INTRODUCTION

Oral Submucous fibrosis (OSMF) is defined as a potentially malignant disorder (PMD) and a crippling disease of the oral mucosa induced by arecanut chewing (Chiu et al., 2002; Sudarshan et al., 2012; Warnakulasuriya et al., 2007). It was first described in ancient traditional medicinal books by Sushruta in 600BC as ‘VIDARI’ (progressive narrowing of the mouth) (Riana et al., 2005) followed by Schwartz who in 1952 named it as ‘atrophia idiopathica mucosae oris’. Pindborg and Sirsat in 1966 defined OSMF as “an insidious, chronic disease of the oral mucosa affecting any part of the oral cavity and sometimes the pharynx. Occasionally it is preceded by and/or associated with vesicle formation, and is always associated with a juxtaepithelial inflammatory reaction followed by fibroelastic changes of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa causing trismus and difficulty in eating” (Rajendran, 1994). Historically OSMF was thought to be a passive and irreversible process replaced by abnormal collagen and deposition of abundant extracellular matrix (ECM) including fibrillar collagen.

The disease is predominantly seen in Asian countries namely India, Pakistan, Bangladesh, Srilanka, Taiwan and China with an incidence ranging up to 0.03% - 3.2% in Indian rural population (Hazarey et al., 2007; Saraswathi et al., 2006) and 0.004% in Srilankan population (Dionne et al., 2015). The evaluated association of areca / betel quid chewing and occurrence of OSMF in Taiwanese population is 17.6% (Yang et al., 2001). A cohort study carried out by Ranganathan et al. in 2004, Chennai, South India concluded that the risk of occurrence of OSMF doubled in individuals below 21 years with a mean duration of chewing arecanut or pan masala for 3.5 years, with male predominance. Similar observations were made in a

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population based study in Delhi by Shah and Sharma in 1998. Approximately 10-20% of world’s population chews areca/betel nut (Paulino et al., 2015). Epidemiological studies have reported a malignant transformation rate of 2.3% - 7.6% (Ho et al., 2009) and 3% - 19% (Sudarshan et al., 2012).

OSMF was earlier defined as a multifactorial condition, after several studies the present understanding claims arecanut to be the key factor responsible for disease causation. Arecanut contains alkaloids such as arecoline (being the most abundant), arecolidine, arecaidine, guvacine, and isoguvacine which are present in minute quantities along with polyphenols like tannins and catechins (IARC, 2004). Exposure of the oral mucosa to arecanut constituents generates reactive oxygen species leading to oxidative stresses (Gupta et al., 2004), creates an imbalanced proinflammatory cytokine secretion and leads to the development of fibrosis (Haque et al., 2000).

Fibrosis is a result of excessive and poorly ordered matrix deposition; which affects normal tissue architecture thereby disabling proper functioning of tissues. Investigations into the molecular events underlying fibrosis after tissue insult implicate infiltrating leukocyte inflammatory response as being the causal agent for fibrosis. Furthermore, epithelial damage in the absence of a blood supply, and thus a source of leukocytes, is insufficient to induce fibrotic repair (Adamson et al., 1988). This suggests that sustained epithelial damage or a deficiency in re-epithelialization may be a driving force that underlies fibrotic progression. Experimental studies have explained that prolonged sustained exposure of alkaloids on buccal mucosal fibroblasts leads to a damage triggered inflammatory response and a loss of regenerative capacity (Stramer et al., 2007).
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Another significant predisposing factor in the development of OSMF is oxidative stress. Oxidative stress plays an important role in tissue fibrosis affecting apoptosis and altering the cytokine microenvironment balance. On evaluation, very low levels of antioxidant vitamins have been observed in OSMF individuals. The extent of oxidative damage caused by reactive oxygen species (ROS) can be exacerbated by a decreased efficiency of antioxidant defense mechanism of the body. The maintenance of high intracellular antioxidants is considered crucial in providing a reducing environment within the cell; further being able to protect the cell against ‘oxidative stresses’ (Bose et al., 2012). Antioxidants are molecules that can mitigate oxidative damage by functioning as reducing agents, hydrogen donators, singlet oxygen quenchers and metal chelators.

The epithelium is capable of producing many of the profibrotic cytokines delivered by inflammatory cells such as TGFβ and PDGF (Antoniades et al., 1990). Cytokines play an important role in regulating fibroblast function, such as proliferation, migration and matrix synthesis. It is the balance of these mediators that is likely to play a key role in regulating the initiation and progression of any fibrotic disease. The two distinct stages involved in OSMF are fibroblast proliferation and the production of extracellular collagen matrix (Haque et al., 2000). Activated macrophages or inflammatory cells are known to produce growth factors such as interleukin 1(IL-1), Tumor necrosis factor alpha (TNFa), platelet derived growth factor (PDGF), interleukin 6 (IL-6), fibroblast growth factor (FGF) and transforming growth factor beta (TGFβ) (Kovacs, 1991). Of these both IL-1 and TNFa have been implicated in fibroblast proliferation, collagen production and accumulation in an in-vitro cell line model and in in-vivo animal model (intradermal injections) (Vilcek et al., 1986). Similarly, both IL-6 (stromal activator) and IL-8 have been implicated in
the development of fibrosis leading to excessive production of type I and III collagens (Hasegawa et al., 1999; Ziegenhagen et al., 1998). Studies done by Zhang et al. in 1993 have illustrated and explained the role of these cytokines in promoting fibrosis by activating the TGF beta signaling pathway. In the normal mucosa, fibroblasts reside in the connective tissue which following chronic injury get activated and acquire contractile, proinflammatory and fibrotic properties. The above phenomenon is described as a vicious cycle in which inflammation and fibrogenic cells stimulate each other to create a fibrotic environment.

Varied group of fibrogenic mediators, hormones, growth factors, lymphokines and cytokines mediate collagen synthesis. TGFβ is a pleiotropic cytokine that exists in three isoforms namely TGFβ1, TGFβ2 and TGFβ3; many physiological processes mainly apoptosis, wound healing, fibrosis, immune regulation and tumor biology are contributed by the TGFβ family (Prud'homme, 2007). A predominant mediator amongst TGF beta isoforms is TGFβ1 (highly fibrogenic) which in earlier reports was shown to be upregulated in OSMF tissues (Khan et al., 2012). Tissue fibrosis occurs through the TGFβ1 / Smad signaling pathway. Downstream signaling pathway Smad 2/3 is activated by TGFβ1 to mediate fibrosis via transactivation of type I and type III collagen, which is negatively regulated by the inhibitor Smad (Smad7) via the ubiquitin-proteosome degradation mechanism (Tang et al., 2012). Due to the constant stimulation of TGFβ1 there is an abnormal deposition and degradation of type I and Type III collagen in the submucosa leading to a complete fibrotic phase. OSMF has also been termed as a collagen metabolic disorder. As the disease progresses there is decreased functioning of the enzyme collagenase and increased
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functioning of the major cross linking enzyme Lysyl Oxidase (Gupta et al., 2008). Ultimately, fibroblasts are the main ECM producing cells in the injured mucosa.

Various treatment options have been established till date (Arakeri and Brennan, 2013; Mehrotra et al., 2011).

i. Conservative therapy: physical therapy.

ii. Medical therapy: steroids, interferon gamma, placental extracts, pentoxyfylline, betacarotene, collagenase, hyaluronidase, chymotrypsin, iron and multivitamin supplements.

iii. Surgical Therapy: Extraoral flaps, Intraoral flaps, Microvascular free flaps and Alloplasts) and laser ablation.

These have been tried time and again for improving the mouth opening; but have proven ineffective as the fibrotic bands recur. Repetitive submucosal injections and multiple needle pricks after a certain time period, lead to trismus which aggravates fibrosis and increases morbidity (Sudarshan et al., 2012). Medicines (pharmaceutical preparations) are chemicals; that cause intolerable short and long term side effects (gastrointestinal symptoms such as dyspepsia, nausea, bloating, bleeding; central nervous system side effects that include headache and dizziness; immunosuppression caused by the progressive use of steroids).

Traditional medicine involving herbal drugs / preparations are available naturally and do not require artificial processing; which makes them cost effective. They have been used for centuries before conventional allopathic medicines were formulated. Herbal medicines are either extracted from or manufactured from an entire plant; the active ingredients in a plant are the natural compounds which lessen the side effects and improve absorption, thereby exhibiting effects that are gentle and safe for long
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term use. Due to the lack of a safe anti-fibrotic drug; investigating a natural mode of treatment such as the use of herbal medicine or phytotherapy would prove beneficial. An ideal antifibrotic agent should be potent, safe, orally bioavailable and inexpensive; such a treatment modality is not yet available (Rockey, 2013).

Evidence suggests that the use of traditional medicinal plants and active secondary metabolites (Siddha, Ayurveda, Unani and Chinese) has proven effective, curative and cost effective in many fibrotic conditions. Studies on Aloe vera in OSMF patients have shown a 5.1mm improvement in mouth opening when compared to steroids (4.6 mm) (Sudarshan et al., 2012). Curcumin an active compound present in turmeric showed potent antioxidant, anti-inflammatory and antifibrotic activity against arecoline induced fibroblasts (Zhang et al., 2012; Zhang et al., 2011). Hazarey et al. in 2015 performed a clinical trial on OSMF patients; he observed that mouth opening in OSMF group (curcumin treated) improved by 5.93 mm whereas in steroid treated group showed an improvement only by 2.66 mm. Lycopene treatment significantly increased the mean mouth opening by 4.46 ± 3.65 mm in OSMF patients when compared to 1.16 ± 1.3 mm in the placebo group (Agarwal et al., 2015). Thus, development of a potent and effective herbal anti-fibrotic formulation is the need of hour.

Centella asiatica Linn. (C asiatica L.) is an age old traditional medicinal plant, which has been used effectively in Ayurvedic and Chinese medicine for the treatment of various ailments. C asiatica L. has been known to demonstrate anti-inflammatory, anti-microbial, anticancer, antioxidant and wound healing potential (Roy et al., 2013). These medicinal properties are attributed to the presence of a vast reservoir of a group of secondary metabolites known as triterpenoids (asiaticoside,
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madecassic acid, madecassoside and asiatic acid). *C. asiatica* L. has been reviewed and listed as an ideal anti-inflammatory and antifibrotic agent. It is known to stimulate TGFβ3, decrease TGFβ1, decrease matrix deposition including collagen and fibronectin, decrease IL-6, increase Smad 7, decrease Smad 2, 3 & 4, complex iNOS and COX-2 inhibition (Widegrow, 2011). Asiatic acid has shown to combat liver fibrosis by inhibiting the TGFβ1 signaling pathway (Xu et al., 2013).

**Ocimum basilicum** Linn. (*O. basilicum* L.) also known as ‘Sweet basil’ in English belongs to the Lamiaceae family, it is a perennial herb with therapeutic properties; used both in Ayurvedic and Unani system of medicine (Bilal et al., 2012). Earlier studies have illustrated its antioxidant, (Politeo et al., 2007) anti-inflammatory (Benedec et al., 2007), and antifibrotic properties (Yacout et al., 2011). The aromatic leaves of *O. basilicum* L. contain a rich reservoir of phenolic compounds, flavanoids and volatile oils (monoterpenoids and sesquiterpenoids). Some of the volatile oils with immense therapeutic potential are eugenol, linalool, cis-geraniol and 1, 8-cineole (Politeo et al., 2007; Bilal et al., 2012). Plants of the Lamiaceae family are known to impart antifibrotic effect through TGFβ inhibition and enzymatic digestion of the fibrillar deposits (Dumitriu et al., 2013). *O. basilicum* L. extracts exhibit antifibrotic effect by downregulating the level of hydroxyproline in liver fibrosis induced by carbon tetrachloride (Yacout et al., 2011). Linalool a plant derived monoterpen alcohol rescues the kidney from diabetes-induced nephropathic changes by decreasing the levels of TNFα, IL-6, TGFβ1 and NF-κB (Balasubramaniam and Anuradha, 2011).

**Origanum vulgare** Linn. subsp *hirtum* (*O. vulgare* L. subsp *hirtum*) belonging to the Lamiaceae family is known to have various medicinal uses. It has...
antibacterial, antifungal, antimutagenic, antioxidant, anti-inflammatory, antiparasitic, antithrombin activities (Lee et al., 2005). It contains volatile and non-volatile phytochemical constituents; the main components of the plant comprise gamma-terpene (0.6–3.6%), P-cymene (17.3–51.3%), thymol (0.4–42.8%) and carvacrol (1.7–69.6%). Liang et al. in 2014 demonstrated a decrease in proinflammatory cytokines such as TNF-α, IL-6 and IL-1β levels on pretreatment with thymol for 48 hours in LPS induced inflammation in mouse mammary epithelial cells. On testing Oregano for its anti-inflammatory activity it was observed that at a dosage of 0.5 mg/mL the secretion of proinflammatory cytokines IL-6 was reduced by 25%, while it increased the secretion of anti-inflammatory cytokine IL-10 (Mueller et al., 2010).

In context with the above reported literature, plants and their active secondary metabolites have immense potential to exhibit anti-inflammatory and antifibrotic activity with no side effects in various in-vitro and in-vivo models of diseased conditions. Therefore, three selected species of plants (C. asiatica L., O. basilicum L. and O. vulgare L.) and their active pure compounds (asiatic acid, linalool and thymol) were taken up for further analysis as they may prove to be promising anti-inflammatory and antifibrotic agents in relation to OSMF.