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Abstract: Background: Scabies is an enervating parasitic infestation of skin caused by Sarcoptes scabiei, affecting besides 130 million people at any time. Globally, this neglected tropical disease is ame-nable for 0.07% of the total burden of disease. Amomum subulatum Rox. (Large Cardamom) plant parts are used in traditional medicine for curing dyspepsia, skin disease, anorexia, dysentery, hyper-cidity, ulcers, wounds, cardiac debility, fever, cough, liver congestion and gonorrhea.

Objective: The objective of this study was the phytochemical characterization of essential oil of A. subulatum leaves and evaluate its anti-scabies potential against S. scabiei.

Methods: Essential oil was collected by hydrodistillation of fresh leaves of A. subulatum using Clevenger apparatus and subjected to Gas Chromatography (GC), gas chromatography-mass spectrometry (GC-MS) for identification and quantification of components of oil. Anti-scabies potential of essential oil of leaves of A. subulatum against S. scabiei was investigated by contact bioassay method.

Results: GC and GC-MS analysis results revealed the presence of 39 constituents, of which terpinen-4-ol (29.87%), eucalyptol (18.69%), β-phellandrene (7.97%), γ-terpinene (6.67%), p-cymene (6.20%), were detected as major constituents. Oxygenated monoterpenes predominated in the A. subulatum essential oil, and constituted 59.03% of the total oil composition. The anti-scabies study demonstrated their scabicidal potential as its 10% concentration caused 100% mortality within 60 min.

Conclusion: The result indicated anti-scabies potential of essential oil of A. subulatum can be used as an alternative for the treatment and effective control of S. scabiei.

Keywords: Large cardamom, essential oil, GC, GC-MS, anti-scabies activity, Sarcoptes scabiei.

1. INTRODUCTION

Scabies is a contagious, ectoparasitic infestation caused by Sarcoptes scabiei var hominis, known as itch mite [1]. These mites lay eggs in stratum corneum for weeks by burrowing into epidermis, that leads to a host immune response and production of antibodies and resulting in pruritic lesions and papules on the surface of skin [2]. Due to insufficiency of currently available treatments for overcoming this insidious disease and its secondary complications such as rheumatic heart disease, impetigo, and acute post-streptococcal glomerulonephritis, the treatment of Sarcoptes scabiei infection is getting hindered in human beings [3, 4].

Large Cardamom (Amomum subulatum Roxb.), family Zingiberaceae, is a perennial herbaceous plant, indigenous to Eastern Himalayan region. It is cultivated in Assam hills, Darjeeling, Sikkim, northern West Bangal, Nepal and Bhutan, widely distributed in south-east Asian countries like Thailand, Laos and Indonesia [5, 6]. Large Cardamom is named as Badi Ilayachi (Hindi), Greater Cardamom (English), Bara Ilachi (Bangla), Bhadraila, Shthulaila (Sanskrit), Peralam (Malayalam), Kattelam, Perelam and Periya (Tamil), Didda Yelakki (Kannada) and Pedda Yelakaya (Telugu) [7]. In Ayurveda and Unani system of medicines, it is used for the treatment of gastrointestinal ailments, gastric ulcers, migraine and also used as a liver tonic, appetiser, hypnotic and diuretic [8]. It possesses various medicinal properties like astringent, stomachic, carminative, aromatic stimulant, diuretic, alexipharmic and cardiac stimulant [9, 10]. Traditionally, it is used for the treatment of abdominal pain, congestive jaundice, indigestion, rectal disease, vomiting, flatulence, malarial disorders, biliousness, throat, respiratory and cardiovascular disorders [9-12]. The seeds are used in flavoring foods such as beverages, confectionaries, liquors, native medicines, etc. due to the presence of volatile oil possessing characteristic aroma [6, 12]. A. subulatum have been reported to possess various biological activities such as anti-inflammatory [5], antibacterial [9, 13], antidiabetic [10], antimicrobial [14-16], anti-oxidant [13, 17-19], fibrinolytic [18], antiaflatoxigenic, antifungal [20], antiulcer [21], larvicidal [22], lipid lowering [19], analgesic [23].
Due to biological and medicinal importance, the present study is carried out to analyze and characterize the bioactive constituents present in the essential oil of leaves of *A. subulatum* by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) technique and further to assess the in vitro anti-scabies potential of essential oil against *S. scabiei*.

2. MATERIALS AND METHODS

2.1. Plant Material

The leaves of *A. subulatum* were collected from Botanical Garden, Khizrabad, Haryana, India. The leaf samples were identified and authenticated by Dr. Satish Kumar, Taxonomist at Department of Botany, Government College of Girls, Bhodia Khera, Fatehabad, Haryana. A voucher specimen (GJUPCOG160015 II) was preserved in the Herbarium of Department of Pharmaceutical sciences, GuruJambheshwar University of Science and Technology, Hisar, Haryana, India. The fresh leaves (100g) were distilled for 6 hr to obtain oil by hydrodistillation method using Clevenger’s apparatus. The oil was collected over anhydrous sodium sulphate in a glass vial to obtain pure oil, without any traces of moisture and stored at 40°C until used [24].

2.2. Analysis of the Essential Oil

The Gas Chromatographic (GC) analysis of essential oil was carried out using a Shimadzu GC-2010 Gas chromatography equipped with flame ionization detector using Rtx 5 MS capillary column (RESTEK Company: crossbond 5% diphenyl/ 95% dimethyl polysiloxane) having dimensions 30m (Length) x 0.25mm (diameter) x 0.25 µm df (film thickness). The sample (0.2 µl) was injected into the column with a split ratio of 1:100. The analytical conditions were: carrier gas (N₂ 1.21 mL/min, 69.0 kPa), injector temperature 260°C, detector (FID) temperature 280°C, oven temperature 50°C (2 min hold) to 280°C (9 min hold) at 3°C/min. The retention indices (RIs) were in relation to homologous series of n-alkane (C9 to C33) on the Rtx 5 MS capillary column under the same chromatographic condition.

GC-MS analysis was performed using GCMS-QP2010 Plus, Shimadzu, system equipped with mass selective detector, having ion source temperature 230°C, Interface Temp. - 270°C, Solvent Cut Time - 2.50 min threshold of 1000ev and mass range was 40-650 m/z, Rtx 5 MS capillary column and aforementioned chromatographic conditions, with He used as a carrier gas.

Compounds were identified by comparison of retention indices (RIs) with those reported in the literature for Rtx 5 MS capillary column with data in NIST or Wiley Library.

2.3. In Vitro Anti-scabies Activity

2.3.1. Collection of Mites

The *S. scabiei* mites were isolated from scabes and ear cerumen of infested legs and ears of rabbits under clinical examination by Dr. Snehal Gupta, Assistant Professor, Department of Veterinary Parasitology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana. The morphologically characterized mites were placed in petri dishes and motile adult mites were collected for testing.

2.3.2. Contact Bioassay

The essential oil was diluted with paraffin oil to get concentrations of 1%, 5% and 10%. In each petri dish, ten adult mites were placed followed by diluted essential oil for contacting directly. For each concentration of oil, three replicates were performed. Permethrin 5% and liquid paraffin were used as positive control and negative control, respectively. The mites were inspected under stereo-microscope (Olympus) 20, 40, 60, 80 min after inoculation. Mites were recognized as dead when no movement was seen even after touching it with needle and no gut movement was observed over 2 min [25].

3. RESULTS AND DISCUSSION

3.1. Essential Oil Composition

The essential oil (3.40%, yellowish-green) obtained by hydrodistillation of fresh leaves of *A. subulatum* was analyzed by means of GC and GC-MS. Thirty-nine compounds accounting for 98.79% of the total oil contents were identified from *A. subulatum* leaves as well as their percentage area and retention indices were determined. Quantitatively, out of 39 constituents (98.79%), in *A. subulatum* essential oil, monoterpenic hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and other constituents were 31.17%, 59.03%, 3.11%, 5.07% and 0.42%, respectively. Oxygenated monoterpenene (59.03%) represented by terpinen-4-ol (29.87%), eucalyptol (18.69%), linalool (4.90%), α-terpineol (2.56%), thujaol (2.21%), constitutes the main fraction of the oil followed by monoterpenic hydrocarbons (31.17%), containing mainly β-phellandrene (7.97%), γ-terpinene (6.67%), p-cymene (6.20%), β-pinene (2.58%), α-thujene (2.14%) as major constituents. Oxygenated sesquiterpenes (5.07%) were mainly represented by caryophyllene oxide (2.57%), β-eudesmol (0.83%) and cis-nerolidol (0.72%), which slightly prevailed over sesquiterpene hydrocarbons (3.11%), constituting caryophyllene (2.18%) as major component. The findings obtained were compared with those reported earlier on *A. subulatum* seeds, fruits, rind, husk (capsule pericarp) essential oil analyzed by GC-MS (Table 1).

Bhandari et al. [26] analyzed essential oil of *A. subulatum* fruits grown in different agro-climate regions of Uttarakhand, India, by FT-IR spectroscopy and reported the presence of 1,8-cineole in essential oil. Noumi et al. [16] reported the phytochemical profile and antimicrobial potential of *A. subulatum* and found the presence of oxygenated monoterpenes representing 51.0% of total oil content, of which 1,8-cineole (41.7%), α-terpinyl acetate (12.5%) were found as major constituents Fig. (1).

Bhandari et al. [11] analyzed essential oil of dried capsules of large cardamom by GC-MS and 1,8-cineole (73.27%), limonene (4.20%), α-terpineol (4.23%), β-pinene (2.12%), α-terpinyl acetate (3.33%), α-pinene (2.90%), terpinen-4-ol (2.82%), were found as major components. Shrestha [13] characterized essential oil of *A. subulatum* fruits and recorded α-terpineol (27.85%), terpinen-4-ol (11.19%), pinocarvone (8.02%), nerolidol (6.90%), pino carveol (6.32%) as major constituents. Oil was found to...
Table 1. Essential oil composition of leaves of *Amomum subulatum* determined by GC/GC-MS analysis.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Name</th>
<th>Area%</th>
<th>RI&lt;sup&gt;a&lt;/sup&gt; (Lit.)</th>
<th>RI&lt;sup&gt;b&lt;/sup&gt; (Exp.)</th>
<th>R. Time&lt;sup&gt;c&lt;/sup&gt;</th>
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<td>1</td>
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<td>1417</td>
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<td>β- Eudesmol</td>
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<td>1654</td>
<td>38.24</td>
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</tbody>
</table>

<sup>a</sup>RI, programmed temperature retention index as determined on Rtx 5 MS capillary column using a homologous series of n-alkanes (C9 to C33);

<sup>b</sup>RI, Identification was based on the compound of retention indices with those of published data (NIST);

<sup>c</sup>Retention Time.
Fig. (1). GC-MS chromatogram of essential oil of leaves of *Amomum subulatum* Roxb.
have remarkable inhibitory potential against tested bacterial strains. Kaskos et al. [27] analyzed essential oil of *A. subulatum* fruits and recorded monoterpenes (90.00%) total volatile such as 1,8-cineole (77.40%), β-myrcene (5.00%), α-terpinenol (4.90%) and terpinen-4-ol (2.30%) and two sesquiterpenes (2.70%) as t-caryophyllene (2.30%) and caryophyllene oxide (0.40%). Analysis of black cardamom essential oil with the help of multivariate curve resolution approach (MCR) showed the presence of 82 components, of which 1,8-cineole (36.6%), α-terpineol (8.44%), β-pinene (8.55%), 1Rα-pinene (5.10%) and limonene (4.51%) were found as major constituents [28].

Joshi et al. [29] studied and compared the constituents of essential oil from seeds of *A. subulatum* grown in different regions of Himachal Pradesh. 4-terpineol, trans-sabinene hydrate, δ-3-carene, 1-phellandrene, 1-terpineol, α-terpinene, bicyclogermacrene, ledenoxxid-II, longifolenaldehyde, isopinocarveol, and α-terpenyl acetate were found as major constituents with quantitative variations which contribute to distinct aroma of *A. subulatum*. α-Pinene and allo-aromadendrene were not present in the oil of *A. subulatum* seeds from Sikkim. Satyal et al. [12] analyzed essential oil of seeds and rind of *A. subulatum* by GC-MS, monoterpenoids were found as major constituents such as 1,8-cineole (60.80% and 39.00%), α-pinene (6.40% and 4.80%), β-pinene (8.30% and 17.70%), and α-terpineol (9.80% and 12.30%). Seed oil showed marginally toxic effect against red fire ant and moderate toxicity potential against nematode and fruit fly while rind oil showed significant antifungal potential against *A. niger*. Naik et al. [6] characterized the volatile components of capsule pericarp (husk) of *A. subulatum* and 1,8-cineole (38.70%), spathulenol (8.30%), α-terpinenol (12.60%), β-pinene (13.60%), 4-terpinenol (4.50%), germacrene-D (3.00%), α-pinene (2.80%) and β-selinene (2.70%) were found as major compounds. GC-MS analysis essential oil of seeds of *A. subulatum* revealed the presence of 1,8-cineole (81.50-86.00%) as a major component [29]. Jafri et al. [21] studied the gastroprotective action of essential oil obtained from dried fruits of *A. subulatum*. Gurudatt et al. [30] showed the presence of oxygenated monoterpenes (75.20%), constituting 1,8-cineole (61.30%), α-terpinenol (7.92), α-pinene (3.79%) and β-pinene (8.85), allo-aromadendrene (3.20%) as most abundant compounds in steam-distilled volatile oil of *A. subulatum* capsules.

### 3.2. Anti-scabies Activity

The in vitro anti-scabies potential of *A. subulatum* essential oil against *S. scabiei* mites was determined through the contact method. % Mean mortality for the mites treated with three concentrations of oil is presented in Table 2. The anti-scabies study revealed that *A. subulatum* essential oil showed 100% mortality at its 10% concentration within 60 min while 5% diluted solution took 80 min to kill all the mites. Based on % mean mortalities study, it was found that Permethrin as a positive control killed all the mites within 60 min but in negative control group, mortality was only 1.58% and most of the mites remained alive after 80 min of treatment.

Previous studies have reported that eucalyptol plays an important role in the protection mechanism of *S. Scabiei* mites by enhancing the superoxide dismutase and glutathione-s-transferase enzymatic activity [31]. Fang et al. [25] reported scabicidal potential of ten essential oils. 1% palmarosa and clove oil killed all the motile mites within 50 and 20 min, respectively. Clove oil (1.56%) killed all the mites after exposure of 15 min while nutmeg oil showed moderate toxicity against scabies mites as studied by Pasay et al. [32] using contact bioassay method. 20% lemon oil caused 100% mortality of mites after 24 hr of treatment and caused elevation in hydrogen peroxide level that led to considerable cellular damage [33]. Zhou et al. [34] reported acaricidal potential of *Elsholtzia densa* against *S. scabiei* and found that at 16 mg/ml concentration *E. densa* killed all the mites within 16 hr period.

### CONCLUSION

Most of the medicinal properties of the fruits have been reported till date, but the leaves oil can also be utilized for medicinal activities as an alternate to fruits as the components of the volatile oil of the leaves and fruits are...
similar to great extent. In vitro anti-scabies activity of Amomum subulatum essential oil can be explored in the future.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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