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A comparative study on antioxidant, anti-inflammatory, genotoxicity, antimicrobial activities and chemical composition of fruit and leaf essential oils of *Litsea cubeba* Pers from North-east India

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ABSTRACT

Litsea cubeba Pers. is a medicinal as well as aromatic plant distributed widely in North-East India. This study was designed to evaluate and compare antioxidant, anti-inflammatory, genotoxicity, antimicrobial activities and chemical composition of Essential oil *of Litsea cubeba* (EOLC) fruit and leaf collected from CSIR-NEIST, Jorhat (Assam) experimental farm. EOLC fruit and leaf showed effective and strong concentration-dependent antioxidant activity which increases with increase in oil concentration. In the in-vitro anti-inflammatory study, using egg albumin denaturation assay, it was demonstrated that EOLC fruit and leaf has strong activity towards protein denaturation assay in an optimum concentration, where diclofenac sodium was used as standard reference drug. EOLC fruit showed stronger activity than the leaf in in-vitro antioxidant and anti-inflammatory tests. Genotoxic effects showed EOLC fruit and leaf has the negligible effect on root growth inhibition, mitotic index and chromosome aberration in comparison to the standard ethyl methanesulphonate. Antimicrobial activity towards eight microbes was evaluated using Disc diffusion assay (DD[a]) and Minimum inhibitory concentration (MIC) test, where no considerable effect was observed. GC–MS analysis of the oil revealed methyl heptenone (30.9%) as the major component in fruit EO and sabinene (25.22%) in leaf EO. This paper gives an overview of the biology, chemistry and toxicity of EOLC fruit and leaf. Since very few evidence available on the biological activity of North-East India *Litsea cubeba* EO in public domain so the possibilities on the exploitation of potential are discussed and tried to explore here.

1. Introduction

Litsea is a genus belonging to the family *Lauraceae*, widely distributed in Asia, South and North America, tropical and subtropical Australia and New Zealand composed of approximately 622 species (Agrawal et al., 2011). In India, the genus has 46 species (Santapau and Henry, 1973). Among them, 27 species are endemic to India (Ahmedullah and Nayar, 1986).

Litsea cubeba (Lour.) Pers., grows wild in South East Asian countries, including India, China, Bhutan, Nepal, Myanmar, Vietnam, Korea, Taiwan and Indonesia. It is cultivated for various purposes such as growing timber, in aromatherapy and traditionally for the treatment of atopic eczema (Anderson et al., 2000), coronary heart disease (Jiang et al., 2005). In northeastern states of India, it is used for rearing (*Antheraea assamensis*) Muga silkworms (Chaudhury, 1981). Flowers are used to flavour tea and the fruit is edible, carminative, also used for dizziness and in paralysis (Anonymous, 1956). In Arunachal Pradesh (India) *Litsea* fruits are sold in the market to be eaten raw or as pickles due to their carminative properties (Srivastava, 2009).

In China, the seeds are eaten to enhance digestion and to treat cough and bronchitis, to cure mental disorders like hysteria and forgetfulness bark and leaf decoction is taken (Perry, 1980). The Ministry of Health China has approved essential oil of *Litsea cubeba* (EOLC) for use as a food additive also it has been used as a crude material for the manufacture of citral, vitamins A, E, and K, ionine, methylionone, and perfumes (Jiang et al., 2009). Extracts of *L. cubeba* have also been used

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in traditional Chinese medicine for the treatment of a variety of infirmity (Mao et al., 2000). In the recent past the EOLC has demonstrated antibacterial (Wang and Liu, 2010), antifungal (Luo et al., 2004; Yang et al., 2010), acaricidal (Pumnuan et al., 2010), insecticidal (Amer and Mehlhorn, 2006; Nossidum et al., 2008), antioxidant (Hwang et al., 2005) and anticancer properties (Ho et al., 2010). In Taiwan, plant extract is used to treat athlete's foot and other skin diseases and in Indonesia, fruits are used as a substitute of cubeb piper, *Piper cubeba* L. (Oyen and Nguyen, 1999).

The reactive oxygen species, such as superoxide (anionic form of O_2), hydroxyl (OH^-), and peroxyl (univalent radical derived from a peroxide, −OOH, R−O−O−) radicals, play vital roles in degenerative or pathological processes like ageing (Burns et al., 2001), cancer, coronary heart disease, Alzheimer's disease (Diaz et al., 1997), neurodegenerative disorders, atherosclerosis, diabetes and inflammation (Chen et al., 2006). Antioxidant protects molecules from oxidation, in food industry synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been widely used but due to the possible toxicities, improvement and use of more effective antioxidants of natural origin is highly desirable (Rodil et al., 2012). Several scientific reports suggest that the genus *Litsea* is the rich source of natural antioxidants (Agrawal et al., 2011) but within a plant species EO composition varies depending on the type of environment and geographical location they grow (Boland et al., 1991) which may increase or decrease the bioactivity of EO.

Inflammation has been known to be associated with certain diseases including hypertension, cancer and stroke (Robak and Gryglewski, 1996). The traditional use of essential oil suggests that they possess potent anti-inflammatory activity: redness, warmth, swelling and pain are the complex inflammatory response of vascular tissue to harmful stimulates, pathogens and irritants (Libby, 2008). Prolonged inflammation leads to the rheumatoid arthritis, atherosclerosis, hey-fever, ischemic heart diseases (Stevens et al., 2005; Black and Garbutt, 2002; Robbins and Cotran, 1979), infectious diseases like leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, vascularitis, celiac diseases and auto-immune diseases (Sherwood and Toliver-Kinsky, 2004). Anti-inflammatory drugs like NSAIDs used to reduce inflammation but prolonged use of these agents carry the risk of gastrointestinal toxicity, cardiovascular and other toxicity (Abramson and Weaver, 2005). Hence, there is a need for anti-inflammatory drugs having less severe side effects to use for chronic inflammatory diseases. Therefore, in recent time, more interest is shown in alternative and natural drugs for the treatment of various diseases, but there is a lack of proper scientific evidence.

Many plants that are used in traditional medicine and as food have genotoxic effects with scientific evidence (Higashimoto et al., 1993; Schimmer et al., 1994; Kassie et al., 1996; Celik and Aslanturk, 2007). Essential oils are composed of different classes of natural products among them terpene has an inhibiting effect towards plant growth and seed germination (Dudai et al., 2000; Singh et al., 2002). In scientific studies to evaluate genotoxicity, including chromosome abnormalities and disturbances in the mitotic cycle onion plant has been widely used (Leme and Marin-Morales, 2009). Genotoxic effects of the EOLC fruit and leaf on root growth, cell division, chromosome aberration on onion roots were evaluated by measuring root length, determining mitotic indexes and the percentages of chromosomal abnormalities. The GC–MS analysis was used for evaluation of EO chemical compositions. On selected bacterial and fungal strains antimicrobial activity was also carried.

Till date very few studies on North-East India EOLC fruit and leaf essential oil are available in the public domain, to best of our knowledge, this is the first report on chemical composition and different biological activities like antioxidant, anti-inflammatory, genotoxicity, antimicrobial activity evaluation and comparison from NE India.

2. Materials and methodology

2.1. Chemicals used in the study

Solvents and reagents used are HPLC grade methanol (CH_3OH), ascorbic acid ($C_6H_8O_6$), ferric-chloride ($FeCl_3$), sodium-carbonate (Na_2CO_3), 2,2-diphenyl1picrylhydrazyl (DPPH)

($C_{18}H_{12}N_5O_6$), potassium ferricyanide ($K_3Fe(CN)_6$), tris-buffer ($C_4H_{11}NO_3$), aluminum chloride (Al Cl_3), ethyl methanesulphonate (EMS) ($C_3H_8O_3S$), acetocarmine, Muellar Hinton Agar (MHA), potato dextrose agar (PDA) were acquired from HiMedia Laboratories Pvt. Ltd. (Dindori, Nashik, India) and trichloroacetic acid ($C_2HCl_3O_2$) was from Sigma-Aldrich Company (Steinheim, Germany). All other unlabelled chemicals sodium diclofenac-100 mg (Ranbaxy Laboratories Mohali, India) and fresh egg albumin were acquired from local market of Jorhat Assam and are of analytical grade.

2.2. Plant material and isolation of essential oil

Fruits and leaves of *Litsea cubeba* were separately collected from the experimental farm of CSIR-NEIST Jorhat. The plants were identified by the breeder of MAEP group and herbarium specimens were deposited at the departmental herbarium of CSIR-NEIST Jorhat. Fresh and separately collected plant materials were dried in shade and 300 g of each, were transferred to two different Clevenger apparatus and added 3 litres of distilled water for extraction of fruits and leaves essential oils separately. The Clevenger apparatus was set at the boiling temperature of water for five and half hours and distilled oils were collected in dry glass vials. The collected oils were treated with anhydrous sodium sulphate (Na_2SO_4) so as to remove the traces of water present and stored in a sealed tube at 4 °C until further used for anti-oxidant, anti-inflammatory, genotoxicity, antimicrobial test and for chemical analysis.

2.3. Chemical analysis of essential oils through GC/MS

Litsea cubeba fruit and leaf essential oils were analysed with TRACE Ultra Gas Chromatograph coupled to an ISQ Mass Spectrometer (Thermo Fisher Scientific, USA), connected with a computer software. The GC instrument equipped with a TG-Wax MS (60m × 0.25 mm i.d., 0.25 µm film thickness) column. The analysis was performed by helium as a carrier gas at a flow rate of 1 mL min^{-1}. The temperature of GC oven was maintained at 40 °C for 2 min, then programmed to 250 °C at 5 °C $min^{-1}$and to up to 300 °C at 30 °C $min^{-1}$and maintained it for 10 min. One µl of dilute sample (in acetone 1:100,v/v) was injected with a constant temperature of 250 °C through a split injector (1:20 during 1 min). The scan range of the GC/MS was 40 to 650 amu. Peaks in the total ion chromatogram profiles tentatively identified by matching their mass spectra data to NIST/Willey mass spectral library and confirmed by comparison with standard samples on TG Wax column. Concentration of identified compounds, expressed as a percentage, was directly calculated from respective peak areas. The authentication of compound was determined by the standard samples. (Standards were purchased from Fluka and Sigma Aldrich, Germany).

2.4. Antioxidant assay

2.4.1. DPPH free radical scavenging assay

Free radical scavenging activity of EOLC fruit and leaves were determined spectrophotometrically. The changes in colour (from deep-blue to light-yellow) were measured at 517 nm. Radical scavenging activity of extracts was measured by the standard method (Blois, 1958). Various concentrations of oil ranging from (1.5–48) µg were prepared in methanol and added 1 mL of 0.2 mM DPPH solution. The mixture was shaken and incubated for 30 min at 37 °C in dark, and then the absorbance was measured at 517 nm with a UV-VIS spectrophotometer.