Publications


In-vitro antioxidant activity of ethanolic extracts of Centella asiatica L., Oregano vulgare subsp hirtum and Ocimum basilicum L. via five model systems

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ABSTRACT

Cells undergo oxidative stress under two circumstances, either when optimal nutrition is lacking or during diseased conditions. Under such circumstances reactive oxygen species are produced that cause damage to DNA, proteins and cell membrane. Synthetic antioxidants cause several side effects when compared to natural antioxidants. Antioxidants in plants, herbs, spices, fruits and vegetables are present in the form of active constituents namely terpenoids, flavonoids and polyphenols which can play a protective role in inactivating harmful reactive oxygen species. The activity of antioxidants largely depends upon their ability to chelate metal ions or scavenge free radicals. In this study, on comparing the antioxidant activity of Ethanolic extracts of Centella asiatica L. (ECA), Oregano vulgare subsp hirtum (EOV) and Ocimum basilicum L. (EOB) via five different model systems we observed that ECA showed maximum activity via Nitric oxide radical scavenging assay and superoxide radical scavenging activity. Erov via Total Antioxidant assay and Reducing power assay and EOB via 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay with maximum activity at 1000µg/ml (p<0.01). The results suggest that Ethanolic extracts of Centella asiatica L., Oregano vulgare subsp hirtum and Ocimum basilicum L. exhibited potent antioxidant activity and their active constituents can be further evaluated and studied for formulating a polyherbal antioxidant drug.

Keywords: Centella asiatica L., Oregano vulgare subsp hirtum and Ocimum basilicum L., antioxidant

1. INTRODUCTION

Oxidative stress is defined as an imbalance between oxidants and antioxidants, which results in subsequent damage to cell molecules constituting to the production of reactive oxygen species (ROS). ROS such as hydroxyl ions (OH·), superoxide radicals (O₂⁻), peroxide (ROO·) and nitric oxide radicals are produced in living organisms during excessive metabolism; causing several pathophysiological states such as cardiovascular diseases, neurodegenerative diseases, cancer, inflammatory conditions and aging. The most effective way to eliminate and suppress the action of ROS is with the help of antioxidants. Antioxidants both endogenous and exogenous, wether natural or synthetic can prove effective in counteracting free radicals by scavenging or suppressing them.

The use of antioxidants was initiated by ancient Egyptians; they used plants having high content of phenolic compounds to preserve dead bodies (John, 2010). In India plants are being used as a source of traditional medicine since the times of Charaka and Shushruta. Medicinal plants have global importance due to the presence of phytoconstituents with effective pharmacological action. Today several synthetic antioxidants like Butylated hydroxytoluene (BHT) and Butylated hydroxynapthole (BHA) are available commercially and are being used as preservatives in processed food. These synthetic antioxidants are carcinogenic and cause several side effects (Ito, 1983). Today natural antioxidants contained in rich amounts in plants, fruits and vegetables have attracted interest due to their nutritional, safety and therapeutic value. Natural antioxidants are present in the form of phenolic compounds in plants (Kahkonen, 1990). Scientific studies in literature have indicated promising phytoconstituents that can be extracted from single or multiple plant extracts for the development of herbal drugs. Nutraceutical formulations are cost effective and have minimum side effects and this is one of the major reasons that the quest for formulating a prime natural antioxidant has become a major scientific research and industrial challenge. Considering the advantages of natural antioxidants over synthetic antioxidants are study aimed at comparing the antioxidant activity of ethanolic extracts of Centella asiatica Linn, Oregano vulgare subsp hirtum and Ocimum basilicum Linn via five different antioxidant assay systems.

Centella asiatica Linn belongs to the ‘Apiaceae or Umbelliferae family’; it is an herbaceous plant native to Sri Lanka, Malaysia and all over India. In Tamil Nadu it is known as ‘Vallarai Keerai’ and is routinely cooked in households. Other well known names are Pennywort, Mandakarni,
Pharmacognostic evaluation of leaves of Ocimum basilicum L: the Lamiaceae family
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ABSTRACT
Pharmacognostic investigation of the leaves of Ocimum basilicum L. was carried out to determine its
morphological, micromorphological, and preliminary phytochemical screening profiles. The anatomy of the
leaves of Ocimum basilicum reveals single, wide and bowl shaped vascular strand, dicotyley type of stomata; peltate
types of glandular trichomes seen on the epidermis of the lamina. The phloem is seen as small discrete masses and
xylem elements are angular and narrow. Under powder microscopy epidermal trichomes are seen predominantly.
Phytochemical analysis shows the presence of triterpenoids, flavanoids and polyphenols. These
observations could be of immense help to validate the several medicinal properties of Ocimum basilicum L. and
would also add value in the botanical identification and standardization of the leaves of the plant in the crude form
for nutraceutical purposes.
Keywords: Ocimum basilicum L., macro morphological, micromorphological and phytochemical screening

INTRODUCTION
Medicinal plants are a blessing to humanity. Traditional medicinal preparations are being used since the
times of ‘Charaka’ and ‘Sushruta’. One such perennial herb with therapeutic potential is ‘Ocimum basilicum L’
popularly known as ‘Sweet basil’ used in both ‘Ayurvedic’ and ‘Unani’ system of medicine (Ahl, 2012). It is a
culinary herb that belongs to the ‘Lamiaceae family’ and is well distributed throughout India. Ocimum is known
for its antioxidant, antimicrobial, antifibrotic and anticancer properties (Pote, 2007). The aromatic leaves of
O. basilicum contain a rich reservoir of phenolic compounds, flavanoids and volatile oils (monoterpenoids and
sesquiterpenoids) (Juliani, 2002). It is used as a treatment modality for various ailments such as poor digestion,
nausea, migraine, depression, insomnia, kidney malfunction and skin infections (Muafia Shafique, 2011; Biljana,
2011). Inspite of the various medicinal uses attributed to this plant, there are not many pharmacognostical reports
on the leaves of this plant in particular. Hence, our work deals with morphological (macroscopic and microscopic
characteristics) and preliminary phytochemical screening of the leaves of basil from Tuticorin, Tamil Nadu. This
information could be of immense help to researchers working with the leaves of O. basilicum in in-vitro and in-vivo
experiments.

MATERIALS AND METHODS
Collection of Specimen: The leaves of O. basilicum were collected from Tuticorin, Tamil Nadu. The plant was
taxonomically identified by Dr. P. Jayaraman, Plant Anatomy Research Centre, Chennai, Tamil Nadu, India. The
macroscopic features were described by Dr. D. Chamundeeswari, Principal, College of Pharmacy, Sri Ramachandra
University, Chennai. Healthy plants were collected in the month of April and processed for evaluation. Different
parts of the leaves were sectioned with 15mm Blood pressure blade and immediately fixed in FA4 (Formalin (5ml)
+ Acetic acid (5ml) + 70% Ethyl alcohol (90ml)). After fixing the specimen for 24 hours; they were dehydrated in
graded series of Tertiary-Butyl alcohol (TBA) following which the specimens were infiltrated with paraffin
wax(melting point 58-60°C) and casted into paraffin blocks.
Sectioning: Rotary microtome was used to section the paraffin embedded specimens. The section thickness was
10-12μm. The dewaxed sections were stained with Toluidine blue and Safranin. Paradermal sections were taken for
studying venation pattern, trichome distribution and stomatal morphology. The leaf sections were then cleared with
5% Sodium Hydroxide (NaOH) or epidermal peeling by partial maceration by Jeffrey’s maceration fluid. Temporary
preparations mounted with glycerine were made for cleared materials. Clearing with NaOH was carried out
for powdered materials which were later mounted in glycerine medium after staining. Different cell components
were studied and measured.
Photomicrographs: Micrographs of tissues were supplemented along with microscopic description wherever
necessary. Photographs were taken with Nikon lab 2 microscopic unit at different magnifications. Bright field
microscopy was used for normal observations.
Annexure

ORIGINAL ARTICLE

Oral Biosciences

Antifibrotic effect of Centella asiatica Linn and asiatic acid on arecoline-induced fibrosis in human buccal fibroblasts

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Keywords
antifibrotic, collagen, herbal medicine, oral submucous fibrosis, transforming growth factor-β1.

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Abstract
Aim: The aim of the present study was to investigate the in vitro antifibrogenic effects of Centella asiatica (CA) and its bioactive triterpene aglycone asiatic acid (AA) on arecoline-induced fibrosis in primary human buccal fibroblasts (HBF).

Methods: An ethanolic extract of CA was prepared, and AA was purchased commercially. High-performance thin-layer chromatography (HPTLC) was performed to quantify AA in the CA extract: colorimetric assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was performed to determine an half-maximal inhibitory concentration. HBF were cultured and stimulated with arecoline. The inhibitory effects of CA and AA at different concentrations were assessed using gene-expression studies on fibrosis-related markers: transforming growth factor-β1, collagen 1 type 2, and collagen 3 type 1. The stimulatory effect of arecoline and the inhibitory effect of AA on fibroblast morphology and extracellular matrix were assessed qualitatively using Masson trichrome stain.

Results: The HPTLC analysis determined 1.2% AA per 100 g of CA extract. Arecoline produced a concentration-dependent increase in the fibroblast markers, treatment with CA significantly downregulated fibrotic markers at higher concentrations, and AA downregulated at lower concentrations. Arecoline altered fibroblast morphology and stained strongly positive for collagen, and AA treatment regained fibroblast morphology with faint collagen staining.

Conclusion: CA and AA can be used as antifibrotic agents.

Introduction
Oral submucous fibrosis (OSMF) is defined as a potentially malignant disorder and a crippling disease of the oral mucosa induced by arecanut chewing.¹ It was first described in ancient traditional medicinal books by Sushruta in 600 BC as “Vidari” (progressive narrowing of the mouth), followed by Schwartz in 1952, who termed it “atrophia idiopathica mucosae oris”.² Pindborg defined OSMF as “an insidious chronic disease of the oral cavity affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and or vesicle formation, it is always associated with 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”.³

The disease is predominantly seen in Asian countries, namely India, Bangladesh, Pakistan, Taiwan, China, and Sri Lanka, with an incidence ranging up to 0.4% in the 

In-vitro human buccal fibroblast cell line model for screening antifibrotic activity of plant compounds in Oral Submucous Fibrosis

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INTERNATIONAL CONFERENCES

1. Attended and presented a ‘scientific poster’ titled ‘Significance of oral evaluation in systemic diseases’, at the XIX National and First International Conference of IAOMP, on 10th & 12th of December 2010 at Mamallapuram, Chennai.

2. Attended the International Conference on Ayurveda at ‘Sai Ram Ayurveda Medical College & Research Center, Chennai, India from 12th – 13th December, 2011.

3. Attended and presented a ‘scientific poster’ titled ‘Expression of transforming growth factor beta receptor 1 in potentially malignant disorder – Oral submucous fibrosis & in Oral squamous cell Carcinoma’, at the International Conference on Cancer prevention, Diagnosis and treatment at Jaipur, on 19th & 20th of January 2011.

4. Attended and presented a ‘scientific paper’ titled ‘In vitro antioxidant activity of Centella asiatica, Ocimum basilicum and Oregano vulgare in Oral Submucous Fibrosis’ at the 25th International Conference of Indian Society for Dental Research, on 5th-7th October 2012, at Chennai, Tamil Nadu, India.

5. Attended and presented a ‘scientific paper’ on “Anticancer potential of thymol, Asiatic acid and linalool in oral squamous cell carcinoma cell lines: An In vitro study”, at the “1st International Congress of Society for Ethanopharmacology” on 8th and 9th of March 2014 at Sri Ramachandra University, Chennai, Tamil Nadu, India.

6. Attended and presented a ‘scientific poster’ on “Wound healing effect of Asiatic acid, Thymol and Linalool on primary oral cultured fibroblasts using scratch assay model”, at the 2nd International Congress of Society for Ethanopharmacology on 20th to 22nd February 2015 at Chitinivas convention center, Nagpur, Maharashtra, India.

7. Attended and presented a ‘scientific poster’ on “In vitro antifibrotic effect of Centella asiatica L. and asiatic acid in arecoline induced human buccal fibroblast cell line model”, at the International Symposium on Oral Submucous Fibrosis on 6th & 7th February 2016 at Hotel Pride, Nagpur, Maharashtra, India.
NATIONAL CONFERENCES

1. Attended and presented a ‘scientific poster’ titled ‘Expression of transforming growth factor beta receptor 1 in oral submucous fibrosis’, at the 30th National conference held on the 18th & 19th of November 2011 at Hyderabad, India.


3. Attended and presented a ‘scientific paper’ on ‘In-vitro Morphological evaluation of Human Buccal Fibroblast cell cultures’, at “MAHER BIOSUMMIT” organized by Meenakshi Dental College and Research Institute on 30th December 2013.

4. Attended and presented a ‘scientific paper’ on ‘In-vitro antifibrotic activity of Ocimum basilicum L. and linalool in arecoline induced human buccal fibroblast cell line model’, at the National Conference ‘HERBESCON’ on Phytochemicals as Biotherapeutics: Unravelling the Mystery of Natural products held on 17th & 18th February 2016 at Sri Ramachandra University, Chennai, Tamil Nadu, India.
AWARDS


2. Received “Best paper award” for presenting “In-vitro Morphological evaluation of Human Buccal Fibroblast cell cultures’, at “MAHER BIOSUMMIT” organized by Meenakshi Dental College and Research Institute on 30th December 2013.

3. Received “Overall 2nd best poster award”, Manjushree Pal Memorial Award for presenting a poster on “Wound healing effect of Asiatic acid, Thymol and Linalool on primary oral cultured fibroblasts using scratch assay model”, at the 2nd International Congress of Society for Ethanopharmacology, held on 20th to 22nd February 2015 at Chitinivas convention center; Nagpur.

4. Received “Overall 1st Scientific presentation award’ for presenting on “In-vitro antifibrotic effect of Centella asiatica L. and asiatic acid in arecoline induced human buccal fibroblast cell line model”, at the International Symposium on Oral Submucous Fibrosis, held on 6th & 7th February 2016 at Hotel Pride, Nagpur, Maharashtra, India.