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Analytical methods for standardization of *Aegle marmelos*: A review

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ABSTRACT

Aegle marmelos **Correa. is a deciduous tree recognized in traditional medicine for the treatment of various diseases such as dysentery, fever, diabetes, asthma, heart problems, ophthalmia, haemorrhoids urinary problems in humans. The plant has been reported to contain several phytoconstituents mainly marmenol, marmin, marmelosin, marmelide, psoralen, alloimperatorin, rutaretin, scopoletin, aegelin, marmelin, fagarine, anhydromarmelin, limonene, â-phellandrene, betulinic acid, marmesin, imperatorin, marmelosin, luvangentin and auroptene. Though the plant is listed in Ayurvedic Pharmacopoeia of India but many reports on its standardization have appeared recently. Hence, the present review is comprehensive compilation of all possible reports on analytical methods for quantification of its constituent(s) which can be employed for standardization of the plant. Additionally the non-therapeutic uses and toxicity of the plant is also discussed.**

Keywords: *Aegle marmelos*, bael, marmenol, aegeline, standardization, HPLC, HPTLC.

INTRODUCTION

Traditional or indigenous drugs used by different ethnic groups of the world for treatment of various diseases have special significance of having been tested on wide population over a long time scale. Traditional herbal therapies coupled with dietary measures are prescribed by Ayurvedic and other complementary systems of medicines as remedies to many ailments in human and animals. A sizeable population in almost all developed countries uses at least one form of unconventional therapy including herbal medicines.[1,2] These medicines are available as single or poly herbal preparations. Because of consumption of these herbal preparations by a large masses of developed as well as developing countries, there is a need to control and assure the quality of such preparations through systematic scientific studies including chemical standardization, biological assays and validated clinical trials.[3] This importance of quality control and quality assurance of herbal products is driven by possible variations in the nature and content of constituents due to different times of harvesting and conditions of storage, processing and formulation methods.[4,5] The United States Food and Drug Administration (USFDA) and The European Agency for the Evaluation of Medicinal Products (EMEA) have drafted guidelines on various aspects of quality control of medicinal plants such as identification, water content, assay of active ingredients, inorganic impurities or heavy metals, microbial limits, mycotoxins, pesticides, etc. for standardization and validation of herbal preparations.[6,7] Amongst these, the chemical standardization of a herbal formulation with respect to its major phytoconstituent (preferably active) has emerged as the most sought after parameter. Numerous separation methods such as high-performance liquid chromatography (HPLC), high-performance thin layer chromatography (HPTLC), gas chromatography (GC) and capillary electrophoresis (CE)[8,9] and analytical methods such as UV-VIS, spectrofluorimetric, diode-array detection (DAD) and mass spectrometric detection (MS) are employed for herbal drug analyses and standardization.[10] Various pharmacopoeie such as United States Pharmacopeia,[11] Chinese Pharmacopoeia,[12] Japanese Pharmacopoeia,[13] Ayurvedic Pharmacopoeia of India[14] have included complete monographs various medicinal plants. In addition to this, WHO have also developed monographs for various medicinal plants.[15,16] International Union of Pure and Applied Chemistry (IUPAC) has also published a technical report on safety, efficacy, standardization and documentation of herbal medicines.[17]

Aegle marmelos Correa (syn. *Feronia pellucida* Roth, *Cratarea marmelos* L., vern. Bael, Vilwam, Kuvalam, Bengal Quince, Golden Apple, Stone Apple and Wood Apple) belonging to family Rutaceae is a handsome deciduous aromatic tree growing upto 8.5 meters height which is widely distributed throughout the Indian peninsula along with Sri Lanka, Burma and Thailand.[18,19] In addition to being regarded as good dietary supplement,[20,21] it has been valued in the treatment of various diseases[22] in complementary systems of medicine. Various parts of the plant have been reported to have a number of bioactive compounds and secondary metabolites belonging to various classes of natural products[23-25] mainly marmenol,[26] marmin,[27] marmelosin, marmelide, psoralen, alloimperatorin, rutaretin, scopoletin,[28-30] aegelin,[31,32] marmelin,[33] fagarine, anhydromarmelin[34,35] b-carotene, limonene, â-phellandrene, betulinic acid, marmesin,[36] imperatorin,[37] marmelosin, luvangentin[38,39] and auroptene.[40] Many reviews on the plant with special emphasis on pharmacological activities and phytoconstituents have been published in literature.[22,25,41-45] However, the most recent aspects of herbal drug research such as standardization, non-therapeutic potential and stability of the plant are not covered in these reports. The plant has been included in the WHO list of herbal drugs wherein a HPTLC fingerprint (Fig. 1) is available for standardization of the herb.[46] The Ayurvedic Pharmacopoeia of India includes the monographs on stem bark[47] and root[48] of the plant. However, extensive research on the plant has generated a lot of data which remained unattended in these reviews. Hence, the present review is attempted to compile numerous reports covering standardization and stability testing of *Aegle marmelos*. Structures of the chemical constituents employed as markers for standardization of the plant by various research groups are given in Fig. 2.

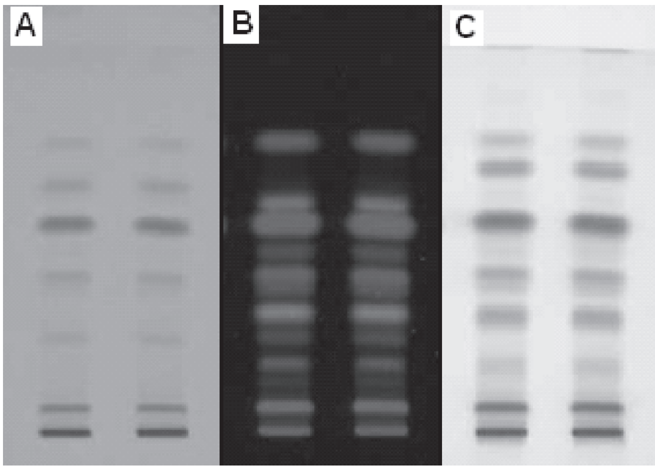

Figure 1: HPTLC fingerprints of *A. marmelos* roots visualized in UV 254 nm (A), UV 366 nm (B) and after derivatization under white light (C). *(Reproduced from "HPTLC- Fingerprint atlas of Ayurvedic single plant drugs mentioned in Ayurvedic Pharmacopoeia"[46])*

Chromatographic Methods

HPTLC

Exploiting marmelosin as one of the major constituents of the plant, Shailajan et al.[49] have developed and validated an HPTLC method for standardization of plant material with reference to marmelosin. Its content is found maximum in ripe fruits followed by unripe fruit, minimum in seeds and almost undetectable in other parts of the plant (Fig. 3). Dhalwal et al.[50,51] have developed and validated simple HPTLC methods for simultaneous quantifications of umbelliferone and psoralen as well as umbelliferone, psoralen and eugenol in fruit pulp.

HPLC and LC-MS

Yadav et al.[52,53] have determined the contents of tannin (0.985%) and riboflavin (0.005%) on a C18 column maintained at 40ºC using mobile phase composed of methanol, water and acetic acid glacial (65:35:0.1, %*v*/*v*/*v*) flowing in isocratic mode at a flow rate of 0.5 mL/min employing 270 nm as detection wavelength. Content of the two components was found to vary in two different samples of the fruit (Fig. 4a). The various organic acids including oxalic (1), tartaric (2), malic (3) and ascorbic (4) acids were separated (Fig. 4b) and quantified using a RP-HPLC method employing a C18 column (set at 40ºC) with $NH_4H_2PO_4$ (0.5% *w/v*, pH 2.8) used as mobile phase running at a flow rate of 0.5 mL/min using 214 as detection wavelength. The method is reported to be linear and precise for ascorbic acid, oxalic acid and malic acid in the concentration range of 0.004 – 0.124 % *w/v* while for tartaric acid in the concentration range of 0.016 – 0.124 % *w/v*. A normal phase HPLC method is also developed for separation of various sugars wherein fructose, glucose and sucrose are separated on an NH_2 column with mobile phase composed of acetonitrile and water (75:25 %*v*/*v*) at a flow rate of 1 mL/min using refractive index detector and the column maintained at 40ºC (Fig. 4c). The method is linear and precise for determination of each sugar in the concentration range of 0.05 – 2.05 % *w/v*. The content of all the components except ascorbic acid are reported to increase with ripening.

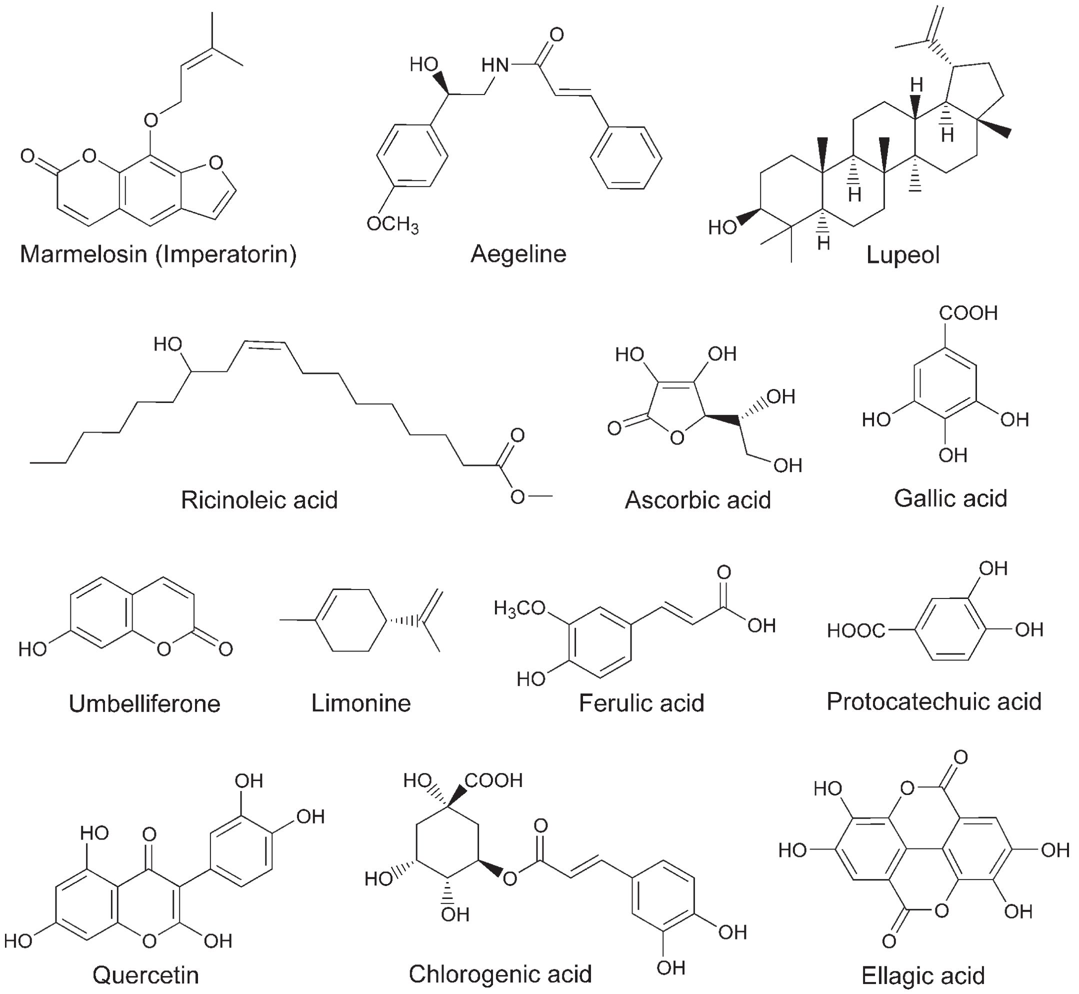

Figure 2: Some chemical constituents employed as markers for standardization of *A. marmelos*

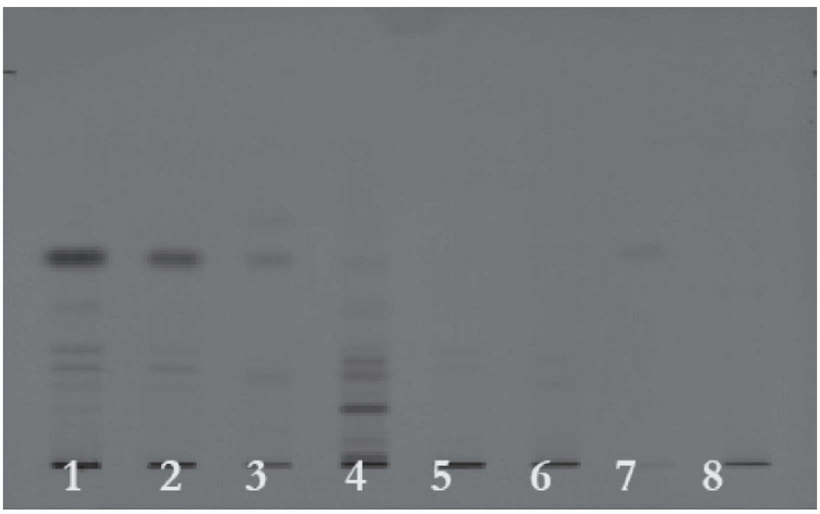

Figure 3: An HPTLC chromatogram showing presence of marmelosin (7) in ripe fruit (1), unripe fruit (2), seed (3), leaves (4), rind (5), inner stem (6) and outer stem (8). *(Reproduced from "Shailajan et al., J Pharm Res 2011; 4:1353-1355")*[49]

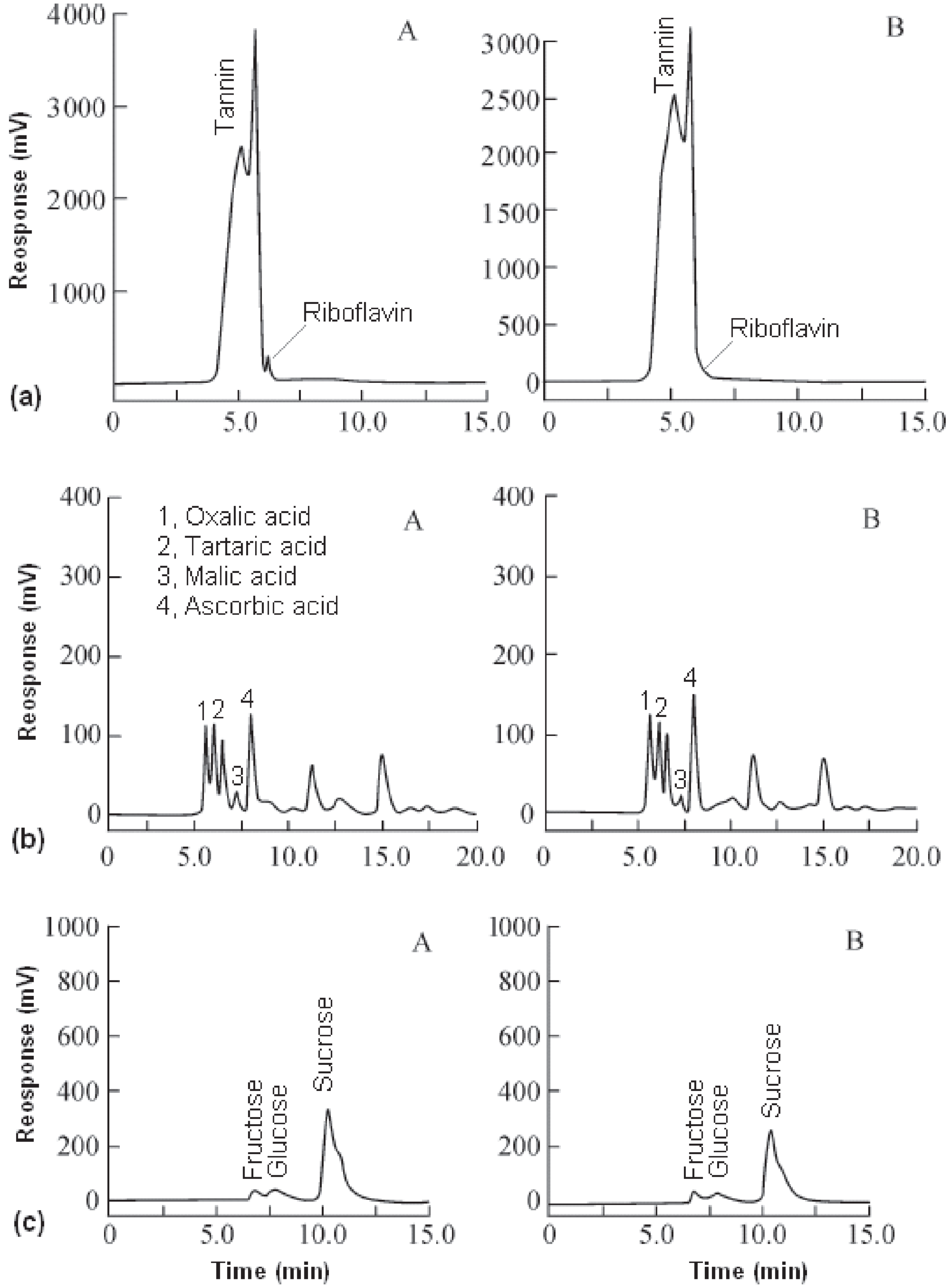

Figure 4: HPLC chromatograms of tannins and riboflavin (a), organic acid (b) and sugars (c) in two different samples (A and B) of bael fruit. *(Reproduced from "Yadav et al.,* Chinese J Nat Med 2011; 9:204-209*")*[53]

Another RP-HPLC method is reported for quantification of umbelliferone in the roots. The method is linear in the concentration range of 0.5 – 6.5 µg/ml and content of unmbelliferone have been found to be 0.044 % *w/w* with the help of a RP-HPLC method.[54] Dhan et al.[55] have characterized the various phenolics in the fruit as chlorogenic acid (136.8 µg/g), ellagic acid (248.5 µg/g), ferulic acid (98.3 µg/g), gallic acid (873.6 µg/g), protocatechuic acid (47.9 µg/g) and quercetin (56.9 µg/g) through LC-MS and LC-MS/MS scans and HPLC studies.

GC-MS

A SPME-GC-MS analysis of volatile compounds from the fruit pulp has helped in identification of 28 volatile compounds (Fig. 5) with monoterpenes and sesquiterpenes comprising the major class.[56] Limonene is the major constituent (32.48% of the total peak area) which is responsible for characteristic fruit flavor. The other major constituent is found to be *p*-cymene (27.19%). A GC-MS analysis of the plant extract has identified lupeol (a triterpenoid) as the major bioactive constituent that stimulates the decoy effect of RA4 DNA sequence and inhibits cell proliferation.[57]

Non-therapeutic Uses

A mucoadhesive material extracted from the fruits provides mucoadhesion better than hydroxyl propyl methyl cellulose (4000M) when used in formulating oral mucoadhesive coated terbutaline sulfate tablets without affecting the release profile of the drug.[58] Aqueous extract of the plant in 1N hydrochloric acid has been found to inhibit corrosion pickling with an efficiency of 96-98% due to a protective film on the surface of mild steel formed by adsorption of the plant constituents. Aegelin has been implicated for the activity.[59] The plant leaves are found as effective bio-adsorbents of lead even in the presence of other heavy metal ions which makes the plant an excellent green alternate in control of pollution due to lead laden effluents from the industries.[60] An activated carbon prepared from non-usable fruit shell is used as an efficient low cost adsorbent to remove the Cr(VI) toxic metal from

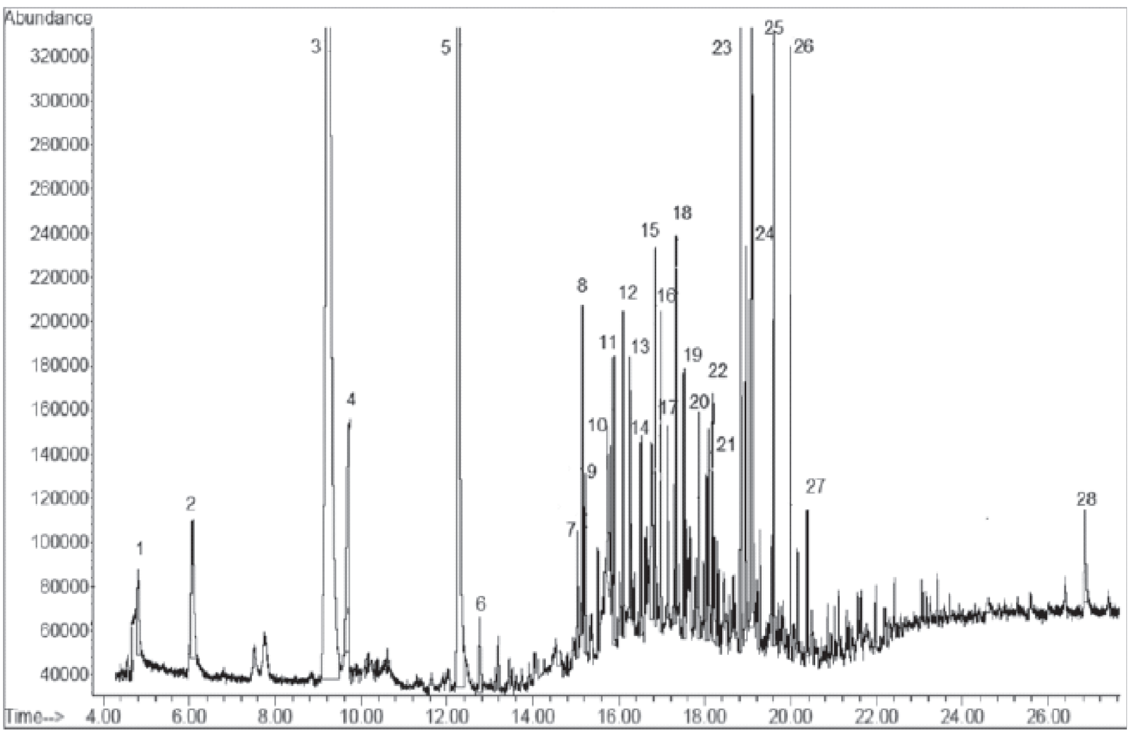

Figure 5: Chromatogram of volatile compounds identified through SPME/GC/MS. (1, Hexanal; 2, Isoamyl acetate; 3, Limonene; 4, β-Phellandrene; 5, *p*-Cymene; 6, Acetoin; 7, (*E*)-2-Octenal; 8, (*E,E*)-2,4-Heptadienal; 9, Dehydro-*p*-cymene; 10, Linalool oxide; 11, 3,5-Octadiene-2-one; 12, α-Cubebene; 13, *trans-p*-Mentha-2,8-dienol; 14, Citronellal; 15, β-Cubebene; 16, β-Caryophyllene; 17, Hexadecane; 18, Pulegone; 19, α-Humulene; 20, Verbenone; 21, Carvone; 22, Carvyl acetate; 23, Dihydro-β-Ionone; 24, (*E*)-6,10-dimethyl-5,9-Undecadien-2-one; 25, β-Ionone; 26, Caryophyllene oxide; 27, Humulene oxide; 28, Hexadecanoic acid). *(Reproduced from "Suvimol and Pranee, Int Food Res J 2008; 15:287-295")*[56]

aqueous phase.[61] An oil from the seeds (49% yield) has been found to contain 12.5 % of an unusual fatty acid, ricinoleic acid along with other normal fatty acids. The oil is found to be a good alternative feed stock for production of bio-diesel as evaluated by prominent parameters for bio-diesel such as cetane number, lower heating value and higher heating value.[62]

Stability and Toxicity

In an attempt to study the effects of desiccation and temperature on the storage of seeds of the plant has revealed that 80% of cryostored seeds dried to 13% moisture remained viable compared with the 68% of those dried to 6% critical moisture content.[63] Borde et al.[64] have extracted alkaloids from dried leaves and generated a TLC profile of the extract using butylated hydroxyanisole and butylated hydroxyltoluene as antioxidants for stability testing of the extracts. The acute toxicity study of AME showed that the drug is non-toxic up to a dose of 1750 mg/kg body weight. The LD_{10} and LD_{50} were found to be 2000 and 2250 mg/kg respectively.[65]

Conclusions

Aegle marmelos is ethnically used in various diseases in humans and animals. With standardization of any herbal drug preparation becoming more important for reproducible and safe therapeutic effects, various research groups are engaged in the area. Various separation methods such as TLC, HPLC, HPTLC, GC coupled with varied detection techniques such as UV-VIS, Refractive index and mass detection have been reported for separation and quantification of numerous specific phytoconstituents in the plant. These methods can be conveniently employed as means for standardization of *A. marmelos* as such as well formulation derived from it. In addition to the therapeutic uses, the plant is also well exploited for non-therapeutic uses such as formulation additive, corrosion inhibitor, etc.

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