SYNOPSIS

1. TITLE

DEVELOPMENT OF CHEMO-GENETIC METHOD OF STANDARDIZATION OF SOME PLANTS OF CHHATTISGARH AND THEIR ETHNO-PHARMACOLOGICAL VALIDATION

2. INTRODUCTION

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. Nature has bestowed our country with an enormous wealth of medicinal plants; therefore India has often been referred to as the Medicinal Garden of the world. Countries with ancient civilizations such as China, India, South America, Egypt, etc. are still using several plant remedies for various conditions. In this regard India has a unique position in the world, where a number of recognized indigenous systems of medicine viz., Ayurveda, Siddha, Unani, Homeopathy, Yoga and Naturopathy are being utilized for the health care of people. No doubts that the herbal drugs are popular among rural and urban community of India. The demand for plant based medicines, health products, pharmaceuticals, food supplement, cosmetics etc are increasing in both developing and developed countries, due to the growing recognition that the natural products are non-toxic, have less side effects and easily available at affordable prices **(Kalia et al.2005)** .Herbal drugs or medicinal plants extract and actives have demonstrated spectrum of biological activities. Such have been used and continued to be used as medicine in folklore or food supplement for various disorders **(Barnes et al. 2004)**.The efficacy of a number of herbal formulations has been tested by valid phyto-pharmaceutical techniques and the number of plant-based drugs or health foods has increased steadily to meet the growing demand. Over the years a new relationship between phyto-chemists and pharmacologists has accordingly developed which in many cases has proved to be very productive **(Acharya 2011)**. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems.

There are about 45,000 plant species in India, with high concentration in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island out of 6000-7000 are estimated to have medicinal usage in folk and documented systems of medicine. India is the largest producer

of medicinal herbs and is appropriately called the botanical garden of the world **(Dasgupta 2010)**.Nearly 44 % geographical area of the Chhattisgarh state is under forests and is very rich in biodiversity because of favorable agro climatic conditions. In the herbal drug technology the botanical materials converted into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. The use of chromatographic techniques and marker compounds to standardize botanical preparations since long time but this marker is affected by several factors. DNA-based molecular markers overcome the drawback of chemical marker and it has utility in the fields like taxonomy, physiology, embryology, genetics, etc. DNA-based techniques have been widely used for authentication of plant species of medicinal importance whereas chemical method used for quantification of secondary metabolites. Thus chemo-genetic developed fingerprinting markers involve the screening of ethno-medicinal plant to ensure its quality, safety and efficacy. Further, it is also an important tool for establish a new ethno-pharmacology prospective for ethno-medicinal plant with high trade potential.

3. OBJECTIVEOF RESEARCH

The present research work will take into account the following broad objectives:

1. Pharmacognostic standardization of selected medicinal plant.
2. Development of chemical and genetic fingerprinting of selected medicinal plant
3. Development of preclinical toxicity profile of selected medicinal plant.
4. Pharmacological screening of medicinal plant.

4. BRIEF REVIEW OF THE WORK ALREADY DONE IN THE FIELD

Bahmani et al.2013 studied the genetic diversity of 25 fennel ecotypes of Iran using RAPD and proved A relationship between fennel ecotypes with their geographical distributions and the climate condition.

Oman et al.2013 determined genetic diversity among six commercial varieties of "Coleus blumei " for identification the best quality of genetic similar and concentration of total phenolic compounds in the leaf tissues of the six varieties.

Pathaket al.2013 developed a protocol for isolation of good quality of DNA that is free from using minimum chemicals. The quality of isolated DNA was confirmed by spectroscopic, electrophoresis and PCR for carrying out genomic studies.

Chesteret al.2013 studied the genetic and metabolic variability in *S. rebaudiana* among accessions of different geographical regions of India using random amplified polymorphic DNA (RAPD) markers and high-performance thin layer chromatography (HPTLC) analysis. The study revealed that two accessions found superior genotype in context to RAPD and HPTLC analysis.

Sairkaret al.2013 studied the nine different methods for DNA isolation Optimized the previous process of DNA isolation and enhancement of RAPD PCR for low quality genomic DNA of *Terminalia arjuna*.

Singh et al.2013 characterized coriander varieties using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and random amplified polymorphic DNA (RAPD). SDS-PAGE of total soluble seed proteins and RAPD based DNA fingerprinting or polymorphism can differentiate the coriander varieties. The polymorphism of the total seed protein can serve as supplementary and RAPD analysis as confirmatory for cultivar identification of coriander genotypes.

Sairkaret al.2013 Studied distributive pattern of genetic variation of both the species W. somnifera and R. serpentine using RAPD analysis which provides an important baseline data for conservation and collection strategies for this species.

Singh et al.2013 studied 4 species of the genus Asparagus, with help of RAPD. This study distinguishes and authentic A. racemosus with other three species, thereby aiding information of drug standardization, its collection, management and conservation.

Gahlautet al.2013 distinguishes *Polyalthia longfolia* is mostly used as substitute of *Saraca asoca* and *Wrightia tinctoria* and *Wrightia rothii*, adulterants/ substitutes of Holarrhena pubescence (Buch. Ham) Wall. ex. G. Don. With the use of RAPD–PCR analysis involving 8 decamer random primers was used to assess the quantum of genetic variation at genomic level.

Kumar et al.2013 evaluated diversity among 46 samples of *C. decidua* using chemical parameters and random amplified polymorphic DNA (RAPD) markers and concluded that the chemical-based diversity will assist in selection of nutritionally rich samples for medicinal purpose, while genetic diversity to face natural challenges and find sustainable ways to promote conservation for future use.

Ali et al.2012 analyzed Genetic and essential oil variations from eight Tunisian natural populations of Thymus algeriensis Boiss were assessed using 47 terpenoids and 154 RAPD markers amplified by seven selected primers. The study revealed that the genetic and chemical structures are in accordance with geography distances indicating isolation by distance.

Ganie et al. 2012 authenticate the plant materials traded as shankhpushpi through RAPD-based markers for Fresh samples of C. pluricaulis, E. alsinoides and C. ternatea, and market samples of crude-drug markets of different geographical regions of India.

Hussain et al.2012 authenticate Picrorhiza kurrooa and its adulterant Lagotis cashmiriana by using randomly amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) marker techniques. The unique bands obtained in RAPD amplification clearly discriminated between the two populations of Picrorhiza kurrooa that had similar morphologies.

Ivonne et al.2012 Investigated the relationship of the carotenoid and phenolic profiles with the RAPD fingerprint of three different commercial cultivars of chilli peppers of seven regions of Mexico and establish a separation and differentiation between the chilli species C. annuum L. and C. chinense Jacq , through the RAPD molecular method by using different primers.

Patel et al.2012 developed DNA fingerprints of the leaves of Croton roxburghii Balak ,for its authentification and standardization with the help of unique bands obtained in PCR amplification.

Ryuk et al.2012 established HPLC and RAPD methods for the quantitative analysis and the quality control. Multi-simultaneous discrimination in Phellodendri Cortex. polymorphism of P. amurense and P. chinense were determined by RAPD , utilizes 48 randomly primers. The three constituents (berberine, palmatine and jatrorrhizine) in the extract of twenty Phellodendri Cortex was quantified by HPLC method.

Khan et al.2011 studied RAPD'' molecular marker for the identification of Senna angustifolia , Senna acutifolia , Senna tora and Senna sophera by using 32 decamer primers. Based on study all 4 species were differentiated from each other and showed more divergence. Thus, this technique may prove and to contribute the identification of these species of Senna having similar morphology sold in the local markets.

Khan et al. 2011 developed reproducible markers for authentication of Ruta graveolens from its adulterant Euphorbia dracunculoides with 42 primers. Out of which 10 gave faint

band, 12 gave species-specific reproducible unique band and the remaining did not amplify the DNA. RAPD could thus, serve as a complementary tool for quality control.

Khan et al. 2011 employed the RAPD molecular marker for the identification of *Sennaangustifolia*, *Sennaacutifolia*, *Sennatora* and *Sennasophera* using 32 decamer primer out of which 6 primers yielded species-specific reproducible bands.

Sheorey et al.2011 explicate DNA based technique,Random amplified polymorphic DNA (RAPD) which has been extensively used for identification of herbs and herbal preparations due to its rapidity, simplicity, economy, and absence of prior genetic information and requirement of less sophisticated equipment.

Dhanya et al.2010reviewed lists some of the adulterants in powdered black pepper, chilli and turmeric and their detection with special reference to selected molecular markers (RAPD and SCAR).

Guo et al.2010 developed efficient molecular method to better distinguished C. armandii and C. montana, the official species of Caulis Clematidis Armandii from other Clematis species adulterants.

Khan et al. 2010 employed randomly amplified polymorphic DNA (RAPD) technique for authentication of Cuscuta reflexa and its adulterant Cuscuta chinensis with the help of Thirty two decamer oligonucleotide primers. The unique bands obtained in PCR amplification clearly discriminated the two species, having similar morphology.

Khan et al.2010identified the dried seed of samples of Piper nigrum (black pepper) from its adulterant Carica papaya using Eight decamer oligonucleotide primers in the RAPD . Five primer gave species-specific reproducible unique amplicons, which could clearly distinguish genuine as well as adulterant samples having similar morphology.

Huet al.2009 described An effective and low-cost protocol for isolating genomic DNA from root of Rheum Tanguticumt involved a modified CTAB method with distilled water pre-treating samples. The A260/A280 absorbance ratio of extracted DNA, free from polysaccharide, polyphenols and tannins contaminants, ranged from 1.85 to 2.0 within the high level of purity.

Khanet et al.2009 authenticated Glycyrrhiza glabra L.from its adulterant Abrus precatorius L. RAPD analysis using fifty two decamer oligonucleotide primers.

Romero et al.2009 identified molecular markers associated with high quercetin accumulation in the leaves of Psidium guajava L. trees by using Genetic linkage disequilibrium analysis.

Shinde et al.2009 determined the genetic polymorphism of different samples of Tinospora cordifolia using one hundred and twenty decamer oligonucleotide primers in Random Amplified Polymorphic DNA (RAPD) analysis.The result revealed A low genetic diversity among the Tinospora cordifolia samples.

Zhen et al.2009studied the combined Molecular genetic and phytochemical methods to investigate 17 Chinese strains of Armillaria mellea. RAPD primers. The fingerprints obtained with HPLC could be readily utilized as a quality-control method for pharmaceutical grade Armillaria mellea.

Liu et al.2008 authenticated Astragalus medicines form Hedysarum polybotrys, common adulterant of Astragalus radix in Taiwan by using sequence characterized amplified region (SCAR) markers

Khan et al. 2007developed an efficient protocol for isolating higher yield of genomic DNA from fresh and dry roots of medicinal plants in lesser time.

Shinde et al.2007 determined the components in an Ayurvedic herbal prescription, Rasayana Churna (dried stem of Tinospora cordifolia , dried fruit of Emblica officinalis and dried fruit of Tribulus terestris) by using One-hundred-and-twenty RAPD primer. They have concluded that Primer OPC-6 simultaneously generated three distinct amplicons, each specific to one component.

5. NOTEWORTHY CONTRIBUTION IN THE FIELD OF PROPOSED WORK

Lack of standards of safety and efficacy of the traditional herbal drugs is issue.These herbal drugs are inconsistent in quality, and their active principles are not known. Further, confusion in the identification of medicinal plants and their substitutes, adulteration, lacking of valid and reliable scientific information for their therapeutic efficacy are some of the major problems for medicinal plants. The proposed work will provide standard documents for its identity along with correlation of its active phyto-constituents qualitative as well as quantitative through amalgamation of chemical and molecular method. After the development of accurate and simple chemo-genetic method of standardization for the medicinal plant, we can achieve the global acceptance of traditional medicines.

6. PROPOSED METHODOLOGY OF THE RESEARCH WORK

The proposed research work will be performed as per the following scheme:

1. Review of literature
2. Collection of traditionally used plant material.
3. Authentication of plant.
4. Extraction and standardization of plant material as per the WHO.
5. Development of chemical and molecular fingerprinting
    A. Spectroscopic and chromatographic analysis.
    B. PCR based method.
6. Toxicity studies
    - Acute toxicity using mice along with behavioral observations and determination of LD50.
    - Long-term toxicity studies.
7. Pharmacological evaluation.
8. Compilation of data and results

7. EXPECTED OUTCOME OF THE PROPOSED WORK

Authenticity of traded medicinal plant is important to assure consumer for quality of medicinal plant which protect fraudulent practices, commonly seen in unscrupulous trade. The proposed research work will lead to exploration of molecular method with combination of chemical method, which has been successfully used for authentication of medicinal plants and its active constituents in commercial preparations. Thus, present work will bring the credibility of chemo-genetic fingerprinting marker for documentation and validation of ethno-pharmacology for its value addition correlation of traditional medicinal plant for betterment of human society.

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