GC-MS | $C_{10}H_{14}O$ | 150.22 | (Kumar et al., 2010) |
| α -Terpinolene | monoterpenoids | 0.74 | HD | GC-MS | $C_{10}H_{16}$ | 136.23 | (Kumar et al., 2010) |

Table 4 Phytochemicals of Holy Basil essential oil with relative abundance > 0.5%.

HD - Hydrodistillation, SD - Steam distillation, GC-MS - Gas Chromatography Mass Spectrometry.

derivatives (76.0%), ten monoterpene hydrocarbons (15.5%), nine sesquiterpene hydrocarbons (6.1%), 6 oxygenated monoterpenes (1.6%), and 1 oxygenated sesquiterpene (0.1%) with eugenol (75.1%) as the most abundant. However, in OSEO, the compositions of the 25 constituents were 3 phenyl derivatives (94.9%), 9 sesquiterpene hydrocarbons (2.6%), 5 oxygenated sesquiterpenes (0.9%), 5 monoterpene hydrocarbons (0.3%), and 3 oxygenated monoterpenes (0.2%) with was methyl eugenol (92.4%) as the most abundant (Joshi, 2013). Thus, phenyl derivatives are the most abundant class in OGEO and OSEO, but the composition of other chemotypes varies in the two species.

Besides, irrespective of the distillation techniques, several studies have identified eugenol and thymol-rich compounds as the most abundant constituents of EO from OG and OS (Chimnoi et al., 2018b). For instance, 55.55 and 74.83 % of eugenol were obtained in OGEO by (Chimnoi et al., 2018b; Melo et al., 2019b), respectively, while 37.15 and 61.30 % of eugenol were identified in OSEO by (Kumar et al., 2010; Saharkhiz et al., 2015), respectively. Eugenol, with 54.0, 34.6, and 73.1, was also reported as the most abundant constituent in OGEO obtained with SD, HD, and SFE, respectively (Prabhu et al., 2009a). However, the second and third compounds may vary. Examples are 1,8-Cineole (Melo et al., 2019b; Mohr et al., 2017), cis-ocimene (Chimnoi et al., 2018b), thymol, p-cymere (Kpoviessi et al., 2014) and β caryophyllene (Kumar et al., 2010). Thus, the variation in EO calls for the chemical characterization of EO to maximize its use. Eugenol has demonstrated antimicrobial and antioxidant potency and thus can control the oxidation of unsaturated lipids and free radical scavenging, thereby enhancing the shelf life of food products (Kumar et al., 2010).

5. Antimicrobial pharmacology of African and Holy Basils essential oil

Pathogenic microorganisms (bacteria, fungi, and viruses) usually cause several diseases and debilitating health states. Efforts have been made for decades to achieve a lasting weapon against these microbes in the form of antibiotics. However, these pathogenic microbes resist these synthetic antibiotics (Sodhi et al., 2021). Antibiotic and multidrug resistance have been the major cause of death due to microbial infections, as treatments usually fail to annihilate infecting pathogens. Scientists have taken the bull by the horn to investigate alternative routes or treatment regimens that would be long-lasting and effective. Natural products have gained tremendous application as antimicrobial agents and have interested the wider scientific population (Okeke et al., 2021). Plant EO is one of many other forms of plant extract that are vastly known for potencies against pathogenic microbes. One interesting fact about plant extract as an antimicrobial is the multi-targeting mode of action of the plant extract due to the numerous phytochemical constituent of the extracts (Ezeorba et al., 2022). Among several mechanisms of action by plants, EO is disrupting the microbial cell membranes, fos-

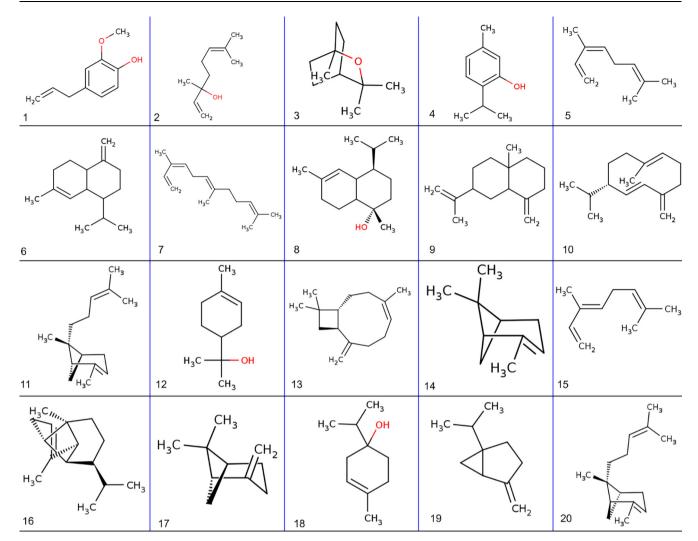


Fig. 3 2D structure of Major phytochemical constituents of African Basil Essential Oil. 1) Eugenol 2) Linalool 3) 1,8-Cineole 4) Thymol 5) *cis*-Ocimene 6) γ -Muurolene 7) (Z,E)- α -farnesene 8) α -Cadinol 9) β -Selinene 10) Germacrene D 11) α -Bergamotene-trans 12) α -Terpineol 13) β -Caryophyllene 14) α -Pinene 15) *trans*-Ocimene 16) α -Copaene 17) β -Pinene 18) Terpinen-4-ol 19) Sabinene 20) α -*cis*-Bergamotene.

tering the increase in ROS for oxidative damage to the microbes, damage of the microbial DNA and its replication machinery, denaturing key proteins for survival, and many more (Fig. 5) (Srivastava et al., 2022; Vaishampayan and Grohmann, 2021). Recent studies showing the antimicrobial activities of African and Holy Basil EO are concisely reviewed in this section (Table 4).

5.1. Antibacterial studies

In a study by Sneha et al. (2022), the EO extracted from African Basil leaves by hydrodistillation and characterized by GC–MS showed antibacterial potencies against *S. aureus*, *S. enteritidis*, *E. coli*, and *P. aeruginosa*. The minimum inhibitory concentration (MIC) ranges from 2 to 4 μ g/mL, and the zone of inhibition (ZI) was between 5 and 10. Moreover, the EO containing majorly p-Cymene (15.1%), camphor (10.1%), Thymol (32.8%), Eugenol (12.3%), and Methyl

Chavico (9.3%) also showed larvicidal activities against *Aedes aegypti* and *Culex tritaeniorhynchus* causing the mortality of the third instar larva with an LC₅₀ of fewer than 50 µg/mL for both mosquitoes (Sneha et al., 2022). Finally, the antioxidant capacity of the EO was estimated using several assays such as the DPPH radical scavenging, Hydrogen peroxide scavenging, ABTS cation radical scavenging, FRAP, Nitric oxide assay, and Lipoxygenase inhibition assay. The IC₅₀ for the antioxidant scavenging activities was reasonable and was presented in Table 5 (Sneha et al., 2022).

In another recent study, procured EO of Basil with specific gravity 0.96456, containing majorly Isopropenyl-4,8a-dime thyl-1,2,3,5,6,7,8,8a-octahedron-naphthalene-2-ol (16.22%); Phenol, 2-methoxy-3-(2-propenyl)- (14.71%); γ -Muurolene (6.57%); 9-Methoxycalamenene (6.51%), was shown to possess antibacterial activities against *E. coli, Enterobacter cloacae* and *Staphylococcus aureus* (Salvi et al., 2022). Using the Disc diffusion methods, the MIC for S. aureus was 12.5 µg/mL, while E. coli was 6.25 µg/mL and Enterobacter cloacae, 25

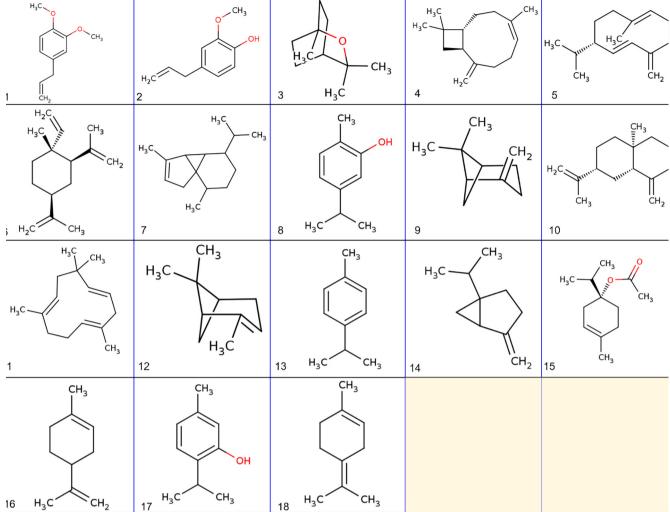


Fig. 4 2D structure of Major phytochemical constituents of Holy Basil Essential Oil 1) Methyl eugenol 2) Eugenol 3) 1,8-Cineole 4) β-Caryophyllene 5) Germacrene D 6) β-Elemene 7) α-Cubebene 8) Carvacrol 9) β-Pinene 10) β-Selinene 11) α-Humulene 12) α-Pinene 13) p-Cymene 14) Sabinene 15) DL-γ-Terpinene 16) Limonene 17) Thymol 18) α-Terpinolene.

µg/Ml.The EO also showed antioxidant activities through the DPPH radical scavenging assay, with an EC50 of 9.43 ng/ml (Salvi et al., 2022). Essential oil of African Basil extracted from steam distillation was also reported with antibacterial activities against gastroenteritis pathogens (Staphylococcus aureus, Escherichia coli, Salmonella Typhimurium, and Shigella flex*neri*) as well as against oral/Periodontopathic/cariogenic bacteria pathogens (Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Streptococcus mutans, and Lactobacillus acidophilus) (Chimnoi et al., 2018a; Ocheng et al., 2015).

Similarly, Holy Basil's EO has also been shown to portray excellent antibacterial activities. By adopting the BD BACTEC MGIT assay method, Jayapal et al. (2021) investigated the anti-mycobacteria activities of the EO fraction. Their results showed that the EO has impressive potencies against isolates of M. tuberculosis and H37Rv with MIC ranging from 2.931 μ g - 5.862 μ g (Jayapal et al., 2021). In another study, the antibacterial activities of Holy Basil were investigated using the Disc diffusion and broth micro-dilution method against S. aureus, S. epidermidis and E. faecalis, P. aeruginosa, and E. coli. The diameter of the zone of inhibition was greater than 15 mm for all organisms and a MIC ranging from 0.03 % to 0.12 % and MBC of 0.12 % to 0.25 %. The Essential oil had an abundance of m-eugenol (69.1 %), according to the GC-MS analysis of the leaves EO (Bugayong et al., 2019). Similarly, as reported in Subramanian, Saumya 2021, the EO from the leaves of Holy Basil should have impressive inhibition against Methicillin-sensitive and resistant S. aureus, as well as other organisms such as E. coli, K. pneumonia, and P. aeruginosa. S. pyogenes, S. agalactiae, Cutibacterium acne, and Bacteroides fragilis. For all the organisms, the Zone of Inhibition diameter estimated from the agar punch well method was grater than 42 mm (Subramanian et al., 2021).

5.2. Antifungal studies

The Essential Oil of African Basil has been mentioned in several studies to be potent against some pathogenic fungi such as Candida, Fusarium, Penicillium, Aspergillus strains, and others. Different fungi species such as A. flavus, A. niger, A.

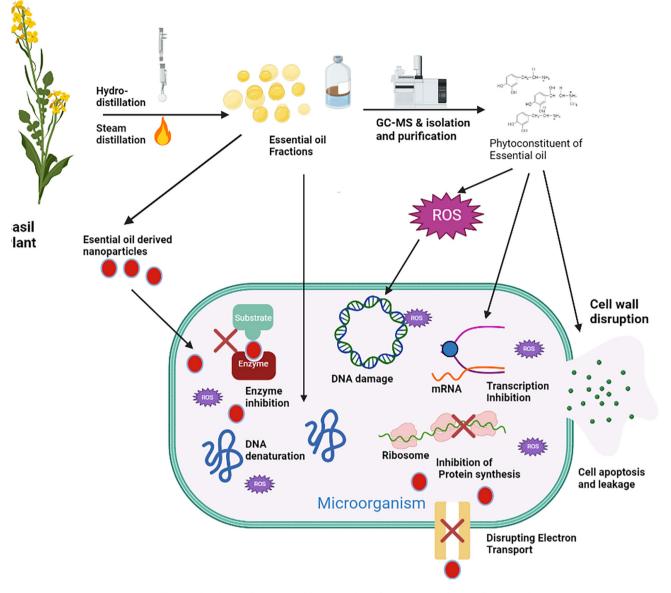


Fig. 5 Possible mechanisms of antimicrobial activities of essential oil from African and Holy Basil.

tamarii, A. terreus, Fusarium poae, F. verticillioides, P. citrinum, P. griseofulvum, Aspergillus aculeatus, A. ustus, Penicillium brevicompactum, and Scopulariopsis brevicaulis are susceptible to the EO from the aerial part of African Basil, having thymol (28.1%). Para-cymene (21.2%), and terpinene (16.5%) as the major components (Sessou et al., 2013). From the study, the mycelial growth inhibition for all the fungi strains fell between 91.1 and 100%, while the minimal fungicidal concentration of 50-100% was also reported (Sessou et al., 2013). In a different study, several strains of Fusarium species were inhibited by the EO from the African Basil leaves containing majorly linalool (32.9%) and 1,8-cineole (21.9%). Using the broth microdilution technique, the MIC for the different strains was between 31.25 and 125 µg/mL (Mohr et al., 2017). In a more recent study, the agar medium assay evaluated the minimum fungicidal concentration of African Basil EO nanoformulation against Penicillium digitatum. The EO

nanoformulation was fabricated from the Basil EO and nonionic surfactant in a 1:1 ratio by ultrasonication (Mahajan et al., 2021). In the study, the nanoformulation showed more potency than the pure EO of African Basil. After 15 days of incubation of the EO with the fungi, a growth inhibition of 85% was recorded for the pure oil, while a 96% inhibition was reported for the nanoformulation (Mahajan et al., 2021). Similarly, Several other studies showing the antimicrobial activities of African Basil EO are summarized in Table 5.

Moreover, a few studies have reported the antifungal activities of Holy Basil EO. The EO of Holy Basil, having eugenol as its major constituent, was packaged in Poly Lactic Acid and used in a fumigation study against *Aspergillus ochraceus* and *Aspergillus westerdijkiae*. It was reported that the EO formulation significantly inhibited the fungal growth as well as its ochratoxin A production (Brandão et al., 2022; Kumar et al., 2020). Similarly, *Aspergillus flavus*, one common cause

| Class of Basil | Biological activities | Purified or characterized compound | Extraction method | Microbes | Assay/Treatment / dosage | Assays and bioactivities | Ref. |
|-------------------|--|---|-------------------|---|--|---|------------------------------|
| African Basil | Antibacterial Larvicidal Antioxidant Capacity/ Radical scavenging activities | EO from leaves having GC/MS characterized compounds (p- Cymene (15.1%), Camphor (10.1%), Thymol (32.8%), Eugenol (12.3%), Methyl Chavico (9.3%) | HD | S. aureus, S. enteritidis, E. coli and P. aeruginosa Aedes aegypti, Culex tritaeniorhynchus | 10 μL of EO on a 6 mm diameter filter paper to 100 μL of microbial isolates (107 CFU/mL) Mortality of the Third instar larva DPPH, HP, ABTS, FRAP, NO, Lipoxygenase inhibition assay | MIC range of 2–4 μ g/ mL and a zone of inhibition of 5– 10 mmLarvicidal (LC ₅₀) – Less than 50 μ g/mL for both mosquito larva IC ₅₀ Values (μ g/mL) – DPPH (44.2); H ₂ O ₂ Scavanging (32.1); ABTS (20.3); others (> 50) | (Sneha et al., 2022) |
| | Antibacterial and Antioxidant capacity | Procured EO with specific gravity of 0.96456- containing majorly Isopropenyl- 4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro- naphthalen-2-ol (16.22%); Phenol, 2- methoxy-3-(2-propenyl)- (14.71%); γ- Muurolene (6.57%); 9- Methoxycalamenene (6.51%). | Procured | <i>E. coli, Enterobacter cloacae</i> and <i>Staphylococcus aureus</i> | Disc diffusion methods DPPH assay | MIC of S. aureus (12.5), E. coli (6.25) and Enterobacter cloacae (25%)DPPH Scavanging (EC50) - 9.43 ng/ml | (Salvi et al., 2022) |
| | Antibacteria | EO from leaves and inflorescences containing majorlyeugenol (43.3%), 1,8- cineole (28.2%) and β -selinene (5.5%) | HD | Aeromonas hydrophila | Microdillution methods | MIC and MBC - 1250- 5000 µg/mL | (Monteiro et al., 2020) |
| | Antibacteria and anti- biofilm | EO from leaves and inflorescences containing majorly Eugenol (74.83%); 1,8-cineole (15.16%) | HD | Staphylococcus aureus and Escherichia coli | Disk diffusion, Microdillution, growth under sub MIC-exposure, | Zone of inhibition diameter – a. E. coli – 12–13 mm b. S. aureus – 15 – 20 mm MIC and MBC – 1000 – 2000 µg/mL | (Melo et al., 2019a) |
| African Basil | Antibacteria (gastroenteritis pathogens) | EO from the leaves containing majorly eugenol (55.6%); <i>cis</i> -ocimene (13.9%), γ -muurolene (11.6%), (Z,E)- α -farnesene (5.6%), α - <i>trans</i> -bergamotene (4.1%), and β -caryophyllene (2.7%). | SD | Staphylococcus aureus, Escherichia coli, Salmonella Typhimurium, and Shigella flexneri | Disk diffusion and membrane disruption | MICs of 1–2 mg ml ⁻¹ Killings at 5 S at 4 times the MICs | (Chimnoi et al., 2018b) |
| | Antibacterial against oral bacteria pathogen | EO from the leaves containing majorly β - Cubebene (10.9%); Eugenol (56.4%); <i>trans-\beta</i> -Ocimene (7.6%) | SD | Periodontopathic Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans and cariogenic Streptococcus mutans and Lactobacillus acidophilus | Broth dilution methods | Less than 10 CFU as % of control at conc of 1.0, 0.1 and 0.01% EO in text solution for all the tested strain | (Ocheng et al., 2015) |
| | Antibacterial | Uncharacterized EO from the leaves | HD | Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, MDR- Acinetobacter baumannii | Agar diffusion methods | MBC ₉₀ of 2 mg/ml against MDR- <i>A</i> . <i>baumannii</i> Diameter for zone of inhibition (6.0–17.3 mm) | (Intorasoot et al., 2017) |

 Table 5
 Antimicrobial (antibacterial and antifungal) activities of Essential oils from African and Holy Basil.

(continued on next page)

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African and Holy Basil - a review of ethnobotany, phytochemistry, and toxicity of their essential oil

| Class of Basil | Biological activities | Purified or characterized compound | Extraction method | Microbes | Assay/Treatment / dosage | Assays and bioactivities | Ref. |
|-------------------|---|--|----------------------|--|--|---|---|
| | Antibacterial | EO from the Aerial parts containing majorly estragol (43.0–44.7%) and linalool (24.6–29.8%) in <i>O. Basilicum</i> oils; carvacrol (12.0–30.8%) and p-cymene (19.5–26.2%) in <i>O. canum</i> oils; thymol (28.3–37.7%) and γ -terpinene (12.5– 19.3%) | HD | L. monocytogenes and S. Typhimurium | Disc diffusion and broth microdilution assays | MICs and MBCs 0.34– 2.5 $\mu L/\ mL$ | (Mith et al., 2016) |
| | Antibacterial | Uncharacterized EO from the leaves | HD | Multiresistant strains of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa | Microdillution technique | MIC against i) E. coli ATCC10536 – 128 µg/ mL, S. <i>aureus</i> ATCC25923 – 512 µg/mL, | (Do Nascimento Silva et al., 2016) |
| | Antibacterial | EO from leaves having majorly camphor (26.2%), Methyl Chavicol (18%), Eugenol | HD | S. aureus, S. enteritidis, E. coli and P. aeruginosa | Disc Diffusion method; | MIC range of 4– 8 µg/ mL and a zone of | (Sneha et al., 2022) |
| Holy | Larvicidal | (29.0%), Thymol (7.2%) | | Aedes aegypti, Culex tritaeniorhynchus | Mortality of the Third instar larva; | inhibition of 6– 10 mmLarvicidal | |
| Basil | Antioxidant Capacity/ Radical scavenging activities | | | Aeaes aegypti, Culex tritaentornynchus | DPPH, HP, ABTS, FRAP, NO, Lipoxygenase inhibition assay | $(LC_{50}) - 50-60 \ \mu g/mL$ for both mosquito larva H ₂ O ₂ Scavenging (39.4); ABTS (26.3); | |
| | | | | | 10 μ L of EO on a 6 mm diameter filter paper to 100 μ L of microbial isolates (107 CEL (mL)) | others (> 50) | |
| | Antibacterial and Antioxidant | Procured EO with a specific gravity of 0.92 Containing majorly Phenol, 2-methoxy-3- (2-propenyl)- (8.52%), γ-Muurolene | Procured | E. coli, <i>Enterobacter cloacae</i> , and methicillin-resistant <i>Staphylococcus aureus</i> | (107 CFU/mL) Disc diffusion methods | MIC (v/v) of S. aureus (12.5), E. coli (12.5) and Enterobacter | (Salvi et al., 2022) |
| | capacity | (7.43%); α-Citral (14.75%); β-Elemene (11.81%); Caryophyllene (8.91%); α- | | | DPPH assay | cloacae (25%) | |
| | | Amorphene (7.78%); | | | | DPPH Scavenging (EC50) – 9.48 ng/ml | |
| | Antibacterial | Uncharacterized EO from the leaves | HD | Anti-mycobacterium against H37Rv and isolates of M. tuberculosis | BD BACTEC MGIT method | The MIC was 2.931 μ g for H37Rv and 0.5 μ L (1.4655 μ g) to 6 μ L (5.862 μ g) for the different isolates. | (Jayapal et al 2021) |
| | Antibacterial and anti- biofilms | EO from the leaves containing majorly Estragole, eugenol, methyleugenol, benzofuran, citral | HD | - | <i>In silico</i> (Molecular docking of compounds to <i>csgA</i> gene | Benzofuran was the most potent with a docking energy of -9.27 K cal/Mol | (Saishree Anchana et al., 2021) |
| Holy Basil | Antibacterial | EO from the leaves with GC/MS analysis Most abundant - <i>m</i> -eugenol (69.1 %) | SD | S. aureus, S. epidermidis and E. faecalis, P. aeruginosa and E. coli | Disc diffusion and broth micro- | Zone of inhibition diameter > 15 mm for | (Bugayong et al., 2019) |

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| Class of | Biological | Purified or characterized compound | Extraction | Microbes | Assay/Treatment / | Assays and bioactivities | Ref. |
|------------------|--|---|------------|---|--|---|--|
| Basil | activities | i united of characterized compound | method | Microbes | dosage | rissays and bioactivities | Kei. |
| | | | | | dilution methods | all organism. MIC ranging from 0.03 % to 0.12 %, and MBC of 0.12 % to 0.25 % | |
| | Antibacterial (Dental protection and mouthwash) | Uncharacterized EO from the leaves | HD | Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum | Agar well diffusion methods | Zone of inhibition diameter (48–55 mm) | (Kalra et al., 2019) |
| | Antibacterial | EO from the flower spikes, leaves with Major bioactive compounds such as camphor, eucalyptol and eugenol | HD | Staphylococcus aureus (including MRSA) and Escherichia coli | Broth micro- dilution method | MIC of 2.25% of E) against the tested strains | (Yamani et al., 2016) |
| | Antimicrobial | EO from the leaves | HD | Methicillin sensitive and resistance S. aureus, E. coli, K. pneumonia, P. aeruginosa S. pyogenes, S. agalactiae, Cutibacterium acne, Bacteroides fragilis | agar punch well method | Zone of inhibition diameter of 42 mm | (Subramanian et al., 2021) |
| African Basil | Antifungal | EO from the leaves containing majorly 1,8-Cineol (23.8%); Eugenol (51.84%); β- Selenine (8.88%); β-Cariofileno (5.79%) | HD | Candida species | modified broth microdilution method Checkerboard methods Crystal violet membrane permeability assay | MIC of 1–8 g/ml, after 24 h resulted to a reduction of 70.1– 80.8% microbial growth | (OLIVEIRA et al., 2016) |
| | Antifungal | EO from the leaves, containing majorly 0.18% essential oil yield containing linalool majorly (32.9%) and 1,8-cineole (21.9%) | HD | Fusarium sp | broth microdilution method | MIC of the essential oil 31.25 to 125 μ g/mL | (Mohr et al., 2017) |
| | Antifungal | EO from the aerial parts majorly with thymol (28.1%) Para-cymene (21.2%) And terpinene (16.5%) | HD | A. flavus, A. niger, A. tamarii, A. terreus, Fusarium poae, F. verticillioides, P. citrinum, P. griseofulvum, Aspergillus aculeatus, A. ustus, Penicillium brevicompactum and Scopulariopsis brevicaulis | Agar medium assay with Minimum Fungicidal concentration and Mycelia growth inhibition | It was reported that the mucelial growth was inhibited 91.1–100% and MFC of 50–100% | (Sessou et al., 2013) |
| | | | | | 800–1000 g/Ml of EO | | |
| | Antifungal | EO from the leaves with nanoformulation by non-ionic surfactant and water by ultrasonication (Oil: Surfactant - 1:1 ration (v/v) | HD | Penicillium digitatum | agar medium assay with Minimum Fungicidal concentration | Growth inhibition of 85% pure oil and 96% nano emulation after 15 days | (Mahajan et al., 2021) |
| Holy Basil | Antifungal | EO from the leaves packaged in Poly Lactic Acid, having its major constituent as eugenol | HD | Aspergillus ochraceus; Aspergillus westerdijkiae | Fumigation test | Inhibit the fungal growth and production of Ochratoxin A | (Brandão et al., 2022; Kumar et al., |

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| Table 5 | Table 5 (continued) | | | | | | |
|-------------------|---|---|-------------------------------|--|---|---|------------------------------------|
| Class of Basil | Class of Biological Basil activities | Purified or characterized compound | Extraction Microbes method | Microbes | Assay/Treatment / dosage | Assay/Treatment / Assays and bioactivities Ref. dosage | Ref. |
| | Antifungal | O.4% yield of EO from the aerial parts with Eugenol (53.3%), Estragole (19.11%), and Camphor (10.01%) as the major component incorporated inside Chitosan Nanomatrix | CH | Aspergillus flavus | poisoned food technique | MIC of 0.75 μL/ml was (Brandão obtained et al., 202 | 2020) (Brandão et al., 2022) |
| | Antifungal | Uncharacterized EO from the aerial parts | П | Penicillium chrysogenum, Aspergilhus tubingensis, A. minutus) | MIC estimation following CLSI M38-A2 protocol | A MIC of 0.25 – 5.04 μg/Ml | (Angelini et al., 2019) |
| HD - Hy | drodistillation, SD | HD - Hydrodistillation, SD - Steam distillation, GC-MS - Gas Chromatography Mass Spectrometry, EO - Essential oils, DPPH - 2,2-diphenylpicrylhydrazyl, NO - Nitric oxide, ABTS - 2,2- | atography Ma | iss Spectrometry, EO - Essential oils, DPP | H - 2,2-diphenylpicryll | hydrazyl, NO – Nitric oxid | le, ABTS – 2,2- |

azino-bis-3-ethylbenzothiazoline-6-sulphonic acid, FRAP - Ferric ion reducing antioxidant power.

of food poisoning, showed a MIC of 0.75 μ L/ml when targeted against EO from the aerial parts Holy Basil incorporated inside Chitosan Nanomatrix, with Eugenol (53.3%), Estragole (19.11%), and Camphor (10.01%) as the major component (Brandão et al., 2022). In summary, the constituents found in the EO of African and Holy Basil confer the antimicrobial activities to the EO, and this can be harnessed as a friend and less toxic alternative to combat disease-causing pathogens.

5.3. Antiviral activities

The use of natural products as antiviral therapy is steadily increasing. Although numerous studies have shown the antiviral potencies of several plants' bioactive chemicals and essential oils, there are limited or no studies revealing the antiviral properties of African and Holy Basil (Ezema et al., 2022). Researchers can consider covering this knowledge void by exploring both plants' antiviral potencies. There are possibilities that findings from such future research endeavors may provide a path toward lasting solutions to several endemic and pandemic viral disturbances. On the other hand, there have been several exciting findings from the few studies which have evaluated the antiviral potencies of solvent extracts of the plant leaves. Ghoke et al. (2018) reported the antiviral activities of holy Basil leaves against the H9N2 virus using an embryonated chicken egg model. The hydro-methanol (1:1) crude extract of holy Basil leaves and its terpenoid-enriched fraction showed the best antiviral properties in limiting the viral replication after 48-72 h post-inoculation. Moreover, a significant decrease in viral genome copy was reported after treatment with the leave extract (Ghoke et al., 2018; Rai et al., 2023). In more recent studies, the cytopathic inhibition and haemagglutination inhibition assay was used to show the antiviral potencies of extracts of Holy Basil against different animal diseases such as Bovine Herpes Virus-type-1 (BVH-1), Foot and Mouth disease virus (FMDV) and Newcastle Disease Virus (NDV). A concentration lower than the non-toxic doses of O. sanctum leaves caused 85.3% and 98.4% protection of BHV-1 and NDV, whereas there were no significant observable effects on the FMD virus (Goel and Bhatia, 2022). Just as the above-discussed illustrative studies, Similar research in the future can perform with the essential oils of Holy and African Basils, and their potencies can be compared.

5.4. Anti-parasitic activities – A focus on mosquitocidal properties of African and Holy Basil essential oil

Mosquitoes are members of a problematic group of insect vectors of human disease causative agents. They belong to the Culicidae family comprising about 3600 flies species. Some diseases and parasites dispersed by mosquitoes are Zika, West Nile, Chikungunya, dengue, and plasmodium (malaria) (Agboli et al., 2021). These disease-causing organisms, transmitted by mosquito vectors, cause serious debilitating health conditions, systemic and metabolic damage, and even death (Chala and Hamde, 2021; Okagu et al., 2022). Essential oil from African and Holy Basil has been shown in several studies to possess mosquitocidal activities, either as a repellent or with insecticidal, larvicidal, or ovicidal activities. This section summarizes key findings from some of these studies to expose the applicability of this oil to reduce the prevalence of some of these diseases, such as malaria, dengue, and zika virus infections (Table 6).

In a recent study, Sharma et al. (2021) reported the mosquitocidal and larvicidal activities of an uncharacterized EO obtained via hydro distillation from the leaves of African Basil against Anopheles subpictus, Aedes aegypti, and Culex quinquefasciatus mosquito species. It was observed that about 94-97% of mosquitoes were knockdown after exposure for 1-2 h and the concentration at 50% lethality (LC₅₀) was 40.08 \pm 1.60 ppm. The EO also caused inhibition of mosquito larva motility (Sharma et al., 2021). In another study, African Basil essential oils acted as an effective repellent against Anopheles gambiae mosquito at an RD₅₀ of 2.77 \times 10⁻⁵ mg cm⁻² (Ywaya et al., 2020). GC-MS characterized the essential oil fractions obtained from the leaves of African Basil. They were found to contain majorly (Z)-Ocimene (29.73%), Eugenol (21.76%), and Germacrene D (9.65%) (Ywaya et al., 2020). On the other hand, Harikarnpakdee and Chuchote (2018) reported the oviposition deterrence activities of African Basil essential oil. In their study, Essential oil from the aerial part of the plants containing majorly eugenol (67.38%) and Z- β ocimene (14.95 %) promoted oviposition deterrence in Aedes aegypti mosquito, for about 8 - 27 d, at a concentration of 0.5 – 3.5 mg/ml (Harikarnpakdee and Chuchote, 2018).

In the same vein, in recent pieces of literature, holy Basil essential oil has shown larvicidal activities, mosquitorepellent, and ovicidal activities against Aedes aegyptii. In a study by Chaiphongpachara et al. (2020), uncharacterized essential oils from Basil, extracted through steam distillation, promoted the mortality (81 - 97%) of Aedes aegyptii larvae within a concentration range of 0.075 - 0.175 ppm. Another study that used hydrodistillation to obtain the EO from Holy Basil leaves showed significant ovicidal effects on Aedes aegypti eggs when exposed to the oil within a 100-1000 ppm concentration 24 to 72 h (Sarma et al., 2020). Finally, an earlier study obtained 100% repellency of Aedes aegyptii adult mosquito, which only had a concentration of 20% EO of Holy Basil in ethanol (Lalthazuali and Mathew, 2017). From these studies, it is very clear that the essential oils of both African and Holy Basils are very potent against mosquito vectors and may have similar applications against other insect pests of humans and agro-produce. Future studies may focus more on improving the potency, which out any harm or toxicity to humans or non-targeted species.

5.5. Other anti-parasitic activities of African and Holy Basil

Basil EO may be potent against other parasites infesting aquaculture and livestock animals. A few studies have recently been performed to investigate the efficacies of essential oils from African Basil as well as its components against several varying classes of parasites (Table 7). However, only sparsely available old studies have investigated similar anti-parasitic activities with Holy Basil Essential Oil (Asha et al., 2001).

Basil EO has been effective in aquaculture as an antihelminthic agent in combating different monogenean parasites affecting various fishes. A study by Meneses et al. (2018) reported an impressive 80% mortality of the monogenean parasite (*Cichlidogyrus tilapiae*) isolated from Nile tilapia after a 2 h incubation with 40.70 mg/l African Basil EO. The major components of the EO were Eugenol (42.3%), 1,8-cineole (20.4), and β -selinene (12.9%) detected by GC–MS analysis after the hydrodistillation extraction (Meneses et al., 2018). Furthermore, in a similar study, the EO of Basil was shown to be efficacious against the monogenean parasite *Gyrodacty-lus sp*, isolated from silver catfish (*Rhamdia quelen*) maxillary barbells. About 50% mortality of the parasite was reported after a 1 h exposure of 10 mg/l EO, whereas the EO content had a significant quantity of eugenol (91.47%) (Bandeira et al., 2017). Other interesting studies on the anti-parasitic activities of Basil EO are summarized in Table 7.

Like aquaculture, many parasites affect livestock animals causing detrimental effects on their health, reproduction, and viability. Essential Oils from Basil are effective phototherapy against some of these livestock parasites, such as fleas, ticks, and other insect parasites, at different stages of their development (egg, larvae, pupa, or adult). A recent study by Oliveira et al. (2022) has shown that African Basil EO and its Eugenolenriched fractions were very effective in causing mortality of the egg, larvae, pupa, and adult of Fleas (Ctenocephalides felis felis) and Ticks (Rhipicephalus sanguineus). About 50-10,000 μ g/ml EO and 100–1000 μ g/ml eugenol were effective in causing significant mortality (>40%) in the mature stage of the parasite (Larvae, pupae, and adult). A 25-1500 µg/ml EO concentration and 2.3-1200 µg/ml eugenol effectively managed the immature stage (ovicidal) of both the Flea and Tick parasites (Oliveira et al., 2022). These results have shown the potencies of African Basil EO act as effective pulicidal and acaricidal agents in causing mortality in all the developmental stages of the parasites. In an older study, Silva Lima et al. (2018a) showed the larvicidal activities of African Basil EO against Rhipicephalus microplus larvae (Cattle Ticks). The larva immersion test was performed, followed by a stereomicroscopic count of dead and live larvae after a 10 min incubation with EO (0.66 to 5.0 mg/mL). The study reported a variation in larvicidal activities based on seasons (rainy and dry), with an LC₅₀ ranging between 0.84 mg/mL to 2.57 mg/ml (Silva Lima et al., 2018). Future studies should consider more research into the crop and animal protection derived from Basil essential oil.

6. Toxicity of African and holy Basils (essential oils)

Toxicity studies determine the short-, medium- and long-term effects of administering specific substances on an organism. Despite the beneficial effects of the essential oils of African and holy Basils, they may have toxic effects. Perhaps, the beneficial effects of the toxicity of the essential oils of African and holy Basils are manifested in its control of pests, as demonstrated by previous research (Cruz et al., 2016; Bhavya et al., 2018; Benelli et al., 2019; Thodsare et al., 2019; Bhavya et al., 2020; Essoung et al., 2020; Kobenan et al., 2021a; Žabka et al., 2021).

The median lethal dose (LD_{50}) of African Basil essential oil in rats was 1750 mg/kg body weight (Fandohan et al., 2008). For the 14-day sub-acute toxicity, rats administered 1500 mg/kg b.w. of the oil died within four days of the experiment, with morphological alterations in the stomach. However, no liver changes were observed even at higher doses (Fandohan et al., 2008). A study by Degla et al. (2021) also showed that 500 mg/kg b.w. of essential oil of African Basil caused a 10% reduction in rats' weight and had a toxic effect

| Class of Basil | Biological activities | Purified or characterized compound | Method of extraction | Experimental model | Treatment/dosage and time | Assays and bioactivities | References |
|-------------------|---|--|----------------------------|--|---|--|--|
| African Basil | Mosquitocidal and Larvicidal activities | Uncharacterized EO from leaves | HD | Anopheles subpictus, Aedes aegypti, and Culex quinquefasciatus | Mosquito knockdown after 1–2h exposure Inhibition of larva | 94-97% of Mosquitocidal activities $LC50 = 40.08 \pm 1.60 \text{ ppm}$ | (Sharma et al., 2021) |
| | | | | | mortality | | |
| | mosquito- repellent and mosquitocidal activities | EO from leaves | HD | Anopheles gambiae | Human bait for evaluation of repellency 30% EO in olive oil | 95.7–97.2% repellency | (Oparaocha et al., 2010) |
| | Mosquitocidal | EO fraction form aerial parts containing majorly eugenol (67.38%) and Z- β -ocimene (14.95%) | HD | Aedes aegypti | Oviposition deterrence 0.5 – 3.5 mg/ml | Oviposition deterrence between 8 and 27 d | (Harikarnpakdee and Chuchote, 2018) |
| | mosquito- repellent | EO fraction from the leaves containing majorly (Z)-Ocimene (29.73%), eugenol* (21.76), and Germacrene D (9.65) | HD | Anopheles gambiae | C, | $ \begin{array}{l} \text{Repellency} \\ \text{RD}_{50} = 2.77 \times 10^{\text{-5}} \text{mg} \\ \text{cm}^{-2} \end{array} $ | (Ywaya et al., 2020) |
| Holy Basil | Larvicidal activities | Uncharacterized EO from the leaves | SD | Aedes aegypti | larva mortality | 81.0–97% larva mortality | (Chaiphongpachara et al., 2020) |
| | | | | | 0.075 – 0.175 ppm | | |
| | mosquito- repellent | Uncharacterized EO from the leaves | HD | Aedes aegypti | 20% EO in ethanol | 100% repellency for 6 h | (Lalthazuali and Mathew, 2017) |
| | Ovicidal | Uncharacterized EO from the leaves | HD | Aedes aegypti | 100–1000 ppm exposure for 24–72 h | LC50 – 8.40 ppm | (Sarma et al., 2020) |

 Table 6
 Bioactivities of Essential oils from African and Holy Basil against mosquito vectors.

| Class of Basil | Biological activities | Purified or characterized compound | Method of extraction | Parasite | Experimental model | Treatment/dosage and time | Assays and bioactivities | References |
|-------------------|--|---|--|--|---|---|---|---|
| African Basil | Anti-parasite (antihelminthic effects) | Eugenol (43.3%), 1,8-cineole (28.2%) and beta- selinene (5.5%) | Hydro- distillation | Monogenean (Aquaculture) | Monogenean parasite count on tambaqui (Colossoma macropomum) fish gills | 0, 5, 10, and 15 mg L^{-1} EO in alcohol | Antihelmintic activities at a minimum conc of 15 mg L^{-1} | (de Lima Boijink et al., 2016) |
| | Anti-parasitic (antihelminthic activities) | Eugenol (91.47%) and <i>Z</i> -β-ocimene (5.93%) | Hydro- distillation | monogenean parasite <u>Gyrodactylus</u> sp (Aquaculture) | monogenean count silver catfish (<i>Rhamdia quelen</i>) maxillary barbells | 5 and 10 mg L^{-1} of EO dissolved by a ratio of 1:10 in 96% ethanol. Exposure time is 1 h before parasite count under a stereomicroscope | Parasite mortality is approximately 35% and 50% in 5 and 10 mg L^{-1} EO | (Bandeira et al., 2017) |
| | Anti-parasite (acaricidal activity) | Thymol (33.4 – 39.5%); γ- Terpinene (26.2 – 35.1%); <i>p</i> -Cymene (6.9 – 17.0%) | Hydro- distillation | Rhipicephalus microplus larvae (Cattle Ticks) (Livestock farming) | Larval immersion test, followed by dead and live larva count | 0.66 to 5.0 mg/mL EO for 10 min | The LC $_{50}$ was 0.84 mg/mL to 2.57 mg/mL | (Silva Lima et al., 2018) |
| | Anti-parasitic (antihelminthic activities) | Eugenol (42.3%); 1,8-cineole (20.4) and β-selinene (12.9%) | Hydro- distillation | monogenean parasite (Cichlidogyrus tilapiae) Aquaculture | Monogenean motality in Nile tilapia | 0-100 mg/l for every 6 h | 80% of parasite mortality at LC_{50} (96 h) of 40.70 mg.L ⁻¹ at 2 h | (Meneses et al., 2018) |
| | Anti-parasitic (pulicidal and acaricide activity) | Eugenol (89.68%) and Apofarnesol (2.68%) | Hydro- distillation and Eugenol purification | Fleas (Ctenocephalides felis felis) and Ticks (Rhipicephalus sanguineus) (Livestock farming) | Mortality of immature and adult stage Flea and Tick | Conc of 25–1500 µg/ml for immature stage (Egg) and 25 to 10000 µg/ml for mature stage (Larvae, pupae, and adult) | Mature stage (Larvae, pupae and adult) – 50–10,000 µg/ml EO and 100–1000 µg/ml EugenolImmature (egg) – 25–1500 µg/ml EO and 2.3–1200 µg/ml Eugenol | (Oliveira et al., 2022) |

 Table 7
 Other anti-parasitic activities of Basil Essential oil – application in aquaculture and livestock farming.

on hematological and biochemical parameters after 14 days of administration.

Estimating oral and intraperitoneal acute toxicities of the essential oil of African Basil in mice and rats revealed intraperitoneal LD_{50} values of 0.27 and 0.43 g/kg, respectively. Similarly, in mice and rats, intraperitoneal LD_{100} values were 0.59 and 0.74 g/kg, respectively. In mice, oral LD50 and LD100 values were 1.41 and 2.50 g/kg, respectively, and 2.29 and 4.07 g/kg, respectively, in rats (Orafidiya et al., 2004). Enlargement in organ weights was observed in the subchronic toxicity study. No deaths were recorded in the group administered orally compared to the intraperitoneally-administered groups within the 30-day test period (Orafidiya et al., 2004).

As revealed by Parandin & Haeri (2010), the oil extracts of African Basil produced negative impacts on male reproductive function and fertility as manifested by the reduction in sperm motility and viability, daily sperm production (DSP), epididymal sperm reserve (ESR), gonadosomatic index (GSI), testosterone concentration and fertility, after 60 days administration at 300 mg/kg b.w.

Fourteen-day acute toxicity study of the essential oil of holy Basil in male Swiss albino rats revealed that the LD₅₀ was greater than 1100 mg/kg b.w (Rangasamy et al., 2012). In mice, the LD₅₀ of holy Basil essential oil was found to be 4571.43 µL/kg (Kumar et al., 2010). Following the Organization for Economic Cooperation and Development (OECD) guidelines 423 in female Wistar rats, the toxic effect of the essential oils of holy Basil was revealed at 2,000 mg/kg bw. However, no mortality was observed (Jayapal et al., 2022). This was in contrast with the acute oral and dermal acute toxicity studies carried out in Sprague Dawley rats by Chil et al. (2017), as no toxic manifestations were observed at 2, 000 mg/kg b.w, following the OECD 423 and 402 guidelines. Only weight gain in male and female Sprague Dawley rats was observed after the dermal toxicity test. The oil was also non-toxic to cardiomyocytes in vitro at 1200 µg/ml (Chil et al., 2017).

7. Conclusion and prospect

According to the findings of this study, EOs from Africa and Holy Basil have a wide range of morphological differences and ethnobotanical applications in treating human diseases. Morphologically, basil leaves are ovate-lanceolate in shape, cuneate, and decurrent at the base, with a coarsely crenate, serrated margin, and glandular trichomes. Whereas Holy Basil differs in having internally glabrous calyces and spreading pedicels, as well as slightly toothed, hairy, sub-ovate, and subserrate leaves. Regarding the stem and flowers, African basil have a brown ark stem and creamy white flowers, while Holy Basils have a purple-green stem and purple-white flowers. This study critically evaluated essential oil phytochemical composition variations and their respective antimicrobial pharmacology. Both essential oils contain diverse phytochemicals with a multi-targeted approach against several microbial strains, making them excellent candidates for developing novel antimicrobial agents. In principle, they exert antimicrobial actions mainly by distorting their cellular architecture, which leads to membrane permeability disruption, denaturation of critical proteins for survival, damage to the microbial DNA/replication machinery, and ultimately cell lysis and organism death. The reports of this study open a window for the food industry to carry out an extensive experiment on the large-scale use of these EOs as food preservatives. Also, more *in vivo* studies, standardization of its dose for application, and the costbenefit ratio must be determined before it can be formulated as an antimicrobial agent. Finally, we recommend that future studies delve more into nano-formulation or nano-derived conjugation of these essential oils products for effective delivery and improved antimicrobial activities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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